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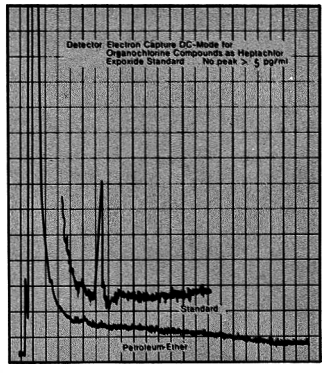

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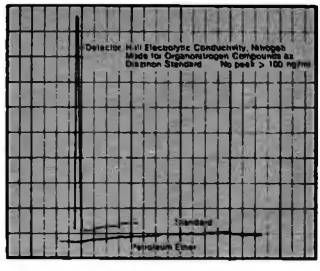

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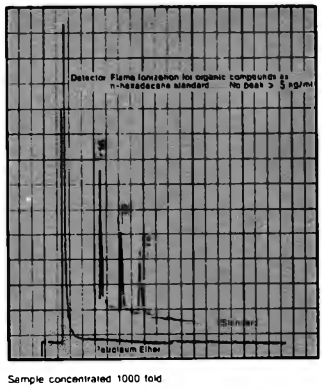

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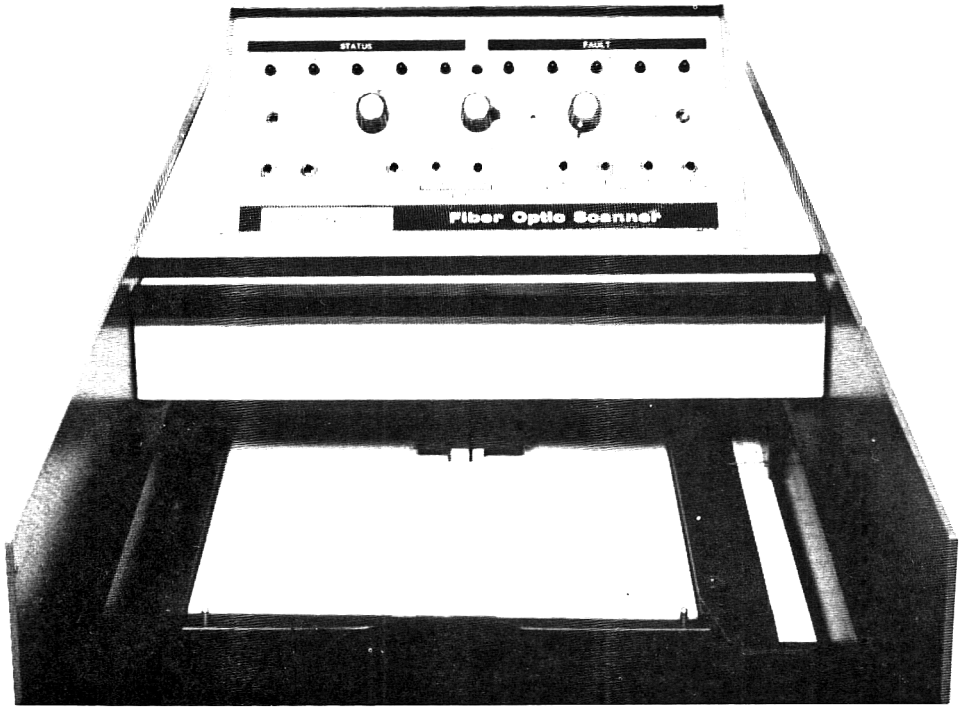

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NOTICE TO READERS ABOUT COPYING

PRESIDENT'S ADDRESS

Growing Pains

HELEN L. REYNOLDS
Food and Drug Administration, Bureau of Foods, Washington, DC 20204

When I was a youngster, adolescents who complained about cramps in their arms and legs used to be reassured by their parents: "Don't worry—those are just growing pains." And it was reassuring to be told that these mysterious aches were a normal part of growing up—of reaching that enviable status of adulthood, which meant freedom from parental control. Growing pains seemed a small price to pay for the advantages of being grown up, independent, able to make decisions, and entitled to certain rights and privileges. As we all instinctively realize, growth is good because it usually signifies life and health; when we stop growing, we begin to die a little.

The AOAC may be said to have enjoyed a long and relatively secure childhood. It began life in 1884 under the protection of the U.S. Department of Agriculture; then from 1927 until the mid-1970's, the Food and Drug Administration acted as parent. As in real childhood, that parental protection was a mixed blessing. The parent was sometimes generous and caring, at other times neglectful or critical. Again, as in real life, as the family fortunes fluctuated, so did the fortunes of AOAC. To extend the analogy still further, we all know that some offspring contribute more to the well-being of the family than others; they do chores, run errands, maybe even assume part of their own expenses through scholarships and part-time jobs. In this respect, AOAC has been a "good kid." It performed a number of very useful chores for its sponsors and earned a substantial part of its own support through sales of its publications.

But every childhood comes to an end some time, and several years ago the Food and Drug Administration decided that the time had come for AOAC to leave the nest. Even though the departure was extended over several years, the severance of the long-established ties provoked a number of "growing pains" for AOAC. Habits and relationships of more than 90 years can't be broken in a few weeks or months, and anyone embarking on independence is bound to make a few mistakes. However, we should think of these growing pains as signs of life and vigor and as a normal stage in AOAC's continued growth and development.

Some of the earliest problems of becoming fully independent are in the process of being solved. They are the obvious ones of finding new quarters, building a professional staff, locating various sources of income, and learning modern techniques of marketing and fiscal management. At first these problems were so pressing that they occupied almost the entire time and attention of AOAC management. In time, however, as progress began to be made with these primary problems, others which were less obvious

Presented at the 95th Annual Meeting of the AOAC, Oct. 19-22, 1981, at Washington, DC.

became more visible. Some of them were closely connected with the new independence of AOAC; others were not, but were late in being recognized because they were developing during a period when the attention of AOAC management was otherwise occupied.

For example, there is the question of membership. What does it now mean to be a member of AOAC? Until relatively recently, the answer was clear. A member of AOAC was a scientist employed by a regulatory agency. That's what the AOAC constitution said. Yet it was apparent that scientists from industry and the academic community play very active roles in AOAC; they publish papers in the Journal, attend the annual meeting and the spring workshop and give presentations, serve as Associate Referees and as collaborators in methods trials, and have been responsible for developing and testing a number of the methods that have been adopted as official. As their contributions have become increasingly important to the life and well-being of AOAC, the concept of Associate Membership has been developed. In return for the payment of modest dues, someone not eligible for full membership can become an Associate Member, with a membership card. This past year, however, that concept became inadequate when, for the first time in its history, AOAC appointed employees of industrial and academic organizations as General Referees and members of Methods Committees. Recently when the Long-Range Planning Committee was discussing the possibility of an increase in dues for Associate Members, it became evident that the old concept of membership is no longer adequate.

What does it really mean to be a member of AOAC today? What are the rights and the responsibilities of membership? Is the distinction between full membership and associate membership still valid? If so, can it still be defined simply by whether the member's employer is or is not a government agency? Shouldn't the individual contributions and qualifications of the member be more meaningful criteria? And what are the benefits of being a member of AOAC? What do members have a right to expect from the Association? What obligations do its members owe to AOAC? Is membership in AOAC essentially different from membership in other scientific and professional organizations? These questions can't be answered by a casual sentence or two. They will probably occupy a considerable share of the work of the Long-Range Planning Committee for the next couple of years and the answers may require further changes in the constitution of the Association.

In the same connection, much more is now required of the officers and standing committees of AOAC. Not long ago, the Board of Directors were able to conduct their share of the Association's management by meeting for one day a year. Now the Board meets four times a year and takes many other actions between Board meetings by phone and mail ballots, yet the Board still has difficulty in completing the agenda. The Long-Range Planning Committee, which didn't even exist 25 years ago, now must meet for a total of at least 3 days a year, besides conducting some of their business by correspondence. Similar commitments are being required of a number of other boards and committees of AOAC, even though the full-time staff of the Association is far larger than it was during AOAC's long and sheltered childhood. Nor is all of this activity a demonstration of Parkinson's Law that work expands to fill the time available to do it.

One of the most serious problems that AOAC is now attempting to solve is that of the decrease in new methods and collaborative studies. This problem has been the subject of long and serious discussions among the Board of Directors, the Long-Range Planning Committee, and the staff. Among the many reasons proposed for this decrease is that regulatory agencies are operating differently now. Whereas in the past their pattern was to gather laboratory evidence and then take legal action, now they tend to rely much more on the promulgation of regulations and standards. This pattern may change again because of the current reaction against new regulations. Meanwhile, however, less emphasis is being placed on the need for validated methods. Even when analytical evidence is still gathered in support of regulatory activity, the traditional mode of the individual analyst in the laboratory performing the various steps of a method is yielding to reliance on automated systems in which several complex instruments are interfaced under the control of a computer that processes and monitors large volumes of data, makes calculations, smooths curves, and

does everything but sign the report for the analyst. Inevitably the nature of analysis and methodology is changing. Will this change mean that fewer new methods are needed? Even though it is not really true that the methods aren't needed, will administrators perceive it to be true, and will they provide less support for development and testing of methods?

By the same token, collaborative studies seem to be changing. Because the new instruments and systems require a different type of expertise to operate, it has become increasingly difficult and expensive to conduct a collaborative study as the AOAC has defined it. The requirement for 6 laboratories to participate has become a pressing problem. Some agencies and large organizations are now conducting their own validation studies with as few as 3 laboratories. Can this arrangement be reconciled with the AOAC criteria for collaborative studies? Is some type of compromise possible? For example, how should we define a "laboratory"? The purpose of specifying several laboratories is surely to reveal the variability among analysts in different locations and under different supervision and to balance out biases to the extent possible. But how far apart must two laboratories be located physically to serve this purpose? We recognize that two field laboratories of the same agency in different cities are separate laboratories, but suppose, for reasons of constricted space, portions of a division of an agency are scattered in several locations in the same metropolitan area, 5 or 10 miles apart? Do they qualify as separate laboratories or not? Or how about the actual situation in the Food and Drug Administration in which several of our scientists are stationed at the National Bureau of Standards because NBS has a reactor but FDA does not? In a collaborative study, would the NBS analysts and the FDA analysts be counted as one laboratory or two? This may seem to be a frivolous question, but if we are rejecting collaborative studies for being one or two laboratories short of the required six, the question is not so frivolous after all.

The original concept of the collaborative study was a small stroke of genius. We tend to take it for granted today, but at the time it was devised, it solved a perplexing problem by offering a solution that was both satisfactory and simple, yet capable of being extended to meet increasingly sophisticated demands. Can this concept be adjusted and extended again to meet present demands? If not, will the collaborative study fall into disuse just as its virtues are being recognized by other organizations and other countries who long refused to accept it? Do we have present-day geniuses in AOAC who will meet this challenge?

On a more mundane level, AOAC has the familiar "growing pain" that is being shared by most organizations today, that of raising funds in a period of high inflation, cutbacks in traditional support, and sharp competition for the limited money that is available. Even mature associations that have been independent and successful for many years are facing this problem. It seems like more of a growing pain for AOAC because the Association is only now beginning to develop the mechanisms and expertise to deal with it. As part of the same problem, many AOAC services that formerly could be provided at cost now must be priced high enough to cover their share of administrative overhead. This comes as a shock to some members who were used to AOAC's low prices and free services, and it has also taken some getting used to for long-time AOAC staff members who were used to operating under the old system. Still another facet of this same problem is the need for the AOAC staff to learn marketing methods and sales techniques, and to convince themselves that salesmanship is really compatible with professional science.

AOAC is sharing in a growing pain that is affecting all people in all phases of modern industrial civilization: the accelerating pace of change. Commentators on the human scene have often remarked on the tremendous changes in daily life that have taken place just since the turn of the century or even since 1950. A large proportion of the things that we use and depend on every day were rare or even unknown 100 years ago: electricity in the home, for example; a safe and readily available water supply; automobiles and airplanes; radio and television; plastics; synthetic fabrics; computers; satellites and space travel; frozen foods; antibiotics. Our very language has had to change accordingly: "a trip to the moon" no longer serves as a symbol of dreams and fantasy. As we all know, despite fears about foreign substances in food and the supposed disadvantages of processed food, human beings now are growing larger and living longer than even

the most visionary prophet would have dared to predict 100 years ago. Paradoxically, increased longevity, like many other advances, has proved to be a mixed blessing with its own problems.

Like daily life, laboratory work has been transformed. Anyone who leafs through past volumes of the AOAC Journal can watch this transformation happening year by year. "Wet" chemical methods for single analytes at percentage levels were gradually replaced by methods centering around the spectrophotometer, then the gas chromatograph, then the mass spectrometer and other sophisticated, complex instruments that determine several or many analytes at levels that have dropped to milligrams, then micrograms, then nanograms, and lower. As I mentioned above, the sophisticated instruments are now being incorporated into automated systems controlled by computers so that to a casual observer the daily routine of the laboratory has changed drastically in just the last generation. Obviously analysis is being done differently today from the way it was done when the AOAC was founded. Moreover, the line between disciplines is dissolving to a large extent. From its earliest days, AOAC has adopted some methods that are not "chemical" but rather involved animals, bacterial organisms, insects, and other non-chemical materials. The number of such methods increased sharply about 10 years ago. Since a large proportion of our Associate Referee topics are concerned with biological tests of some kind, AOAC is no longer exclusively an association of chemists. Moreover, AOAC plans to become more active in the fast-growing area of biological and toxicological methods. Thus one of our problems is how to reflect this shift in emphasis in the name of the Association, its governance and management, its programs, and its membership. We are taking some definite steps to resolve this problem. First, the AOAC is entering into a contract with the Federation of American Societies for Experimental Biology to undertake a study of AOAC programs and operations with special emphasis on greater participation in areas related to the biological and life sciences. Second, because no one has suggested a suitable name for the Association that incorporates the concept of participation by scientists other than chemists and yet retains the familiar initials AOAC, we are planning to sponsor a contest to find a new name. Details will be announced in *The Referee*.

These actions represent first steps but not solutions. I have no instant solutions for all of these problems I have listed. It has been my observation that most advances don't come as a blinding flash that transforms the world in an instant. They usually start with an idea in the form of a question—"What if. . .?"—which then must be played with, tried out, reworked, put into words or sometimes an equation, discussed, criticized, sent back to the drawing board, modified, tried out on a larger scale, challenged, debated, strengthened, supported by evidence, until a long time later it begins to look like a solution to a problem. That's a capsule description of how an AOAC method gets developed, validated, and adopted; how the AOAC and indeed most organizations work in practice; how revolutionary discoveries and inventions come into being; and in general how the world gets its work done. Occasionally the process is interrupted by some drastic upheaval, such as a war, an economic depression, or 40 days and 40 nights of rain. These upheavals slow down the process in some areas and speed it up in others. Meanwhile, growth and its accompanying growing pains continue.

But we don't believe that the AOAC should grow up aimlessly, without purpose or direction. We are constantly examining our growing process, deciding whether we should let go of the old and take on the new, and always seeking improvements in our goals, our structures, and our operations. We hope that all of our members and the users of our products will take part in this examination. Let us known how we can grow better and serve you more effectively. Give us a portion of your time and your thought to help us through this difficult period of our history. As Kermit the Frog in the "Muppet Show" says, "It's not easy being green." But by the end of his song he has decided that green is what he really wants to be. It's not easy being the AOAC just now, either, but the AOAC is what we really want to be—a strong, effective, growing AOAC, rendering service to our members and to the public by providing reliable methods for use in the sciences related to agriculture and the public health.

WILEY AWARD ADDRESS

The Total Picture

LEONARD STOLOFF
Food and Drug Administration, Division of Chemistry and Physics, Washington, DC 20204

I take note that the Harvey W. Wiley award is given for past achievements, not future promise, and that it has been the custom of most recipients to recount the highlights of the work that brought them to this podium. For most of this audience that subject could be of limited interest. Let me instead, in this period when the AOAC is searching for its own identity and for support from a wider community than state and federal regulatory agencies, draw on my experience, and that of Dr. Wiley as presented in his autobiography, to develop a viable strategy for the future, a strategy to develop support and participation at all levels of the organization, one that includes the views of the regulated as well as the regulator—in sum, a strategy of the Total Picture.

This is a strategy, probably utopian, of science in its true sense, the search for knowledge and truth in the physical world, unconstrained by the boundaries of practicing disciplines, legal artificialities, and political compromises. The end will still be that product for which the AOAC is properly held in high regard, the compilation of validated methods of analysis, but the charge to each Referee should be broadened: He must be responsible for more than method development; he must be aware of more than the chemistry, biology, mechanics, and electronics of the analysis; he must also be familiar with the purpose for which each method is to be used, and the consequences arising from the reported values.

This is a strategy that depends more on the members than on the officers. It is they who set the tone of the AOAC; it is they to whom new members come to talk and listen; it is they, through the programs they present and the papers they publish in the pages of the *Journal*, who provide the image of the AOAC to new and prospective members. It is also the members in administrative capacities with control of monetary support for meeting attendance who influence participation. Those who venerate the memory of Dr. Harvey Wiley might heed his advice given in an address to the World's Chemical Congress in 1892: "The chemist is a social being, and there is a life outside of the laboratory as beautiful and as useful as the life within. The narrowness of an idea and the flattening of isolation are to be avoided as the purpose on an investigation is to be pursued. . . And thus to widen his horizon and broaden his views the chemist must leave his desk and seek the acquaintance of his fellows. Every time you take a brother chemist by the hand you enlarge your life and extend your strength, and the farther apart the field of your activities, the greater the benefit." Dr. Wiley's feelings were sufficiently strong in this regard that as a young professor at Purdue he saw fit to cover meeting attendance costs out of his own pocket when the university refused to pay his expenses.

To be realistic, it is obvious that no more than a small fraction of the membership of any organization gets to any one meeting, and it is the unusual group that can boast that every member has attended at least one meeting. Members and prospective members for the most part develop their ideas on the relevance of the Association to their needs from the pages of the newsletter (*The Referee*) and the *Journal*.

Presented at the 95th Annual Meeting of the AOAC, Oct. 19-22, 1981, at Washington, DC.

Cecilia Cassidy has done a remarkable job in editing *The Referee*, since she has of necessity performed as both editor and reporter. It has been an excellent vehicle for communication from the Association executive team to the members. Unfortunately, although the lines are open, the members have not responded by using this medium for communicating with the executives and, more important, with each other. In many journals personal communications on technical subjects are provided for in the "Letters to the Editor" columns, which may be the most interesting part of an issue. Lacking such a provision in the *Journal of the AOAC*, and considering the availability of *The Referee* for letters from the members, I urge those who feel the desire to communicate their views or needs to consider *The Referee* as their proper outlet.

The *Journal of the AOAC* is the continuing face of the Association to the outside world. The research presented as papers and reports in its pages may be the only contact, outside of *Official Methods of Analysis*, that we have with the greater scientific community. It is the quality, relevance, and continuity of these papers and reports that will attract others performing similar investigations to publish in the *Journal*. Having made contact with the Association through its editorial and publishing staffs, they may feel impelled to join us.

This, then, is advice on how to display the most attractive face: not a cosmetic job, but that ruddy glow that comes from a healthy body. The scope of papers in the *Journal* is not necessarily restricted to the presentation of analytical methods; papers on applied methodology, such as surveys, and metabolism or stability studies have been published in the past. Perhaps a separate section on applied methodology could make that point more obvious to those looking for an outlet for their research papers. The same consideration applies to papers on the physical properties and reaction chemistry of the analytes under investigation. Why shouldn't we attract those developing the fundamental data on which our methods rest? But format without content is only an empty shell. The quality of the review process is the insurance that only quality papers are published. Those who are called upon to judge whether a paper merits publication must take that task seriously. Their review of what the Association publishes as science ultimately reflects on them. In that regard the introduction of a paper may need as much attention as the body. I have observed that all too often the introduction to a research paper contains a selling job, usually marked by hyperbole, on the importance of the work. As part of the total picture the reason for attempting a piece of research should be presented just as accurately as the work itself. Moreover, a potential reviewer who receives a paper, any part of which is outside his field of expertise, should not be too proud to seek assistance or to refer the paper to someone more competent to judge its quality.

For those members who do get to the meetings there must be more than the camaraderie and exchanges of information in the corridors to compensate for the time and expense. The attendance and audience participation at the spring meetings that I have seen and have been told about argue forcefully for an action-oriented program. Altogether too much of our annual meeting time in the past has been taken up with routine Referee reports recommending nothing more than "continued study." Furthermore, a report on a collaborative study, although essential to our acceptance process, is tedious fare for oral presentation. I suggest that all Referee reports, particularly those containing recommendations for action, be prepared for publication in the September issue of the *Journal*; that all reports of collaborative studies be published as expeditiously as possible and distributed to the appropriate Committee members at the time of submission for publication; and that formal action for adoption at the meeting be taken on only those methods for which the collaborative study has appeared in print for all interested parties to study. This process should allow the Committees more time to consider each proposal for action and, should they desire, eliminate the Sunday premeeting marathon sessions. Method adoption could thus be made a deliberate process for those who are interested, and the Committees of the Methods Board could hold hearings on request for those opposed to a particular recommendation.

The Association has not, insofar as I know, ever turned down a member with a technical report to present, a wise policy that should continue even though it results in unstructured sessions. This is the material from which serendipity is born. Structured symposia with invited speakers can, however, be planned, and I am pleased to see the

Association taking a positive step in that direction with a call for topic recommendations. As might have been anticipated, most of the recommendations I have seen cover broad areas and are much too general to produce a lively forum. Activity at the cutting edge of science is what we should be seeking to uncover, and like the edge of a saw the areas can be sharply defined. The presentations are then more likely to be novel and not a review of old material. The subject matter can be as fundamental as the theory and techniques of tandem mass spectrometry or ion-selective membranes, and as applied as the sources of variance in the routine monitoring of drug dosage forms by AOAC-approved methods, or distribution and impact of dioxins in the environment. In any case, a symposium is not built on a wish for information, but on reportable work. Planning for a symposium should start when the awareness develops that a number of investigators are working in the same area toward the same or similar goals. Activity is invariably a sign of interest at some level of responsibility. An alert Referee should recognize the signs of activity and steer these investigators to report in a common forum—in essence, organize a symposium around work in progress. To this core, contributions on background, impact, and recommendations for future activity can be added.

Having come full circle back to the AOAC Referee, it is now appropriate to amplify my opening remarks. I stated that the Referee must be responsible for more than method development. In this context there can be no differentiation between General and Associate Referees since the title bears no relation to the scope of responsibility, an organizational problem that affects only the pride of the title bearer and certainly has no relation to professional judgment or importance of the task assigned.

The key word for any Referee is "relevance." It is the attribute of a study or project that attracts attention and guides direction. But relevance is an empty word without a statement of the target: in our case, the physical and economic well-being of the public that pays the bill either through taxes or through the price on the item purchased. As we invite those in industry to join with the "official analysts" in the broadening of our Association, remember that the industrial analysts perform many more determinations than are performed by government analysts in monitoring the adequacy of industry controls. Regardless of our roles, the target of relevance is the same, even though it is not susceptible to easy definition.

To develop a feeling for what is relevant, in analyte, in procedures, and in method attributes, a Referee must develop a knowledge of the industry involved, its mode of operation, and the economic processes that support it; he must recognize the purpose of the analysis in its economic or public health aspects; and he must be familiar with the regulatory or grading processes under which the method will be used. Each Referee, whether chemist, microbiologist, or toxicologist, in developing the total picture must rely on advice from the supporting disciplines, but not relinquish to them the responsibility for putting the picture together. There is no discipline so arcane that its ultimate rationalization cannot be explained in common language to a reasonably erudite individual trained in the physical or biological sciences. A Referee with this background is better able to select those analytes for which methods are needed and select or develop those methods most appropriate to the need. There is at present no need to go as far as did Dr. Wiley. As Chief of the Bureau of Chemistry, he required biological evidence of the dangers he perceived in the use of certain food preservatives. Having no supporting Bureau of Toxicology, he set up his own experiments with human volunteers, which I doubt modern toxicologists could improve upon.

Through the dialogues that are required to develop the total picture, a Referee will have established contacts among those having various interests in the analyte and varied approaches to the analysis. A broader audience will have been exposed to the AOAC and its purposes, and a pool of potential symposium participants will have been developed. There is no telling what will happen when different groups with common interests interact. The result could be the fruitful one in Dr. Wiley's favorite apocryphal story: To be able to crop an otherwise arid acre, a farmer lit on the device of planting alternate rows of potatoes and onions; the onions caused the eyes of the potatoes to water to the extent that both crops were adequately irrigated. The result of interaction could be as beneficial for AOAC.

BANQUET ADDRESS

Science and Public Policy

ARTHUR HULL HAYES, JR
Commissioner of Food and Drugs, Food and Drug Administration, Rockville, MD 20852

I was fortunate to have become the commissioner of Food and Drugs just as FDA was beginning its 75th anniversary celebration. I could not help but be impressed by the sense of history I see among so many people who work for FDA or whose work brings them in touch with the Agency.

From my own crash course in FDA history, I can readily perceive that the problems that daily besiege the Agency are not isolated in this time and this setting, but have historical antecedents. As a result, at virtually all of my initial meetings with FDA staff I made clear that I would not decide any issues or make any changes in existing policies and procedures until I understood the historical basis for them.

I have discovered from my study of FDA history that many of the principles applied by FDA to its everyday work in fact stem from policies and concepts nurtured by Dr. Harvey Wiley some 75 years ago. Probably foremost among these ideas is that regulation must have its base in good science. That principle is as true today as it was when Dr. Wiley was enforcing the Food and Drugs Act. Therefore, I would like to discuss science and how it relates to public policy.

I believe that public policy—as reflected in our laws and regulations, or in our societal attitudes—is inseparable from science. Science not only is a critical element in creating public policy, but must form the basis for sound public policies, including regulation. Our permanent dilemma is that each step forward in science, in a sense, destroys a fragment of our existing public policy, and major scientific advances sometimes generate the need for entirely new policies that inevitably must be effected after they are needed.

We need go no further than the history of this organization to demonstrate how this works. Again, I return to the wise Dr. Wiley,

who wrote in his autobiography: "Up to the formation of the Association the methods used in the chemistry of agriculture were crude, inconclusive and in many cases erroneous. An agricultural product analyzed by two or more chemists would present, very frequently, many important variations. If products were sold on an analysis of this kind the purchaser might be defrauded in one case and the seller might be defrauded in another. In either case chaos reigned. The work of the AOAC has brought order out of this chaos."

The work of the AOAC, and the more refined forms of analysis devised and diffused by its members, have brought more than just order. It has also brought a realization of the need for new laws.

There is an interesting section of the *Congressional Record* from the turn of the century in which Congressman Mann demonstrated to his colleagues that new chemical detection methods were revealing new concerns about the food supply. These new methods, he said, found milk containing formaldehyde, boric acid, borax, and sodium bicarbonate; sausages stuffed with sulfurous acid, salicylic acid, and boric acid; baking power adulterated with calcium acid phosphate, alum, tartaric acid, bitartrate of potassium, and calcium sulfate; etc., etc.

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Congressman Mann's list, and the kind of knowledge that it reflected, created the necessary awareness, the realization, needed in a free society to change the then-current public policy and to substitute a new form of policy in keeping with this new knowledge.

And so the Nation found itself in 1906 with a new law that banned from interstate commerce any adulterated or misbranded foods or drugs. In short, what came first was the new science, the new methods of detection; then came the public policy changes to reflect that new knowledge.

This same theme has been repeated in subsequent FDA history. Public policy in food and drug regulation has evolved as it has because analytic capability forced that evolution. Thus, as the Nation gained an increasingly sophisticated ability to detect hazards, the public policies have had to keep up.

For the majority of this century, we have had only a limited capacity to prevent adulteration of our food and drug supply because our methods were, in a sense, weak. They could detect only gross contamination. By the 1950's, however, good toxicology based on colorimetric methods was actually able to detect quantities of various chemical residues in the microgram range, signifying that the barrier between the gross and the subtle had been penetrated. The 1938 law that replaced the pioneer statute of 1906 gained new effectiveness and new meaning each day because of such analytical advances.

Then in the 1960's the curve of analytical progress seemed to become hyperbolic. Analytical appetite grew by what it fed on, as we moved to gas and then thin layer chromatography, and to even greater refinement, sensitivity, and resolving power in the 1970's with radioimmunoassay, mass spectrometry, and other techniques.

Thus, between 1930 and the present the minimum amount of material that routinely could be quantified diminished by many orders of magnitude, a simply staggering advance in resolving power. The chicken-and-egg-like cycle of progress in measurement and reaction in policy seems endless.

Instead of the gross determinations reflected in those early articles in the AOAC Journal, we now see articles that deal with such matters as "Gas-Liquid Chromatographic Determination of Sulfamethazine in Swine and Cattle Tissues;" "High Pressure Liquid Chromatographic-Thermal Energy Determination of *N*-Nitrosodiethanolamine in Cosmetics;" "Spectrophotometric Determination of Phosphorus in Certifiable Straight Color Additives;" "Gas-Liquid Chromatographic Determination of Aniline Metabolites of Substituted Urea and Carbamate Herbicides in Aqueous Solution;" to cite but a bit of the rich fare offered in the July issue of the Journal.

Moreover, this is only the beginning. Recently, FDA initiated a study to identify technologies likely to emerge over the next 15 years, technologies that will have a significant impact on Agency operations and policy. This study, which reflected the thinking of 190 highly qualified professionals, included not only new disease treatment methods, new approaches to diagnosing disease, and new food sources, but also new and emerging methods of detecting hazards and contaminants, such as computer-assisted advances in chromatography in conjunction with such analytical tools as mass spectrometry.

All of these advances might almost be called an embarrassment of riches, for once again science is creating the need for new public policies. Each step in molecular intimacy brings us past the question: "Is it there?" to another, more difficult question: "What does it mean?"

One thing it does mean is that we are facing a challenge that would have warmed the heart of Harvey Wiley. We have to fashion a *public* policy that responds to and accommodates contemporary scientific reality and we must accomplish this through open debate.

Already there is evidence that public policy is being reshaped. In last year's appropriation process, Congress decided that the time had come to have an outside body such as the National Academy of Sciences examine questions about risk assessment procedures and the institutional means of using and acting on them. FDA also asked the Select Committee on GRAS (Generally Recognized As Safe) Substances of the Federation of American Societies for Experimental Biology to consider, based on its 10 years of experience in a massive review of GRAS substances, the processes of scientific evaluation for regulatory decisions.

In addition, I fully support procedures through which scientific questions before FDA scientists receive the closest scrutiny by

scientific peers inside and outside government. We do this now in a variety of ways, for example, through advisory committees which aid in devising scientific protocols or testing guidelines to ensure that our test requirements represent the "state of the art" in the field of toxicology and safety evaluation. We are also doing this now by publishing notices in the *Federal Register* to secure input from the scientific community as well as the general public about critical issues.

A second category of responses to the question, "What does it mean?" is that FDA, and all scientific regulatory agencies, must be able to create flexible regulatory mechanisms to accommodate science's proliferating challenges.

We do this by working with other regulatory agencies through the Interagency Science/Health Coordination Group, which includes the Department of Agriculture, the Environmental Protection Agency, Occupational Safety and Health Administration, and the Consumer Product Safety Commission. This cooperative effort has sought to develop general guidelines for evaluating carcinogenesis data, estimating risks, and producing uniform testing guidelines that enjoy the approval and consensus of both the Government and the public.

We are also doing more to address specific agency problems. For example, on September 9, I appointed a high-level drug approval task force to recommend, by March 15, 1982, specific improvements that go beyond past efforts and address not only the regulatory process but also wide-ranging scientific policy issues. Included in the scope of this group's efforts are:

- the scientific criteria for new drug approval;
- the role of expert advisory committees;
- deregulation of early phases of clinical research;
- improved post-marketing monitoring of drug effects;
- acceptance of scientific studies conducted abroad;
- pediatric testing; and
- needed legislative changes.

Behind every one of those phrases, pushing at the frontier of public policy formation, are tough, new, controversial, science-based decisions that your Association's members' work in part caused and in part will solve. I hope that I can count on your expert opinions as well as your technical expertise to help ensure the quality of the new policies which FDA must fashion.

An analogous arena of policy construction is in place to update this Nation's food safety law. Both Houses of the Congress have bills before them. Both will hold hearings. Many fine reports have been undertaken in the recent past and have well prepared the stage for this legislative renovation. But it is science's advances that show public policy to be rusty. Indeed, the most frequently cited reason for food safety reform—though clearly one among many—is the inflexibility of the Delaney Clause, which requires banishment from our food supply of any added substances shown to cause cancer, when the ability to measure parts per trillion is becoming accepted as commonplace laboratory work.

Already fully in view on FDA's horizon are similar examples within other categories of products for which we are responsible. If certain kinds of low-level, long-term radiation exposure are harmful, either to some people or to all of us, new public policy will need to be erected squarely in the path of the fastest growing industry in the world—the computer revolution that would seem bent on wiring every last one of us into all humanity.

New forms of disease and newly resistant old medical Nemeses are being confirmed more frequently, even as the number of manufacturers of some of the most important biologics weapons known are *dwindling*. There will have to be new public policy addressing manufacturer indemnification *and* new wonders produced by medical science.

Finally, I would propose a third, more philosophical answer to the question of what all this means for both regulators and scientists. Physicist Freeman Dyson gives us such an answer in his biography, *Disturbing the Universe*. He tells how when he was 8 years old, someone gave him a children's book, *The Magic City*, by Edith Nesbit. He carried the memory of that book throughout his life, saying that even as a boy he understood that it was not just another story about some crazy kids, but rather a fable about a crazy universe—one that, as he grew older and learned more, he felt bore a strong resemblance to the universe we happen to reside in.

One of the main themes in *The Magic City* is technology. As Dyson put it, "It is a law of

life in the Magic City that if you wish for anything you can have it. But with this law goes a special rule about machines. If anyone wishes for a piece of machinery, he is compelled to keep it and go on using it for the rest of his life."

Well, beginning with the Greeks we gained a technology, the technology of measurement. Rather than fantasize and speculate about the nature of the world around them, proto-physicists such as Archimedes measured through experiment. The human species had evolved to a point where it wished for a machine to understand the working of the universe, and that wish was granted.

But, just as in *The Magic City*, humankind has been compelled to keep it and to go on using it. Thus, science, from which we once expected sure, quick, and easy answers, now appends to each answer ever more difficult questions. That does not mean, however, that we should, or we can, turn away from science. It means only that we have the machine and we must find better ways to use it *and* to accommodate to what it tells us.

If we have to accept a machine that discommodes society, even as it unlocks door after door to the nature of our crazy universe, what about that aspect of public policy called regulation?

What we ought to seek, I contend, is regulation attuned to the real world. The kind of regulation suitable for a period in which we were detecting arsenic in molasses and slate in coffee cannot be applied to a time in which the 95th Annual Meeting involved some 200 papers on new techniques, methods, and instrumentation for analysis of foods, drugs, pesticides, cosmetics, feeds, fertilizers, mycotoxins, hazardous substances, air pollutants, and other dimensions of public health. Regulation, as a tool of public policy, is also a machine in our magic city. It too has ancient origins. Regulation stems from free people, freely deciding how to limit their own activities for the public good. This was once called the *polis*, named for the body of citizens who participated in government, and from which we get the name for the art of government, politics, as well as the primary form of self-regulation, the police. The Greeks felt that only through such self-limiting, self-regulating, self-government could humans be truly free. I believe there is profound wisdom in that belief.

The real question, then, is not whether to abandon science. We in the Magic City cannot do that and remain civilized. The real question is how to use science and regulation more effectively for the benefit of our society.

Scientists are asking ever more penetrating questions put with increasing precision. We in the regulatory area of public policy must find ways to respond with greater flexibility and more discretion. As the scientist's work becomes more challenging, the regulator's job will just as surely become more complex. To the degree that this must involve, not the automatic response required by abominations in the food and drug supply, but the exercise of judgment upon the subtle, half-understood facts the scientists bring to light, regulators will find the burden of responsibility increasing.

The Bible tells us: "Ask, and it shall be given you; seek, and ye shall find; knock, and it shall be opened unto you." Each of us, in our own sphere, is deeply involved with the tasks of asking, seeking, and opening doors. And, equally, our common task is to improve the quality of those imperative gifts in the Magic City.

MYCOTOXINS

Comparison of Rapid High Pressure Liquid Chromatographic and CB Methods for Determination of Aflatoxins in Corn and Peanuts

JONATHAN W. DeVRIES and HENRY L. CHANG
General Mills, Inc., James Ford Bell Technical Center, Medallion Laboratories, 9000 Plymouth Ave N, Minneapolis, MN 55427

A method is described for the rapid determination of aflatoxins in corn and peanut samples by high pressure liquid chromatography. The method was compared with the current CB method (AOAC 26.026). For 7 samples of corn and 14 samples of peanut meal and peanut butter, the correlation between methods is 0.991, and no significant difference exists between methods, using the Student's *t*-test at 15.7% α-risk.

The determination of aflatoxins is of great interest to food analysts, particularly in samples of corn and peanuts and their respective products. The number of methods in recent issues of this journal, as well as in *Official Methods of Analysis* (1), verifies this interest and provides the reader with adequate background on this subject. In particular, success has been achieved in quantitating aflatoxins by using thin layer chromatography (2, 3), normal phase high pressure liquid chromatography (HPLC) (4–6), and reverse phase HPLC (7–9). Thin layer chromatography coupled with 2 different extraction procedures (CB method, **26.026,** and BF method, **26.032**) is currently the AOAC official method (1). A recent study (10), which couples reverse phase liquid chromatography with each of the 2 extraction procedures, indicated that the CB method provided more efficient extraction of the aflatoxins present in the sample. The CB method is lengthy, in part due to the necessary column chromatographic cleanup of the sample, and therefore a method was pursued that would provide the extraction efficiency of the CB method in a shorter time.

METHOD

Reagents

(a) *Mobile phase.*—Combine 180 mL acetonitrile, 820 mL water, and 10 mL glacial acetic acid. Stir slowly and continually during use.

(b) *Injection solvent.*—Combine 100 mL acetonitrile, 900 mL water, and 10 mL glacial acetic acid. Shake or stir before use.

(c) *Aflatoxin standards.*—0.025 ng B_1 and G_1 and 0.0075 ng B_2 and G_2/μL. Pipet 100 μL aflatoxin stock solution (containing 1.0 μg B_1 and G_1 and 0.3 μg B_2 and G_2/mL, Supelco, Inc., Bellefonte, PA 16823) into 1 dram vial. Evaporate to dryness under stream of nitrogen and add 50 μL trifluoroacetic acid (TFA). Stir with vortex mixer. Add 4.00 mL injection solvent, and again stir with vortex mixer. Store in freezer until ready to use. Bring to room temperature protected from light just before loading autosampler. Figure 1 is a chromatogram of the derivatized (7) aflatoxin standards.

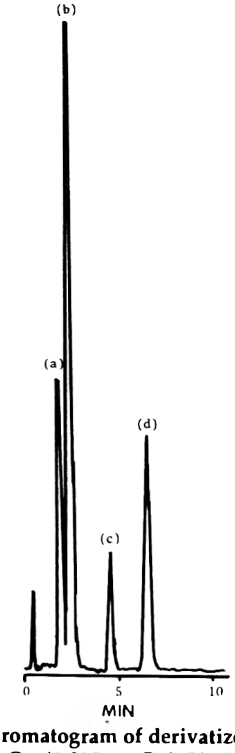

Figure 1. Chromatogram of derivatized (7) aflatoxin standards: (a) G_{2a} (0.025 ng G_1/μL), (b) B_{2a} (0.025 ng B_1/μL), (c) 0.0075 ng G_2/μL, (d) 0.0075 ng B_2/μL. Peak height is fluorescence intensity with fluorescence detector set at 0.3. Time is in minutes.

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Apparatus

(a) *High pressure liquid chromatograph.*—Equipped with Model 110 constant flow pump (Altex, 1450 Sixth St, Berkeley, CA 94710), or equivalent; injector, Model LC 420 autosampler with 20 μL loop (Perkin-Elmer, Norwalk, CT 08856), or equivalent; detector, Model 650-10LC fluorescence spectrophotometer (Perkin-Elmer), or equivalent. Set excitation for 365 nm with 12 nm slit width. Set emission for 440 nm with 12 nm slit width.

(b) *HPLC column.*—Reverse phase C_{18} 5 mm radial compression column fitted in an RCM 100 compression module (Waters Associates, Milford, MA 01757), or equivalent.

Extraction

Weigh 25 g properly homogenized sample (AOAC **26.003**) into 250 mL glass-stopper Erlenmeyer flask. Add 20 mL water and disperse sample evenly by stirring with spatula. Add 100 mL methylene chloride and again disperse by stirring with spatula. Add 5 g cupric carbonate basic, stopper flask, and shake 30 min on wrist-action shaker. Alternatively, at this stage mixture can be extracted 3 min with tissue-homogenizer. Centrifuge if necessary to aid filtration. Filter through 24 cm 2V paper until sufficient filtrate has been collected to allow pipetting 30.0 mL of methylene chloride extract into 250 mL round-bottom flask. Evaporate to dryness under vacuum and immediately transfer residue to 250 mL separatory funnel, using 3 portions of ace-

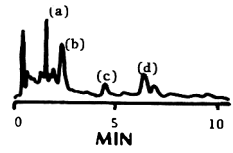

Figure 2. Chromatogram of derivatized extract from samples of peanut butter: (a) G_{2a} (G_1), (b) B_{2a} (G_2), (c) G_2, (d) B_2. Peak height is fluorescence intensity with fluorescence detector set at 0.3. Time is in minutes.

Table 1. Determination of aflatoxins in corn (μg/kg) by the rapid method and the CB method

Sample	B_1	B_2	G_1	G_2	Total
			Rapid Method		
1	48.05	4.37	<dl[a]	<dl	52.42
	46.88	4.302	<dl	<dl	51.18
2	446.76	49.58	15.13	2.90	514.37
	462.21	51.82	14.02	2.21	530.26
3	10.1	0.82	<dl	<dl	14.27
	14.25	<dl	<dl	0.38	14.63
4	22.16	2.55	<dl	<dl	24.72
	22.66	2.65	<dl	<dl	25.31
5	31.22	2.62	7.61	2.47	41.92
	33.14	2.63	8.45	0.36	44.59
6	202.48	25.82	25.62	5.4	259.35
	185.39	21.55	21.87	3.66	232.47
7	146.06	14.15	26.38	3.79	190.37
	194.63	20.27	34.67	7.97	257.54
Mean					160.96
			CB Method		
1[b]	42.88	3.67	<dl	0.28	46.83
2	313.93	52.83	9.71	2.65	379.13
	347.66	50.28	13.35	3.31	414.60
3	12.75	1.19	<dl	0.5	17.98
	12.58	1.0	<dl	0.19	17.44
4	16.60	2.22	0.65	0.37	19.84
	17.70	2.26	<dl	<dl	19.95
5[b]	34.85	2.93	8.87	0.51	47.15
6	221.93	20.44	21.67	<dl	266.69
	172.48	18.55	18.05	2.48	211.56
7	147.07	12.67	22.93	3.14	185.30
	194.42	18.74	32.97	5.05	251.18
Mean					140.83

[a] Detection limit (dl) at 2× noise is 0.21, 0.08, 0.15, and 0.08 μg/kg for aflatoxins G_1, B_1, G_2, and B_2, respectively.
[b] Insufficient sample for second determination.

Table 2. Determination of aflatoxins in peanut meal and peanut butter (μg/kg) by the rapid method and the CB method

Sample	B_1	B_2	G_1	G_2	Total
			Rapid Method		
1	38.13	4.26	<dl[a]	<dl	42.29
	37.45	4.35	<dl	<dl	41.80
2[b]	10.61	1.25	12.70	2.19	26.75
3	8.49	1.17	7.33	1.34	18.33
	8.26	1.17	6.94	1.28	17.65
4	11.25	1.64	11.99	2.31	27.19
	11.22	1.69	12.32	2.41	27.64
5	7.09	0.99	8.41	2.21	18.70
	7.04	0.81	7.31	2.26	17.42
6	8.32	1.22	8.83	1.71	20.08
	8.73	1.17	9.52	1.79	21.21
7	6.26	1.26	<dl	<dl	7.52
	6.62	1.34	<dl	<dl	7.96
8	5.11	0.77	3.13	1.51	10.52
	4.71	0.71	2.75	2.53	10.70
9	14.3	1.6	7.0	1.0	23.8
	19.3	2.7	8.8	2.5	32.2
10	7.3	1.2	6.8	1.5	16.8
	11.2	2.0	10.8	2.9	26.8
11	0.5	0.3	0.3	<dl	1.1
	1.8	0.8	0.8	0.7	4.1
12	2.2	0.8	2.0	1.2	6.2
	2.1	0.7	1.1	0.8	4.6
13	1.79	0.74	1.14	0.83	4.5
	2.26	0.97	1.24	0.99	5.45
14	50.22	9.15	19.94	2.18	81.49
	50.86	8.83	19.94	2.10	81.73
Mean					22.52
			CB Method		
1[b]	34.74	3.86	<dl	0.71	39.31
2	9.18	0.81	12.00	1.43	23.43
	9.11	0.69	11.56	1.20	22.56
3	8.45	1.34	8.05	1.40	19.24
	7.56	1.19	6.74	1.11	16.60
4	10.74	1.63	11.03	2.05	25.45
	10.75	1.71	11.47	2.00	25.93
5	6.26	0.92	7.06	1.16	15.40
	6.18	0.82	6.74	0.98	14.72
6	7.78	1.21	8.87	1.47	19.33
	8.12	1.20	7.70	1.27	18.29
7	5.97	1.27	<dl	0.37	7.61
	5.59	1.15	<dl	0.40	7.14
8	4.95	0.90	3.53	0.70	10.08
	4.97	0.80	3.78	0.83	10.38
9	14.2	1.8	7.4	1.2	24.6
	18.2	1.9	14.0	1.9	35.6
10	7.4	1.3	8.3	1.5	18.5
	6.4	1.2	6.6	1.4	15.5
11	3.7	1.1	1.1	<dl	5.9
	3.4	1.0	1.1	<dl	5.5
12	5.3	1.0	6.9	1.2	14.4
	3.1	1.0	2.7	1.0	7.8
13	3.5	0.9	4.4	1.0	9.8
	2.44	0.8	3.73	0.9	7.87
14[b]	49.26	9.26	18.40	3.25	80.16
Mean					22.16

[a] Detection limit (dl) at 2× noise is 0.21, 0.08, 0.15, and 0.08 μg/kg for aflatoxins G_1, B_1, G_2, and B_2, respectively.
[b] Insufficient sample for second determination.

tonitrile (50 mL total). Wash acetonitrile solution with two 50 mL portions of petroleum ether, and transfer to 250 mL round-bottom flask. Evaporate acetonitrile solution to dryness, immediately transfer residue to 1 dram vial, using minimal amount of methylene chloride. Evaporate to dryness under stream of nitrogen, add 50 μL TFA, and mix 20 s with vortex mixer. By pipet, add 4.0 mL injection solvent, mix on vortex mixer, and load into autosampler vial.

HPLC Quantitation

Set flow rate at 3.0 mL/min and let column equilibrate for 30–45 min. Repeat injections of standard solution until component peak heights are constant. Inject sample extracts interspersed with standards as frequently as necessary to assure accurate quantitation.

Calculation

$$\text{Aflatoxin, } \mu g/kg = C \times PH_{sam} \times (V + 50)/(PH_{std} \times 0.3 \times W)$$

where C = concentration of aflatoxin in standard, ng/μL; PH_{sam} and PH_{std} = peak heights of sample and standard, respectively; V = μL injection solvent used to dissolve sample (usually 4000); W = weight of sample, g (use weight × 0.9 for peanuts and peanut butter to correct for 10% fat content of these samples); and 0.3 = fraction of extraction solvent taken after first filtration.

Results and Discussion

The extraction and cleanup method described was efficient and required a minimal amount of analyst time and labor. The effective removal of fluorescent interferences by the petroleum ether wash of the sample extract dissolved in acetonitrile is evidenced in the chromatogram shown in Figure 2, which shows that peaks representing only 0.8, 1.8, 0.7, and 0.8 μg/kg of G_1, B_1, G_2, and B_2, respectively, in the original sample are free of interfering peaks. This cleanup method compares favorably with column cleanup in terms of effectiveness but requires significantly less time to carry out.

Seven samples of corn and 14 samples of peanut butter and peanut meal, all known from previous analyses to be naturally contaminated, were selected for study. Each corn sample was analyzed in duplicate by this method; however, because of insufficient sample, only 5 samples could be analyzed in duplicate by CB method **26.026** (1). The results are shown in Table 1. The mean total aflatoxin value by the rapid method was 160.96 μg/kg, and by the CB method, 140.83 μg/kg, a difference of 20.13 μg/kg. One heavily contaminated sample of corn showed a large difference in results between the 2 methods (Sample 2, 522.31 vs 396.87 μg/kg). If this sample is eliminated from the comparison, the mean by this rapid method is 100.73 μg/kg vs 98.16 μg/kg for the CB method.

Of the 14 peanut butter and peanut meal samples, sufficient sample was available to analyze 11 samples in duplicate by both methods. Two of the remaining samples were analyzed in duplicate by the rapid method, the other by the CB method. The results are shown in Table 2. The mean total aflatoxin content by the rapid method was 22.52 μg/kg, and by the CB method, 22.16 μg/kg.

For samples for which duplicate determinations were carried out, the relative standard deviation for total aflatoxin by the rapid method was 16.8%, and by the CB method, 25.3%.

Sample-by-sample comparison of the methods for all samples shows a correlation of 0.991 and no significant difference between methods (Student's t-test, 15.74% α-risk). If corn sample No. 2 is eliminated from the data set, the correlation is 0.999 and no significant difference exists at 2.8% α-risk.

Results of this study indicate that the rapid method described provides results equivalent to currently accepted methodology. In our laboratories, we have found that the method described requires substantially less time than does the CB method.

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PESTICIDE RESIDUES

Simultaneous Electron Capture Detection of Chlorpyrifos and 3,5,6-Trichloro-2-pyridinol Residues in Peach and Bahia Grass Field Samples Following Gel Permeation Cleanup

RICHARD A. GUINIVAN, NEAL P. THOMPSON, and PROMODE C. BARDALAYE

University of Florida, Food Science and Human Nutrition Department, Pesticide Research Laboratory, Gainesville, FL 32611

A recently published method involving the simultaneous extraction, gel permeation cleanup, and electron capture gas chromatographic detection of residues of the insecticide and acaricide chlorpyrifos and its principal metabolite 3,5,6-trichloro-2-pyridinol is successfully applied to Lorsban-treated peach and Bahia grass samples. Previous quantitation of these compounds has required 2 separate analytical procedures. Multilevel temperature programming and manipulation of the gel permeation column effluent rate are the only adjustments of experimental parameters necessary to avoid large amounts of gas chromatographic interferences. Chromatographic peaks are more clearly defined and easier to integrate with the multilevel program than they are with 2-step or linear programs. This is one reason that recovery data are improved over those reported for the method as applied in the pea vine work. Residue analyses conducted on field-treated peaches and Bahia grass show the newly published procedure to be sensitive and effective.

Lorsban pesticides (manufactured by The Dow Chemical Co.) are presently being studied for promising insecticidal and acaricidal activity on a variety of agricultural commodities. These pesticides are registered for use on a number of crops, and tolerances have been established for others. Current work involving the effectiveness of these products must include crop residue analyses for chlorpyrifos [*O,O*-diethyl *O*-(3,5,6-trichloro-2-pyridyl) phosphorothioate], the active ingredient of the formulation, and for 3,5,6-trichloro-2-pyridinol, this compound's principal metabolite. These compounds have usually been quantitated by 2 separate extraction, cleanup, and detection procedures (1–7). Recently, a method has been published which allows the simultaneous ethyl acetate extraction, gel permeation cleanup, and electron capture detection of both compounds in Southern pea vines (8). The successful application of this new procedure to the study of chlorpyrifos and 3,5,6-trichloro-2-pyridinol residues in peaches and Bahia grass is reported in this study.

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Experimental

Reagents

(a) *Solvents.*—Pesticide grade ethyl acetate and toluene (Mallinckrodt, Inc., St. Louis, MO 63134).

(b) *Silylation reagent.*—*N,O*-Bis(trimethylsilyl)acetamide (BSA) (Pierce Chemical Co., Rockford, IL).

(c) *Sodium sulfate.*—Anhydrous (Mallinckrodt, Inc.).

(d) *Standards.*—Chlorpyrifos, obtained from the Pesticide Reference Standards Section of the U.S. Environmental Protection Agency; 3,5,6-trichloro-2-pyridinol, a gift from The Dow Chemical Co., Midland, MI.

(e) *Standard solutions.*—Separate stock solutions of 200 ng chlorpyrifos and 200 ng 3,5,6-trichloro-2-pyridinol/μL ethyl acetate; prepare and dilute as needed.

Apparatus

(a) *Gas chromatograph.*—Automated (integration, sampling, temperature control) Hewlett-Packard Model 5840A gas chromatograph, equipped with ^{63}Ni electron capture detector and 183 cm × 4 mm id glass column packed with 10% DC-200 on 100–120 mesh Gas-Chrom Q (Applied Science Laboratories, Inc., PO Box 440, State College, PA 16801). Parameters: argon–methane carrier gas (95 + 5), flow 60 mL/min; injection volume 1 μL; detector 300°C; injection port 190°C; column oven initially held at 105°C for 35 min and then programmed sequentially at 15°/min to 150°C for 10 min, 180°C for 15 min, 200°C for 10 min, and 220°C for 15 min.

(b) *Gel permeation apparatus.*—AutoPrep Model

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1001 (Analytical Biochemistry Laboratories, Inc., Columbia, MO), used with column provided. Column (containing only glass and Teflon parts) is packed with 200–400 mesh Bio-Beads S-X3 (Bio-Rad Laboratories, 32 & Griffin, Richmond, CA).

(c) *Spectrophotometer.*—Beckman DU-8 microprocessor-controlled instrument equipped with 4 mL, 1 cm path length quartz cuvets to monitor fractions of optically absorbent compounds collected from gel permeation column eluate.

Chlorpyrifos Application and Crop Harvest

Spray Lorsban 4E (1.5 lb active ingredient per 378.5 L water) on peach trees, using portable hydraulic John Bean Sprayer (300 psi) (IR-4 (Federal minor use pesticides registration program) project No. 85). Harvest mature fruit at 15 day and 56 day post-application intervals and freeze samples until residue analyses are performed.

Apply 0.5% bait formulation by hand to plots of Bahia grass at rates of 2 and 4 lb active ingredient/acre (IR-4 project No. 69). Harvest grass 30 days after application and freeze until analyzed.

Calibration of Cleanup Column

Use 53 × 2.5 cm Bio-Beads S-X3 column for cleanup of both peach and Bahia grass samples. Determine elution of pesticide and metabolite in crop samples relative to the bulk of absorbent eluting compounds by comparing absorbance of each fraction with gas chromatographic quantitation of chlorpyrifos and 3,5,6-trichloro-2-pyridinol in the same or corresponding fractions. Collect 6 mL fractions for peach samples at 3 mL/min, and 4 mL fractions from Bahia grass samples at 2 mL/min.

Preparation of Samples

Spike 50 g peach and Bahia grass samples to various residue levels ranging from 0.01 to 1.00 ppm. Extract spiked samples and 50 g field-treated samples twice each with 100 mL ethyl acetate. Combine extracts and dry by passage through anhydrous Na_2SO_4. Manipulate sample into ethyl acetate–toluene (3 + 1) by reducing volume to less than 10 mL by rotary evaporation and adding 2.5 mL toluene. Adjust final volume to 10 mL with ethyl acetate. Process samples through AutoPrep gel permeation apparatus (ethyl acetate–toluene (3 + 1) eluant, flow 3 mL/min for peaches and 2 mL/min for Bahia grass). This instrument requires 10 mL volumes for injection but only 5 mL (representing 25 g crop) is actually injected on the column; the other 5 mL is used for internal machine rinses. For peach samples, collect 100 mL eluate after discarding first 146 mL; for Bahia grass, collect 100 mL after discarding first 173 mL. Evaporate collected eluates to 3–4 mL by rotary evaporation; then transfer to 10 mL conical test tube and take to dryness under nitrogen.

Derivatization of 3,5,6-Trichloro-2-pyridinol

Dissolve sample residues in 100 μL *N,O*-bis-(trimethylsilyl)acetamide, cap tube, and let stand at room temperature 10 min. Add 900 μL toluene, mix, and analyze by gas chromatography. Concurrently, evaporate appropriate aliquots of standard solutions to dryness, dissolve residue in 100 μL *N,O*-bis(trimethylsilyl)acetamide, and proceed as for samples.

Results and Discussion

Versatility inherent in the new method is demonstrated by the effectiveness of simple adjustments in 2 experimental parameters which facilitated the quantitation of the 2 compounds among increased levels of interfering substances. Ethyl acetate extracts of peaches are effectively analyzed by replacing the single-step temperature programming system with a discontinuous multilevel program. In Bahia grass, reduction of the gel permeation column elution rate enables good quantitation and detectability of the 2 compounds. Automated gel permeation and gas chromatographic systems allow these 2 simple alterations to be accomplished easily and rapidly.

Peach and Bahia grass samples both develop into multicolored bands when applied to the Bio-Beads column. Figure 1a shows a plot of absorbance at 429 nm vs fraction number, and a plot of integrated gas chromatographic areas for chlorpyrifos and 3,5,6-trichloro-2-pyridinol vs fraction number for the peach calibration of the S-X3 column. Figure 1b shows the corresponding plots for Bahia grass. In both cases the pesticide and its metabolite elute separately from the bulk of absorbent compounds. The effect of the slower flow rate is to increase the elution point of the 2 compounds from 146 mL to 179 mL and to eliminate excessive Bahia grass interferences. The peaks do not seem to broaden with later elution because they elute in approximately the same volume at both flow rates.

Figure 2 shows chromatograms for both peach and Bahia grass samples spiked at 0.5 ppm with

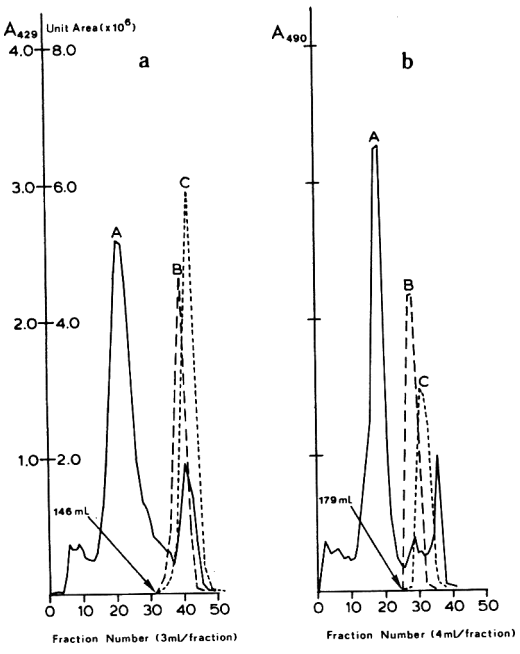

Figure 1. Cleanup column elution profile of (a) 25 g peach sample spiked to 0.5 ppm with chlorpyrifos and 3,5,6-trichloro-2-pyridinol (flow 3 mL/min, 3 mL fractions); and (b) 25 g Bahia grass sample spiked to 0.5 ppm with the same 2 compounds (flow 2 mL/min, 4 mL fractions). Three parameters plotted against fraction number for both crops are (A) absorbance, (B) integrated gas chromatographic area quantitation of chlorpyrifos, and (C) integrated gas chromatographic area quantitation of derivatized 3,5,6-trichloro-2-pyridinol.

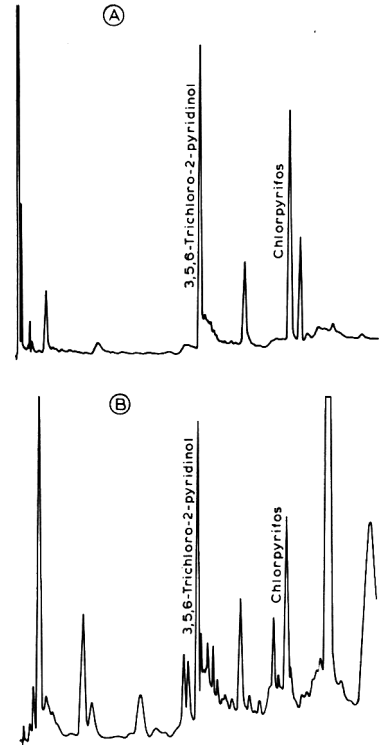

Figure 2. Gas chromatography (on 10% DC-200, attenuation 2^{12}, chart speed 0.3 in./min) of A, peach; and B, Bahia grass samples spiked to 0.5 ppm with 3,5,6-trichloro-2-pyridinol and chlorpyrifos. Retention time of 3,5,6-trichloro-2-pyridinol is 40.74 min and of chlorpyrifos, 60.43 min.

both compounds. 3,5,6-Trichloro-2-pyridinol elutes just as the second temperature step is initiated and therefore the peak is integrated on the decreasing side of the resultant baseline shift. This does not hinder the quantitation because the standard curves for both the pesticide and metabolite are linear through 0.6 ppm. The minimum detectability is 2.5 pg injected for chlorpyrifos and 12.5 pg injected for the derivatized metabolite. Some minor interferences are found in the control samples ranging from 2.5 ng injected to 7.5 ng injected (Figure 3). The integrated area of these peaks is subtracted from the crop sample peaks before quantitation by linear regression analysis.

Recoveries range from 73 to 102% (peaches) and 95 to 122% (Bahia grass) for the metabolite and 73 to 107% (peaches) and 98 to 108% (Bahia grass) for chlorpyrifos (Table 1). These recoveries are higher than those reported for the Southern pea vine study and are probably due to increased resolution and sharper peaks as a result of the multi-step temperature program. The Bahia grass data indicate good recovery but should only be used to supplement the peach data, because little control sample was available and spiking duplicates was not possible.

The initial gel permeation cleanup study for chlorpyrifos and its metabolite in Southern pea vines (7) resulted in a time-saving procedure with increased limits of detectability. This study on peaches and Bahia grass supports these conclusions and demonstrates that incorporation of simple procedural adjustments provides a flexibility in the methodology that makes it applicable to samples with greater amounts of extractable electron-capturing substances. The slower gel permeation elution rate and the 80 min temperature program increase the length of the procedure; however, these time expansions do not require additional analyst time because both involve adjustments in the automation. Also,

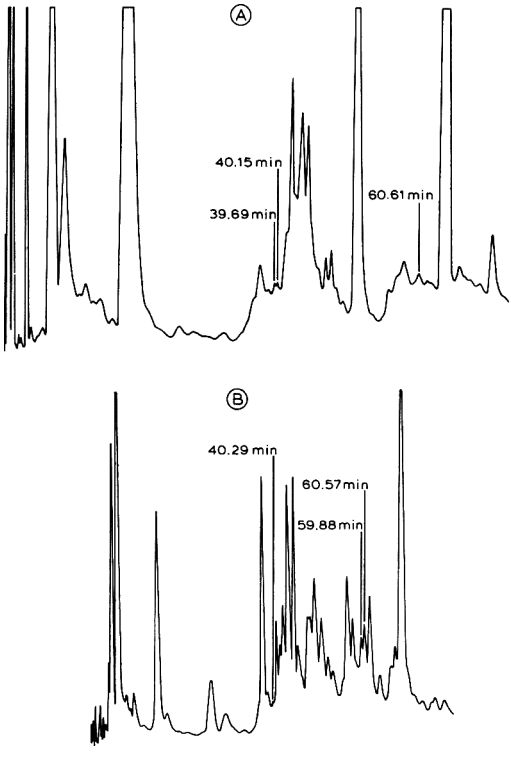

Figure 3. Control samples for A, peach (on 10% DC-200, chart speed 0.5 in./min, attentuation 2^{10}); and B, Bahia grass (on 10% DC-200, chart speed 0.2 in./min, attenuation 2^{12}) samples showing minor chromatographic interferences. Attenuation has been adjusted to allow interferences to be easily seen. Chromatogram has been expanded to 4 times actual size compared with spiked chromatograms.

analyst activity is eliminated by the use of a simplified derivatization procedure which eliminates a high temperature incubation and the use of 2 derivatization reagents.

Residues of chlorpyrifos found in the peaches were highest for fruit sprayed 15 days before harvest (0.056–3.151 ppm, 4 duplicate applications). Lower levels of the metabolite were detected (0.002–0.01 ppm). Chlorpyrifos levels of 0.015–0.256 ppm were found in the fruit sprayed 56 days before harvest, but no metabolite was detected.

Bahia grass samples also showed residues for chlorpyrifos (0.063–0.029 ppm, 4 duplicate applications) and the metabolite (0.060–0.13 ppm) at the higher application rate. It would be expected that residues would be lower than detected on peaches because the pesticide was applied as a bait formulation and not sprayed on the crop.

Conclusion

The gel permeation procedure previously reported for the simultaneous analysis of chlorpyrifos and 3,5,6-trichloro-2-pyridinol in spiked ethyl acetate extracts of Southern pea vines is demonstrated to be effective in the analysis of actual residues in field-treated crops. Simple adjustments (temperature programming and column elution control) are the only modifications necessary to adapt the procedure to crops with increased levels of electron-capturing substances.

Acknowledgments

The authors thank Tim Allen for expert technical assistance and Melissa Michaels for help in the preparation of this manuscript. This work has been performed at the Pesticide Research Laboratory of the Food Science and Human Nutrition Department at the University of Florida. This laboratory is the IR-4 (Federal minor use pesticides registration program) Region 4 leader laboratory. These studies are part of IR-4 projects.

Table 1. Recoveries (%) of 3,5,6-trichloro-2-pyridinol and chlorpyrifos from spiked, representative 25 g samples

Spiking level, ppm	Peaches[a] (av. ± SD)		Bahia grass[b]	
	Metabolite	Chlorpyrifos	Metabolite	Chlorpyrifos
1.00	102.0 (4)	99.0 (1)	121.7	99.5
0.50	94.8 ± 4.1 (4)	89.2 ± 11.3 (6)	102.4	97.9
0.20	98.0 (1)	107.0 (1)	—	—
0.05	90.3 ± 27.6 (4)	89.0 ± 9.0 (2)	95.2	108.2
0.02	88.0 (1)	66.0 (1)	—	—
0.01	73.0 ± 7.55 (3)	73.0 ± 10.7 (3)	—	—

[a] Based on the number of determinations specified in parentheses.
[b] Spiking duplicates were not available because of limited supply of control material.

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Steam Distillation and Gas-Liquid Chromatographic Determination of Triallate and Diallate in Milk and Plant Tissue

LEONARD W. COOK, FRED W. ZACH, and JAMES R. FLEEKER
North Dakota State University, Department of Biochemistry, Fargo, ND 58105

A procedure based on steam distillation is described for the determination of residues of the thiocarbamate herbicides diallate and triallate. The herbicides are steam-distilled directly from aqueous suspensions of milk and plant samples and trapped in hexane. After column cleanup on either activated Florisil or silica cartridges, samples are quantitated by gas-liquid chromatography. Recoveries of diallate and triallate from milk, lettuce, peas, corn, canarygrass seed and straw, and flax straw ranged from 77 to 96%.

Steam distillation is a useful technique for the cleanup of volatile substances from biological samples before analysis. The technique requires that the substance to be recovered is stable in boiling water and has sufficient vapor pressure. Methods for estimation of residues of several thiocarbamate herbicides by using steam distillation cleanup and gas chromatography have been described (1–4). Present methods reported for determination of the thiocarbamate herbicides diallate [*S*-(2,3-dichloroallyl) diisopropylthiocarbamate] and triallate [*S*-(2,3,3-trichloroallyl) diisopropylthiocarbamate] use the techniques of organic solvent extraction and column chromatographic cleanup (5–10). This paper describes the direct steam distillation of diallate and triallate from milk and plant tissue and subsequent gas-liquid chromatography (GLC) for quantitation.

METHOD

Reagents and Apparatus

(a) *Reagents.*—Hexane and 2,2,4-trimethylpentane (TMP) were pesticide analytical quality. Ethylene glycol, *n*-butanol, 2-(2-ethoxyethoxy)-ethanol, NaCl, and anhydrous Na_2SO_4 (12–60 mesh) were reagent grade.

(b) *Antifoaming agents.*—(*1*) 5–10 mL *n*-butanol; (*2*) 10 mL 2-(2-ethoxyethoxy)ethanol–ethylene glycol (71 + 29, v/v); or (3) NaCl sufficient to bring concentration in boiling flask to 2.5M.

(c) *Column chromatography.*—Florisil, PR grade, 60–100 mesh, activated overnight at 130°C before use. Prepacked silica cartridges (Sep-Paks) purchased from Waters Associates, Inc.

(d) *Analytical standards.*—Standards of diallate and triallate were obtained from the U.S. Environmental Protection Agency, Health Effects Research Laboratory, Research Triangle Park, NC.

(e) *Special equipment and glassware.*—Tekmar homogenizer, Model SD-45 (Tekmar Co., Cincinnati, OH 45222). Steam distillation apparatus as described by Fang and Theisen (1). Kjeldahl connecting bulb (Corning No. 1982).

(f) *Gas chromatographs.*—Tracor Model 222 equipped with ^{63}Ni electron capture detector and 1.83 m × 2 mm id glass columns. Temperatures (°C): inlet 225, detector 275; detector purge: 55 mL nitrogen/min; packings: 5% SP-2401 on 100–120 mesh Supelcoport, 45 mL nitrogen/min at 140°C, or 3% OV-101 on 100–120 mesh Chromosorb W HP, 30 mL nitrogen/min at 160°C.

Barber-Colman series 5000 equipped with Coulson conductivity detector operated in halogen mode. Temperatures (°C): inlet 220, oven 195, transfer line 230, furnace 840; column: 1.83 m × 2 mm id glass packed with 10% OV-1 on 80–100 mesh Gas-Chrom Q; gas flows: column 50 mL nitrogen/min, detector reaction gas 80 mL hydrogen/min.

Procedure

Sample preparation.—Grind seed samples in seed grinder. Place 10 g sample of milk or ground seed in 1 L round-bottom flask and add magnetic stir bar and 250 mL water. Shred straw or lettuce into 1 in. sections and homogenize 10 g samples in 200 mL water 2 min. Transfer homogenate to 1 L round-bottom flask containing magnetic stir bar. Rinse homogenizer with 50 mL water and add rinse to round-bottom flask. Add antifoaming agent.

Steam distillation.—Place round-bottom flask in heating mantle and attach Kjeldahl bulb and steam distillation apparatus. Add 50 mL water and 20 mL hexane to distillation sidearm and attach water-cooled condenser. Steam-distill 2 h at 1–2 mL/min with stirring.

Extraction and cleanup.—Drain water and hexane from sidearm into 125 mL separatory funnel

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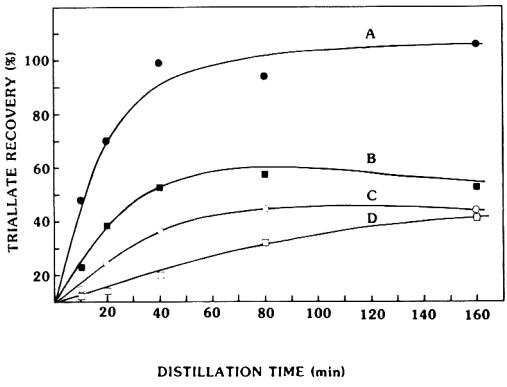

Figure 1. Recovery of triallate vs distillation time for 10 g samples fortified with 10 μg triallate. A, flax straw; B, flax meal; C, flax seed; D, linseed (flax) oil.

and wash condenser and sidearm with 10 mL hexane. Shake 1 min; then drain aqueous layer into second 125 mL separatory funnel and re-extract with 10 mL hexane. Add 1-5 g NaCl to break emulsions if necessary. Pass extracts through 1 × 20 cm glass column plugged with glass wool and containing 3 g Florisil topped with 5 g anhydrous Na_2SO_4, or collect the extracts in 10 mL glass syringe and force through silica Sep-Pak at ca 1 mL/min. Collect eluates in 100 mL volumetric flask and wash column or cartridge with additional 10 mL hexane. Dilute to volume with TMP and analyze by gas chromatography.

Results and Discussion

The length of the distillation period was important for obtaining adequate recovery of the herbicides. Triallate (10 μg) could be quantitatively recovered after 10 min distillation if only water and analyte were added to the boiling flask. The presence of sample material reduced the rate of the herbicide distillation to an extent depending on the sample matrix (Figure 1). Flax seed, oil, and meal retarded the triallate distillation much more than flax straw; flax oil had the strongest retarding effect. The presence of lipid in the sample matrix appears to have a detrimental effect on the rate of triallate distillation. This may reflect partitioning of the herbicide into the lipid phase. A 2 h distillation period was chosen to obtain recoveries greater than 70% for all sample types except flax seed, flax meal, and linseed oil.

Recoveries of diallate from 4 sample types ranged from 79 to 96% (Table 1). Both isomeric forms were recovered to essentially the same extent. Recoveries of triallate ranged from 77 to 96% for most sample types tested (Table 2).

Foaming of the suspension during distillation was a problem with some samples, especially seed. The Kjeldahl bulb, inserted between the boiling flask and the receiving head, helped to

Table 1. Recovery of diallate from milk and plant samples[a]

Sample type	Recovery, % (mean ± SD) Isomer 1[b]	Isomer 2[c]
Whole milk	89 + 2	93 + 6
Head lettuce	96 ± 1	96 ± 3
Dried peas	86 ± 5	79 ± 10
Dried corn	86 ± 4	94 ± 17

[a] Three analyses per sample; fortified at 0.1 ppm diallate.
[b] Retention time = 4.2 min.
[c] Retention time = 5.0 min.

Table 2. Recovery of triallate from milk and plant samples[a]

Sample type	Fortification, ppm	Recovery, % (mean ± SD)
Whole milk	0.1	96 ± 2
Head lettuce	0.1	96 ± 5
Dried peas	0.1	82 ± 3
Dried corn	0.1	86 ± 1
Canarygrass seed	0.1	77 ± 4
Canarygrass straw	0.05	93 ± 4
Flax seed	1.0	26 ± 5
Flax straw	1.0	90 ± 6

[a] Three analyses per sample, except 6 analyses for canarygrass seed and flax seed.

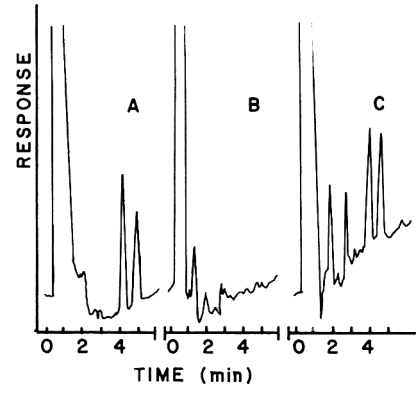

Figure 2. Gas chromatograms of diallate measured by ^{63}Ni detector. Amounts injected: A, 0.1 ng diallate (both isomers); B, 0.5 mg dried pea control; C, 0.5 mg dried pea control fortified with 0.1 μg diallate/g.

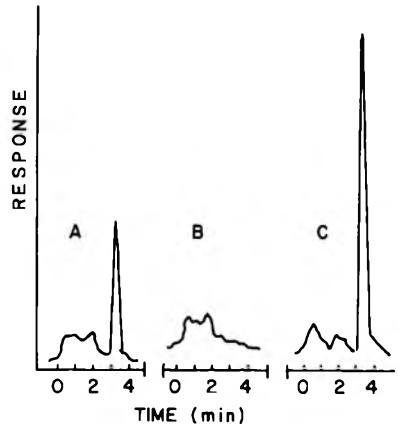

Figure 3. Gas chromatograms of triallate measured by Coulson conductivity detector. Amounts injected: A, 5 ng triallate standard; B, 50 mg canarygrass seed control; C, 50 mg canarygrass seed control fortified with 0.1 μg triallate/g.

prevent foam from reaching the distillate. We found that *n*-butanol, 2-(2-ethoxyethoxy)ethanol-ethylene glycol, and 2.5M NaCl all reduced foaming when added to the sample and that the effectiveness of these varied with the type of sample. The presence or absence of antifoaming agent had no effect on recoveries of the pesticides.

Triallate may be analyzed on any of the 3 gas chromatographic columns listed, which allows flexibility for samples with high sample background. The SP-2401 chromatographic column was used for the determination of diallate beause it gave the greatest resolution of the 2 isomers (Figure 2). Greater selectivity is offered by the use of the Coulson conductivity detector, as shown by chromatograms of triallate in canarygrass seed (Figure 3).

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ALCOHOLIC BEVERAGES

Density Meter Determination of Proof of Ethanol-Water Solutions: Collaborative Study

DUANE H. STRUNK, JOHN C. AICKEN, JACK W. HAMMAN, and ARTHUR A. ANDREASEN

Joseph E. Seagram & Sons, Inc., Research and Development Department, Box 240, Louisville, KY 40201

Collaborators: J. Cherolis; W. D. Daggs; F. J. Feeny; J. W. Hamman; R. N. Holmes; J. C. Jones; F. G. Mark; G. E. Martin; W. C. O'Bryan; R. E. Olson; A. W. Perrault; F. L. Rapp; B. W. Rehn; B. M. Timmel; D. Worden

Density meter procedures and the official AOAC pycnometer method to measure proof of ethanol-water solutions in the 25-79° proof range were collaboratively studied. Measurement of proof by density meter is simpler, requires less time and smaller samples, and gives more reproducible results than the AOAC pycnometer method. Differences of −0.05 to +0.02° proof between averages of results reported for density meter and pycnometer are acceptable, considering that proof of finished alcohol products is reported to 0.1°. The density meter method has been adopted official first action.

Several papers have been published describing use of a density meter for determining proof of distilled alcoholic beverages. Strunk et al. (1) first developed a procedure to measure proof of ethanol-water solutions with a density meter operated at 25 ± 0.01°C. They noted that proof values obtained by pycnometer, which is the official AOAC method (2), were 0.08-0.24° higher than density meter results in the 40-190° proof range for a density meter standardized on air and water. Good agreement of proof results was obtained by the 2 methods for 1-190° proof ethanol-water solutions, vodkas, whiskies, brandies, and liqueurs when the density meter was standardized on air and water for samples under 40° proof and on air and ethanol standards within ±30° proof of samples being tested for samples above 40° proof.

Mark and Vaughn (3), using air and water as standards and sample temperature controlled at 15.56°C, reported excellent agreement for 20-100° proof samples for density meter vs pycnometer and/or hydrometer. The greatest difference between density meter and pycnometer measurements was 0.07° proof for the samples of specialty products, cordials, and distilled beverage products tested. Density meter results were reproducible within 0.02° proof.

Results of a collaborative study for determining proof of ethanol-water solutions by a density meter standardized on air and water at 20 ± 0.01°C were reported by Strunk et al. (4). This temperature was selected because (*1*) the metric system, as used by EEC countries and Canada, reports concentration of ethanol in alcoholic products in percent at 20°C; (*2*) all volumetric glassware is calibrated at 20°C; and (3) as a result of faster temperature stabilization, more samples can be processed per hour by the density meter at 20°C than at 15.56°C. This study showed that the values of 1-190° proof samples as determined by a pycnometer were 0.00-0.17° proof higher than those obtained by a density meter.

Proof by Density Meter
Official First Action

Principle

Meter dets sp. gr. by measuring change in frequency of oscillating U-tube filled with sample compared with frequencies of oscillation when filled with 2 stds.

Apparatus and Reagents

(a) *Density meter.*—Mettler/Paar DMA 55D, with adapter No. 5771 which permits continuous flow of sample thru U-tube (Mettler Instrument Corp., Hightstown, NJ 08520).

(b) *Water bath.*—Const temp., controlled at 20.00 ± 0.01°.

(c) *Syringe.*—10 mL, with Luer fitting, No. 15 needle.

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The recommendation of the Associate Referee was approved by the Referee and Committee D and was adopted by the Association. See *J. Assoc. Off. Anal. Chem.* (1982), this issue.

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(d) *Water.*—Double-distd or treated by ion exchange resin and filtered, freshly boiled (ion exchange cartridge, Research Model, sold by Illinois Water Treatment Co., 840 Cedar St, Rockford, IL 61105; Gelman Capsule Filter, Cat. No. 12106, 0.2 μm, Gelman Sciences, Inc., 600 S Wagner Rd, Ann Arbor, MI 48105).

(e) *Barometer.*

(f) *Thermometer.*—No. 116-C, 18.9 to 25.1°, 0.01° scale divisions (H & B Instrument Co., American and Bristol St, Philadelphia, PA 19140).

Standardization of Density Meter

Rinse U-tube with stream of acetone, and completely dry with air stream. Set switches on density meter in the following positions: Power—on, display—T, sampling rate—either 2 or 3. With clean, dry U-tube at 20 ± 0.01°, note and record T for air.

Turn on light and open shutter to view U-tube. Fill U-tube with freshly boiled double-distd (or ion exchange-treated) H_2O by dipping plastic tube connected to inlet (lower) end of U-tube into H_2O std and slowly pulling plunger on syringe, equipped with No. 15 needle, connected by plastic tubing to outlet (upper) of U-tube. View U-tube to ensure that it is full of H_2O and contains no bubbles. Leave end of filler tube submerged in H_2O std and syringe connected while taking reading. Turn off viewing light and close shutter. T value of H_2O on digital display will continue to change until sample temp. reaches equilibrium with const temp. bath (ca 2–3 min). Record T value for H_2O.

Calc. app. consts A and B according to following formulas:

$$A = [T^2_{water} - T^2_{air}]/[\text{sp. gr.}_{water} - \text{sp. gr.}_{air}]$$

$$B = T^2_{water} - [A \times \text{sp. gr.}_{water}]$$

Sp. gr. of air = 0.00119 at 20.00° and 746–752 torr, and 0.00120 at 20.00° and 753–758 torr. Sp. gr. of water = 1.00000 regardless of barometric pressure.

Enter calcd values of consts A and B into app. memory by rotating appropriate dials. Reset display switch to ρ (sp. gr.), and check reading for sp. gr. of H_2O. Then drain U-tube and dry, and check sp. gr. of air. Numerical displays should be 1.00000 for H_2O, and 0.00119 or 0.00120 for air, depending on barometric pressure. If display values differ >1 in fifth decimal place from correct sp. gr. values, recheck temp. of H_2O bath and T values for air and H_2O.

Specific Gravity Measurement of Samples

Turn on light and open shutter to view U-tube. Slowly fill U-tube with sample by same method used to fill with H_2O std, being careful not to introduce bubbles. Turn off viewing light and close shutter. Sp. gr. of sample on digital display will continue to change until sample temp. reaches equilibrium with const temp. bath (ca 2–3 min). Record sp. gr. of sample, and draw another sample into U-tube. Replicate readings should vary ≤ ±0.00001 sp. gr. unit. Refer to Table **52.003** to convert sp. gr. at 20° to percent alcohol at 15.56°.

Changing Samples

Turn on light and open shutter to view U-tube. Lift inlet end of plastic tubing from below surface of old sample and slowly pull syringe plunger to empty tubing and U-tube. Disconnect syringe from plastic tubing and discard syringe contents. Reconnect syringe, submerge tip of tubing below surface of new sample, and slowly draw 8–10 mL sample thru U-tube into syringe to eliminate air bubbles and to rinse system with new sample. Turn off viewing light, close shutter, and take first reading of new sample after temp. equilibrium.

After each 10 samples, or when erratic digital displays are noted, rinse U-tube with acetone and dry with air stream. Let empty U-tube stabilize at 20 ± 0.01°.

Experimental

Possible Source of Errors

Experience has shown that inaccurate results are caused by the following conditions:

Water Bath Temperature.—We currently use a Braun Thermomix temperature controller, Model 1460, to control the temperature of a water bath at 20 ± 0.01°C and to pump water through the density meter. Before water is returned to this bath, it passes through a small heat exchanger which is cooled by water from a second bath maintained at 19.7°C. If inadequate temperature control is suspected, the clean U-tube should be filled with CCl_4 as recommended on page 19 of the instruction manual, and the sample should be allowed to reach constant temperature. Because of a larger coefficient of expansion, larger fluctuations in readings will occur if the thermostat is not adequately controlling the temperature.

Inadequate Rinsing between Samples.—When differences larger than ±1 occur in the fifth decimal place of replicate samples, the problem

Table 1. Collaborative proof results by pycnometer

	Sample					
Coll.	4	3	5	2	1	6
1[a]	24.82	35.28	53.63	53.93	79.06	79.11
2	24.92	35.40	53.60	53.96	78.64	79.23
	24.86	35.28	53.64	53.86	78.60	79.22
3	24.90	35.34	53.58	53.88	79.12	78.78
	24.92	35.38	53.52	53.90	79.16	78.84
4	24.88	35.36	53.74	54.00	79.14	79.28
	24.82	35.52	53.58	54.02	79.00	79.36
5[b]	24.88	35.36	53.64	53.92	79.04	79.38
	24.70	35.28	53.56	53.74	79.26	79.38
	25.08		53.66	53.94	79.16	79.38
	24.70	35.50	53.64	53.98	79.02	79.44
6	24.79		53.70	53.85	79.16	78.05
	24.66	35.21	53.68	53.83	79.12	78.04
7	24.80	35.34	53.68	53.91	79.10	79.35
	24.80	35.32	53.66	53.91	79.09	79.38
8[a]	24.84	35.32	53.64	53.88	79.12	79.32
9	24.84	35.36	53.59	53.78	79.10	78.71
	24.80	35.34	53.58	53.76	79.10	78.68
Mean	24.854	35.362	53.632	53.899	79.045	78.977
SD	0.094	0.081	0.060	0.078	0.190	0.476
CV, %	0.38	0.23	0.11	0.15	0.24	0.60

[a]Single results not included in statistical evaluation.
[b] Duplicate results selected randomly.
Statistical evaluation, pooled results:
Variance ratios: between-labs, $MS_L/MS_{LS} = 1.72$
lab.-sample interaction, $MS_{LS}/MS_O = 25.49$*
*Significant at 95% confidence limit
Repeatability SD = 0.060
Reproducibility SD (estd) = 0.231
CV of repeat. SD, % = 0.11
CV of reprod. SD, % = 0.42
Std error of mean = 0.226

could be caused by inadequate rinsings between samples or by the presence of bubbles in the sample. Bubbles usually occur if the sample is drawn too fast into the U-tube or if a freshly distilled sample has not been degassed. It should take about 15 s to fill the U-tube. To rinse the U-tube sample adequately, the previous sample should be evacuated from the U-tube before the next sample is introduced, and the U-tube should be rinsed with 8–10 mL of the new sample. After the specific gravity reading is recorded, a second portion of the same sample can be drawn into the U-tube and tested without evacuating the U-tube.

Evaporation of Ethanol during Analysis.—Loss of ethanol due to evaporation can be controlled by running the sample tube through a hole in, or along side of a rubber stopper used to cover the top of the sample bottle. The stopper should not restrict sample flow.

Collaborative Study

This study was planned so that collaborative results for the AOAC pycnometer method and the density meter method for measuring proof could be statistically evaluated by AOAC recommended methods written by Youden and Steiner (5). Seven samples of ethanol–water solutions from 25 to 79° proof were submitted to each collaborator together with the recommended procedure. This is the proof range of most alcoholic products on which federal tax is paid in the United States. Samples 1 through 6 consisted of 3 pairs of samples randomly numbered with respect to proof. The pairs were: Samples 4 and 3, Samples 5 and 2, and Samples 1 and 6. Sample 7, the standard (practice sample), was 69.5° proof as determined by pycnometer.

The collaborators were requested to report duplicate density meter results on each sample. They were also requested to determine proof of these same samples by pycnometer only if they had the expertise to use it.

Results and Discussion

Table 1 presents the results obtained by 9 collaborators using the pycnometer to determine

Table 2. Collaborative proof results by density meter

	Sample					
Coll.	4	3	5	2	1	6
1	24.76	35.36	53.62	53.87	79.02	79.02
	24.76	35.36	53.63	53.87	79.02	79.01
2	24.82	35.32	53.63	53.88	78.65[a]	79.31
	24.82	35.32	53.62	53.88	78.64[a]	79.31
3	24.90[a]	35.34	53.52	53.86	79.12	78.78
	24.92[a]	35.36	53.52	53.88	79.10	78.78
4	24.80	35.40	53.66	53.92	79.10	79.28
	24.80	35.40	53.64	53.92	79.14	79.28
5[b]	24.85	35.41	53.74	53.90	79.16	79.42
	24.86	35.42	53.74	53.90	79.16	79.44
6	24.80	35.32	53.34	53.82	79.10	77.94[a]
	24.78	35.28	53.34	53.80	79.10	77.94[a]
7	24.83	35.36	53.63	53.86	78.97	79.33
	24.83	35.36	53.63	53.86	78.97	79.33
8[c]	24.82	35.32	53.64	53.86	79.12	79.30
10	24.81	35.30	53.62	53.84	79.12	79.18
	24.80	35.30	53.62	53.84	79.12	79.16
11	24.80	35.32	53.52	53.79	78.93	79.15
	24.81	35.30	53.52	53.80	78.93	79.15
12	24.79	35.36	53.48	53.86	79.14	79.34
	24.79	35.36	53.48	53.86	79.12	79.34
13	24.76	35.28	53.18[a]	53.52[a]	79.08	79.18
	24.78	35.30	53.18[a]	53.52[a]	79.10	79.18
14	24.70[a]	35.32	53.62	53.83	79.07	78.60[a]
	24.70[a]	35.32	53.62	53.83	79.07	78.60[a]
15	24.66[a]	35.30	53.56	53.66[a]	79.02	79.08
	24.66[a]	35.26	53.56	53.64[a]	79.04	79.08
Mean	24.800	35.328	53.589	53.855	79.068	79.177
SD	0.024	0.035	0.055	0.034	0.065	0.166
CV, %	0.095	0.099	0.103	0.064	0.082	0.210

[a] Results eliminated as outliers by Dixon test.
[b] Results eliminated by Youden's ranking test.
[c] Single results not included in statistical evaluation.
Statistical evaluation, pooled results:
Variance ratios: between-labs, $MS_L/MS_{LS} = 1.43$
lab.-sample interaction, $MS_{LS}/MS_O = 19.38$*
*Significant at 95% confidence limit
Repeatability SD = 0.009
Reproducibility SD (estd) = 0.186
CV of repeat. SD, % = 0.02
CV of reprod. SD, % = 0.35
Std error of mean = 0.18

proof of 6 different ethanol-water samples. Collaborators 1 and 8 did not submit duplicate results, and were not included in the statistical evaluation summarized in Table 1. Collaborator 5 submitted more than duplicate results for each sample, necessitating the random selection of 2 results for each sample. None of the remaining data was eliminated by Dixon's test for outliers or Youden's ranking test.

Several collaborators commented about the problem of obtaining reproducible proof results by the official AOAC pycnometer method (2). Collaborator 5, using a 100 mL pycnometer, reported that "the accuracy and reproducibility were on the order of 0.1° proof." Collaborators 6 and 9 also were unable to obtain satisfactory reproducible results with the recommended pycnometer. A survey of the collaborators indicated that Collaborators 2, 4, and 5 followed the AOAC pycnometer method (2), Collaborator 1 used a vacuum-jacketed pycnometer, and Collaborators 6, 7, and 9 used pycnometers equipped with side arms and thermometers.

The standard deviation of the proofs for each sample obtained by pycnometer (Table 1) ranged from 0.060 (Sample 5) to 0.476 (Sample 6). The variance ratio MS_L/MS_{LS} (between laboratories) of 1.72 was not significant at the 95% confidence level, while MS_{LS}/MS_O (laboratory-sample interaction) of 25.49 was significant. The relative size of the reproducibility standard deviation (0.231) confirms that larger variations occur

Table 3. Comparison of average pycnometer and density meter proof results for Collaborators 1–8

	Sample					
Method	4	3	5	2	1	6
Pycnometer	24.86	35.34	53.64	53.92	79.04	79.02
Density meter	24.81	35.36	53.62	53.87	79.02	79.01
Diff.	−0.05	+0.02	−0.02	−0.05	−0.02	−0.01

when more than one laboratory performs the analysis. No consistent laboratory bias appears to exist for the pycnometer results.

Results obtained by density meter are shown in Table 2. Single results submitted by Collaborator 8 were not included in the statistical evaluation. Collaborator 9, listed in Table 1, did not submit density meter results. Youden's ranking test (5) of the remaining data resulted in the elimination of the results reported by Collaborator 5. Dixon's test for outliers resulted in 9 pairs being outside of the 95% confidence limit. Analysis of the variance as outlined by Steiner (5) was performed on the remaining data. The variance ratios MS_L/MS_{LS} (between laboratories) and MS_{LS}/MS_O (laboratory–sample interaction) were 1.43 and 19.38, respectively. The between-laboratory ratio is not significant at the 95% confidence level, while the laboratory–sample interaction is significant. Since the between-laboratory variance (MS_L/MS_{LS}) is not significant, no consistent laboratory bias exists. The repeatability and reproducibility standard deviations were 0.009 and 0.186, respectively.

Results obtained by the density meter have better repeatability than those obtained by the pycnometer as shown by the repeatability standard deviation of 0.009 for the density meter vs 0.060 for the pycnometer results. By both methods, the between-sample variance ratios are not significant at the 95% confidence level; between laboratory–sample interaction ratios are significant. These results indicate that higher degrees of variations are obtained by both methods when performed by different operators as opposed to a single operator.

Collaborators 1–8 reported proof results for these samples obtained by both the pycnometer and density meter methods. Averages of these results obtained by each method are shown in Table 3. Although averages for each sample of the 2 methods are similar, pycnometer values, which are considered the true proof, are 0.05–0.1° proof higher than density meter results, except for Sample 3 where the average reported for the pycnometer is 0.02° proof lower than the density meter average. Differences of −0.05 to +0.02° proof between averages of results obtained by pycnometer and density meter methods are acceptable considering that this is the accuracy of the pycnometer method, and the proof of ethanol in distilled alcoholic products is reported to 0.1°.

Conclusions and Recommendation

Compared with the pycnometer, the density meter method is simpler, requires less time and smaller samples, and gives more reproducible results. Density meters are currently being used to measure proof by all regional Bureau of Alcohol, Tobacco and Firearms laboratories, most research laboratories of U.S. distilleries, and some bottling plants in Scotland. Also, the density meter has the capability of being automated. We have developed an automated method using the Mettler/Paar DMA 55 density meter to measure proof of our finished products in bottling plants. Accessory equipment needed for these tests includes an automatic sampler SP2 and a Hewlett-Packard 97S calculator.

It is recommended that the density meter method to measure concentration of ethanol in aqueous solutions be adopted official first action.

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G. E. Martin, Bureau of Alcohol, Tobacco and Firearms, Rockville, MD

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B. W. Rehn, Bureau of Alcohol, Tobacco and Firearms, Atlanta, GA

D. Worden, Heublein, Menlo Park, CA

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Spectrophotometric Determination of Color Intensity of Whisky: Collaborative Study

DUANE H. STRUNK, BERTHA M. TIMMEL, JACK W. HAMMAN, and ARTHUR A. ANDREASEN

Joseph E. Seagram & Sons, Inc., Research and Development Department, Box 240. Louisville, KY 40201

Collaborators: D. A. Bodnar; J. Cherolis; W. D. Daggs; A. Daun; F. J. Feeny; L. Gardner; J. W. Hamman; R. N. Holmes; J. C. Jones; W. E. Kampf; F. G. Mark; G. E. Martin; W. C. O'Bryan; A. W. Perrault; B. W. Rehn; R. D. Steinke; G. A. Stone; B. M. Timmel

A method for measuring color intensity of whisky was developed to replace the present AOAC method, which has become obsolete. The new method was collaboratively studied by 20 persons. Color intensity of whisky was measured as absorbance by a spectrophotometer, using a 1 cm cell, a bandwidth ≤10 nm, and a wavelength of 525 nm. Water was used as reference. Collaborator results appear acceptable for whisky samples that vary in color intensity from 29 to 374 CIU (absorbance × 1000 = Color Intensity Units (CIU)). The maximum standard deviation and coefficient of variation were 5.8 CIU and 6.68%, respectively. The method has been adopted official first action.

The present AOAC colorimetric procedure for whisky (1) was reviewed by Strunk et al. (2) who reported several technical defects: (*1*) It measures absorbance at 430 nm, making it impossible to correlate instrumental and visual results; (*2*) it specifies a spectrophotometer with a bandwidth ≤1 nm, which is a complicated and expensive instrument not available in most industry laboratories; and (3) it uses a ½ in. cell, which is not available for most spectrophotometers. In addition, the AOAC colorimetric procedure for whisky differs from that used by caramel manufacturers, whose products are used to color many alcoholic and food products.

Strunk et al. (2) reported a comparison of color intensity values of aged and caramel-colored distilled alcoholic products measured with colorimeters and spectrophotometers at wavelengths of 430, 525, 560, and 610 nm. The recommended procedure, for which the collaborative study is reported here, uses a spectrophotometer with a bandwidth ≤10 nm at 525 nm. Results are determined in absorbance (A) units for a 1 cm cell path and reported in Color Intensity Units (CIU) (CIU = $A \times 1000$). This method permits all laboratories to uniformly measure color intensity of whisky and other aged alcoholic products with any spectrophotometer meeting specifications.

Collaborative Study

Nine whisky samples at about 80° proof, with absorbance values varying from 0.029 to 0.374, were submitted to each collaborator with the recommended procedure. Sample 1 was the standard with an absorbance of 0.148. Samples 2–9 consisted of 4 closely matched pairs of samples randomly numbered with respect to color intensity: Samples 2 and 5, 3 and 7, 4 and 6, and 8 and 9. Samples 2–7 were blended whiskies which contained caramel; Samples 8 and 9 were bourbons with no caramel. Samples with low absorbance values were prepared by diluting blended whisky with 80° proof vodka.

Samples were shipped in amber bottles to protect them from exposure to light. Collaborators were requested to store these samples in the dark and test them within 30 days. Color intensities of duplicate samples stored in our laboratory showed no appreciable change in absorbance (−0.008 to +0.004) during 60 days of dark storage.

Color

Official First Action

Definition

Whisky Color Intensity Units are defined as 1000 × A at 525 nm of turbidity-free sample measured by spectrophtr or colorimeter with bandwidth of ≤10 nm, using 1 cm cell and distd H_2O as ref.

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The report of the Associate Referee was approved by the General Referee and Committee D and was adopted by the Association. See *J. Assoc. Off. Anal. Chem.* (1982), this issue.

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Table 1. Collaborative results for determination of color intensity of whisky [a]

Coll.	Instrument; bandwidth	Group 1 Samples				Group 2 Samples			
		2	5	3	7	8	9	4	6
1	Hitachi 100-80	28	34	134	150	247[b]	250[b]	328[b]	369[b]
	2 nm	29	35	135	153	251[b]	251[b]	329[b]	370[b]
2	Beckman 25	29	35	134	154	255	258	333	376
	2 nm	29	36	135	155	255	258	333	376
3	Turner 350	30	35	137	152	250	253	332	376
	9 nm	26	32	133	148	246	248	328	372
4	Beckman M-25	29	34	138	154	254	257	334	377
	2 nm	30	34	138	154	255	258	334	377
5	Acta C11	29	34	137	153	252	255	331	375
	1 nm	29	34	138	153	253	256	333	375
6	Cary 15	31[b]	38[b]	142[b]	158[b]	257	260	336	380
		30[b]	37[b]	140[b]	158[b]	254	257	338	380
7	Spectronic 210	31[b]	36[b]	141[b]	157[b]	258[b]	261[b]	337[b]	381[b]
	1 nm	31[b]	36[b]	142[b]	158[b]	258[b]	260[b]	337[b]	381[b]
8	Acta C11	29	34	138	152	254	256	330	374
	1 nm	29	34	139	153	256	257	331	375
9	Hitachi 200	28	36	140	155	256	262	334	378
	2 nm	29	35	139	154	257	260	333	377
10	Perkin-Elmer 550	30	36	138	155	255	258	332	377
	2 nm	30	36	139	156	257	260	334	377
11	Spectronic 20	30	32	135	150	248[b]	250[b]	324[b]	368[b]
	20 nm	29	32	135	150	250[b]	250[b]	325[b]	370[b]
12	Beckman 24	30	36	136	154	253	255	327	371
	2 nm	30	35	135	152	252	255	327	371
13	Beckman 25	25	29	136	151	253	255	330	373
	2 nm	25	30	135	151	253	255	330	373
14	Beckman 25	29	33	135	150	252[b]	252[b]	326[b]	368[b]
	2 nm	30	34	135	150	250[b]	252[b]	324[b]	368[b]
15	Spectronic 20[c]	33	39	135	151	246[b]	250[b]	315[b]	358[b]
	20 nm	31	34	132	148	244[b]	245[b]	311[b]	357[b]
16	Coleman 124D	25	29	136	154	255	256	336	378
	1 nm	27	30	135	154	255	256	337	380
17	Cary 15	30[b]	37[b]	145[b]	155[b]	257	260	330	370
	9.5 nm	32[b]	35[b]	137[b]	155[b]	257	260	330	370
18	Beckman 25	26	31	135	152	254	257	330	374
	2 nm	26	30	135	151	255	257	331	375
19	Beckman 25	30	35	139	154	256	259	330	373
	2 nm	30	35	140	154	256	259	330	374
20	Beckman DU	30[b]	34[b]	130[b]	148[b]	243[b]	248[b]	315[b]	355[b]
	1.5 nm	30[b]	35[b]	130[b]	150[b]	245[b]	248[b]	320[b]	355[b]
Mean		28.8	33.7	135.9	152.2	253.7	256.8	331.0	373.8
SD		1.8	2.3	2.5	2.2	3.5	3.1	4.6	5.8
CV, %		6.39	6.68	1.84	1.44	1.39	1.20	1.39	1.55

[a] Reported as CIU (= absorbance × 1000).
[b] Result discarded based on AOAC ranking and outlier tests.
[c] ½ in. cell used; results converted.

Calibration of Spectrophotometer and Procedure

Check accuracy of wavelength scale of spectrophtr or colorimeter with didymium or Ho_2O_3 glass filter or Hg lamp. Use manufacturer's operating procedure to calibrate and operate instrument. Place turbidity-free sample in 1 cm cell and det. *A* at 525 nm against distd H_2O as ref. Color Intensity Units (CIU) = 1000 × *A*.

Results and Discussion

Twenty collaborators submitted results (Table 1). To facilitate data handling, the paired data were divided into 2 groups: (*1*) Samples 2, 5, 3, and 7 covering the range of 29–152 CIU, and (*2*) Samples 8, 9, 4, and 6 covering the range of 254–374 CIU. Using the AOAC ranking criteria, results of Collaborators 6, 7, 17, and 20 in group 1 and Collaborators 1, 7, 11, 14, 15, and 20 in group 2 fell outside the critical score at the 95% confidence level. Therefore, these results were eliminated from the statistical evaluation.

Results in Table 1 appear acceptable for whisky samples that vary in color intensity from 29 to 374 CIU. Maximum standard deviation and coefficient of variation are 5.8 and 6.68%, respectively.

Table 2. Statistical evaluation of collaborative results [a]

Statistic	Group 1	Group 2
Number of colls.	20	20
Number of coll. results discarded	4	6
Between-lab. variance ratio (*VR*)	3.265S	1.628NS
Lab–sample interaction (*VR*)	3.959S	9.943S
Reproducibility SD	2	3
Repeatability SD	1	1
Reproducibility CV	10.9	14.1

[a] S = significant at 95% confidence level; NS = not significant at 95% confidence level.

An analysis of variance of the acceptable data was made as recommended by Steiner (3) and results are summarized in Table 2. The reproducibility and repeatability standard deviations are acceptable, ≤3, but variance ratios indicate that significant differences occur in results reported between different laboratories analyzing identical samples. However, these results are acceptable for product control.

Many of the 14 different instruments used are more than 7 years old and no longer manufactured; some have a bandwidth ≥10 nm. No attempt was made to correlate results reported with the instrument used.

Results for group 2 samples obtained with a Bausch & Lomb Spectronic 210 and two Spectronic 20 instruments (Collaborators 7, 11, and 15) were eliminated by the ranking test. Collaborator 15 converted results obtained with a 1/2 in. cell to values that would be obtained with a 1 cm cell.

Collaborator 4, using a Beckman M-25 spectrophotometer, reported that "the absorbance of these samples is relatively sensitive to temperature and if the sample residence time in the spectrophotometer was more than 2 or 3 min, the absorbance value of the sample appeared to diminish by 2 or 3 points." We did not encounter this problem and feel that it may be related to instrument design. This was investigated on 2 different instruments. In a Klett-Summerson colorimeter, the color intensity of a whisky sample decreased 1 Klett unit after 1 min exposure and 1 additional unit during the next 9 min. The absorbance value of the same whisky sample did not change during a 10 min exposure in a Beckman Acta C11 spectrophotometer.

This method offers advantages over the current AOAC method (1) for determining color of whisky and possibly other similarly colored, aged alcoholic products. By using a spectrophotometer with the wider bandwidth of ≤10 nm, a medium-priced instrument suitable for plant laboratories can be employed. By taking readings at 525 nm, the CIU values can be compared with visual results.

Recommendation

It is recommended that this method for measuring color intensity of whiskies be adopted official first action.

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DRUG RESIDUES IN ANIMAL TISSUES

Electron Capture Gas-Liquid Chromatographic Determination of Morantel-Related Residues in Bovine Liver

MARTIN J. LYNCH and S. RICHARD BARTOLUCCI
Pfizer Inc., Central Research, Drug Metabolism, Groton, CT 06340

A gas-liquid chromatographic assay has been developed to determine major residues of morantel in bovine liver, a target tissue, at levels of 0.2-0.8 ppm. The method is based on hydrolysis of the *N*-methyltetrahydropyrimidine portion of morantel and its metabolites to *N*-methyl-1,3-propanediamine, and conversion of the diamine to an *N,N'-bis*-(2-nitro-4-trifluoromethylphenyl) derivative. The addition of an internal standard, *N*-ethyl-1,3-propanediamine, to the tissue sample circumvents any potential problem that could arise from variable reaction yields, and eliminates the true recovery as a factor affecting the accuracy and precision of the procedure. The concentrations of the derivatives are determined by pulsed electron capture gas-liquid chromatography over a linear dynamic range equivalent to 0.2-0.8 ppm morantel. The method has been evaluated at 0, 0.2, 0.4, and 0.8 ppm levels in fortified bovine liver, and in a withdrawal sample containing physiologically incurred morantel residues. Mean values of 0.22 ± 0.015, 0.40 ± 0.033, and 0.79 ± 0.045 ppm were found for fortified samples, 0.02 ppm for control liver, and 0.56 ± 0.050 ppm for the withdrawal sample.

Development of analytical methods for enforcement of tissue residue tolerances of drugs that are rapidly metabolized to intractable products is a formidable task when proposed metabolic and assay criteria are to be met (1). What is ideal is often at odds with practical considerations. In our experience, physico-chemical techniques proposed for isolating and identifying bound or intractable residues are not compatible with development of routine analytical methods for sub-microgram residues (2). In this case, liberating the bound residue from tissue by chemical means is justified if analytically useful information is to be obtained. However, this chemical releasing process may convert the residue to one or more moieties, contribute to some losses, and create background problems depending on the similarity of the products to natural constituents. Analytical methods for tissue residues of arsenicals (3), carbadox (4), pyrantel (5), ethopabate (6), arprinocid (7), and cambendazole (8) are examples of methods where the tissue matrix and other similar unavoidable analytical problems were overcome. Morantel tartrate is another example of an animal health product that is rapidly metabolized to poorly extracted residues that require vigorous chemical steps to liberate and convert the tissue residue to one or more measurable moieties. This procedure has been termed the common fragment approach to tissue residue analyses (5).

Morantel tartrate is an anthelmintic compound that belongs to a family classified as tetrahydropyrimidines. Chemically it is 1,4,5,6-tetrahydro - 1-methyl-2-[*trans*-2-(3-methyl-2-thienyl)-vinyl]pyrimidine tartrate. This drug is formulated in a bolus or a feed premix and is administered to cattle in a single dose for removal and control of mature gastro-intestinal nematode infections that include worms of the stomach and small and large intestines. The drug is administered in a bolus or in feed to cattle at the single dose of 9.7 mg/kg. A homolog, pyrantel tartrate, has been approved for prophylactic use in swine in many countries including the United States.

Radiotracer metabolism studies have shown that morantel is rapidly metabolized to poorly extracted, bound residues; digestion and hydrolysis are required to liberate and convert tissue residues to one or more measurable and identifiable moieties (J. K. Faulkner and A. G. Davidson, Drug Metabolism, Pfizer Ltd, Sandwich, UK; private communication). Oxidative biotransformation has been described for pyrantel (5, 9), and is similar to the metabolism of morantel. Radioactive residues of morantel were primarily found in liver, a target tissue, followed by kidney and muscle. Little or no radioactivity was found in fat. However, no single chemical represented all tissue residues of morantel. With alkaline hydrolysis, morantel-related residues were converted to *N*-methyl-1,3-propanediamine (MAPA) and 3-(3-methyl-2-thienyl)acrylic acid (CP-20,107) as major (50%) and minor (10%) components of total radioactivity. Reverse isotope dilution studies,

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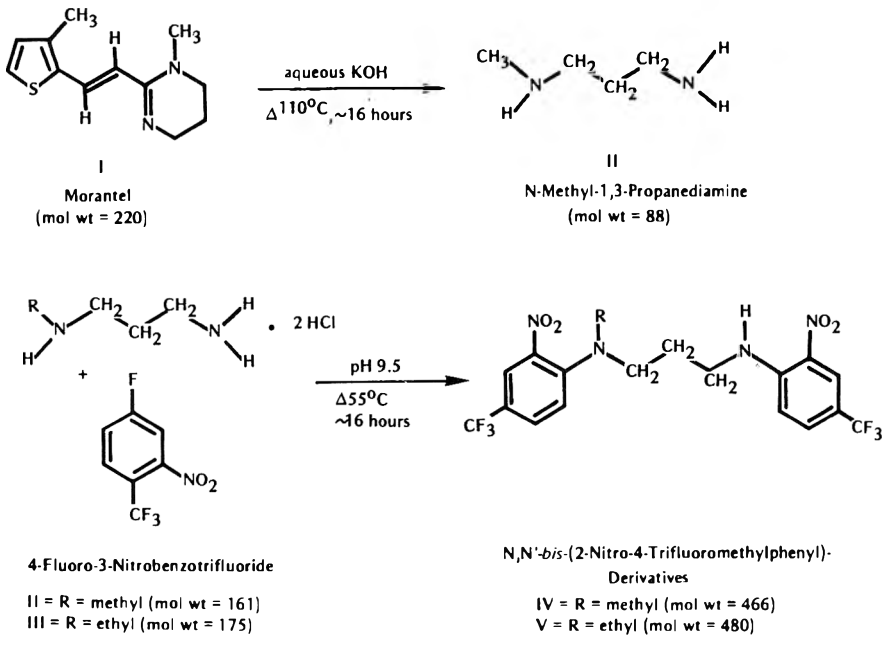

Figure 1. Chemical conversion of morantel to *N*-methyl-1,3-propanediamine and derivatization of this amine and its *N*-ethyl analog to the corresponding *N,N'-bis*-2-nitro-4-trifluoromethylphenyl derivative.

in which cold carrier morantel is added to tissues containing radioactive residues, demonstrated that MAPA represented half of the total radioactivity in liver over a 4 week withdrawal period. The lower abundance of CP-20,107 indicated that metabolism had occurred in the thienyl portion of morantel.

On the basis of this radiotracer metabolism work, an assay was developed which measures MAPA. This method is capable of measuring the clearance of morantel-related residues in bovine liver, and is the subject of this report.

METHOD

Principle

Bovine tissues containing morantel (I, Figure 1) and metabolites are hydrolyzed in aqueous KOH to yield *N*-methyl-1,3-propanediamine (II), a product common to drug-related residues. *N*-Ethyl-1,3-propanediamine dihydrochloride (III) is added as an internal standard to the sample before the hydrolysis step at a concentration equivalent to 0.4 ppm morantel in tissue. Following hydrolysis, both diamines (II and III) are extracted into toluene, back-extracted into dilute aqueous acid, and converted to their *bis*-2-nitro-4-trifluoromethylphenyl derivatives (IV and V) with 4-fluoro-3-nitrobenzotrifluoride in a borax-buffered solution. The diamine derivatives are separated from the reaction mixture by extraction into *n*-hexane and purified by thin layer chromatography before evaluation by pulsed electron capture gas-liquid chromatography.

Accurate results are obtained by referring the relative peak heights of the diamine derivatives of the morantel-related compound (CP-17,347) and of the internal standard (CP-17,462) to a standard curve. This curve is constructed by relating, in a linear manner, the relative peak height response of calibration solutions of the diamine derivatives to 0.5, 1.0, 1.5, and 2.0 molar ratios of each. The concentrations of the calibration solutions are selected to optimize the working range of the GLC instrument and to reflect residue levels ranging from 0.2 to 0.8 ppm. A broader dynamic range may be used, but the recommended range simplifies GLC calibration and assay of tissue extracts. From the established relationship of the relative response of the 2 diamines to a weight ratio, and the known amount of internal standard added to the samples, the analyst can obtain the unknown concentration of morantel by a graphical extrapolation or a linear regression calculation.

Table 1. Concentrations of standard solutions for calibration of GLC instrument

Soln	CP-17,347[a]		CP-17,462[b]		Molar ratio	Morantel, ppm[c]
	μg/mL	μmole/mL	μg/mL	μmole/mL		
1	0.0400	8.58×10^{-5}	0.0206	4.29×10^{-5}	2.0	0.8
2	0.0300	6.43×10^{-5}	0.0206	4.29×10^{-5}	1.5	0.6
3	0.0200	4.29×10^{-5}	0.0206	4.29×10^{-5}	1.0	0.4
4	0.0100	2.14×10^{-5}	0.0206	4.29×10^{-5}	0.5	0.2

[a] *N,N'-Bis*-(2-nitro-4-trifluoromethylphenyl)-*N*-methyl-1,3-propanediamine.
[b] *N,N'-Bis*-(2-nitro-4-trifluoromethylphenyl)-*N*-ethyl-1,3-propanediamine.
[c] When the internal standard is added to tissue at a level equivalent to 0.4 ppm morantel.

Reagents

(a) *Solvents.*—All solvents were glass-distilled, or equivalent. No special precautions were taken with glassware.

(b) *Chemicals.*—All chemicals were reagent grade. 4-Fluoro-3-nitrobenzotrifluoride (ICN Life Sciences Group (K & K), Plainfield, NY 11803, or Marshallton Research Laboratories, Inc., Winston-Salem, NC 27106).

(c) *Analytical standards.*—Morantel tartrate (CP-12,009-18); *N*-ethyl-1,3-propanediamine dihydrochloride (CP-45,991-1); *N,N'-bis*-(2-nitro - 4-trifluoromethylphenyl)-*N*-ethyl-1,3-propanediamine (CP-17,462); and *N,N'-bis*-(2-nitro - 4-trifluoromethylphenyl)-*N*-methyl-1,3-propanediamine (CP-17,347) all provided by Central Research, Pfizer Inc., Groton, CT 06340).

Solutions

(a) *Potassium hydroxide.*—4M. Dissolve 264 g 85% KOH in water and dilute to 1 L.

(b) *4-Fluoro-3-nitrobenzotrifluoride.*—0.2%. Dilute 0.135 mL (ca 200 mg) 4-fluoro-3-nitrobenzotrifluoride to 100 mL with distilled acetone. Fresh solutions were prepared.

(c) *Thymol blue.*—0.1%. Dissolve 100 mg thymol blue in 100 mL 0.1M NaOH.

(d) *Toluene–ethyl acetate (85 + 15).*—Dilute 150 mL ethyl acetate to 1 L with toluene.

Preparation of Standard Solutions

Morantel tartrate solutions.—Dissolve 105.07 mg Reference Standard Morantel Tartrate (equivalent to 62.5 mg morantel) in enough water to make 100.0 mL (625 μg/mL as morantel; 2.84 μmole/mL). Dilute 4.0 mL of this stock solution to 50.0 mL with water (50.0 μg/mL as morantel; 0.227 μmole/mL). Dilute 2.0, 4.0, and 8.0 mL aliquots of latter solution to 100 mL with water (1.0, 2.0, and 4.0 μg/mL as morantel; 0.0045, 0.0091, and 0.018 μmole/mL).

N-Ethyl-1,3-propanediamine dihydrochloride internal standard solutions.—Dissolve 39.7 mg Reference Standard *N*-Ethyl-1,3-propanediamine Dihydrochloride in enough water to make 100.0 mL. Dilute 1.0 mL of this stock solution to 250.0 mL with water (1.59 μg/mL; 0.0091 μmole/mL). (Note: Stock solutions of *N*-ethyl-1,3-propanediamine dihydrochloride and morantel tartrate are stable at least one month when stored at 5°C.)

Gas Chromatography Calibration Solutions

N,N'-Bis-(2-nitro-4-trifluoromethylphenyl)-N-ethyl-1,3-propanediamine.—Dissolve 5.15 mg *N,N' - bis*-(2-nitro-4-trifluoromethylphenyl)-*N*-ethyl-1,3-propanediamine in 200.0 mL toluene (stable at 20–25°C at least one month if protected from light). Dilute 10.0 mL to 100.0 mL with toluene (2.58 μg/mL).

N,N' - Bis - (2- nitro-4-trifluoromethylphenyl)-N-methyl-1,3-propanediamine.—Dissolve 5.00 mg *N,N'-bis*-(nitro-4-trifluoromethylphenyl)-*N*-methyl-1,3-propanediamine in 200.0 mL toluene (stable if protected from light). Dilute 10.0 mL to 100.0 mL with toluene (2.50 μg/mL).

Working standard solutions 1, 2, 3, and 4.—Dilute 4.0, 3.0, 2.0, and 1.0 mL aliquots, respectively, of *N,N' - bis* - (2-nitro-4-trifluoromethylphenyl)-*N*-methyl-1,3-propanediamine (2.50 μg/mL) with 2.0 mL *N,N'-bis*-(2-nitro-4-trifluoromethylphenyl)-*N*-ethyl-1,3-propanediamine (2.58 μg/mL) and adjust each solution to 250.0 mL with toluene. Cover flasks with aluminum foil. Concentrations and molar ratios of these calibration standards are presented in Table 1.

Thin Layer Chromatography Marker Solution

Dissolve 2.00 mg each of the *bis*-(2-nitro-4-trifluoromethylphenyl) derivatives of *N*-methyl- and *N*-ethyl-1,3-propanediamine in 10.00 mL ethyl acetate.

Apparatus

(a) *Gas-liquid chromatograph.*—Micro-Tek Model 220, or equivalent, equipped with ^{63}Ni electron capture detector, and 1.8 m × 4 mm id all-glass column with on-column injection and packed with 3% OV-25 on 100–120 mesh Supelcoport. Condition packed column ≥24 h at 290°C with carrier gas flow. Column may be primed with silanizing agent (ReJUV-8) to maintain sensitivity and peak shape. Conditions: column 260°C; injection port 285°C; detector 300°C; argon–methane (9 + 1) carrier gas 80 mL/min. Power supply pulse parameters: RF mode, voltage output 55 eV; pulse rate 270 μs; pulse width 3.0 μs. Under these conditions, retention times are 3.7 and 3.0 min for *N*-methyl- and *N*-ethyl derivatives (CP-17,347 and CP-17,462), respectively.

(b) *Precoated thin layer plates.*—5 × 20 cm, 250 μm thickness, silica gel 60 F254 (EM Reagents, distributed by Brinkmann Instruments, Inc., Westbury, NY 11590).

(c) *Multiplate developing tank.*—For ten 5 × 20 cm TLC plates (Desaga, distributed by Brinkmann Instruments, Inc.).

(d) *Centrifuge tubes.*—Heavy duty, 50 mL graduated (60 mL capacity), equipped with glass stoppers (Lab Glass Inc., or equivalent).

(e) *Screw-cap test tubes.*—25 × 150 mm with Teflon liners (e.g., A. H. Thomas Co., No. 9447-E50, Philadelphia, PA 19105).

Procedure

Dissolution, internal standardization, and hydrolysis.—Transfer 5.0 g sliced tissue to screw-cap tube, add 1.0 mL *N*-ethyl-1,3-propanediamine dihydrochloride working internal standard solution (1.59 μg/mL), and 10 mL 4M KOH solution. Cap tube and immerse in 110°C oil bath so contents of tube are just below oil level. Digest samples 16–18 h. Cool hydrolysates in ice bath. (Note: Control liver samples, 25 g each, are prepared by subdividing homogenized specimen (1–3 kg) of whole liver. Thinly sliced portions of these samples, 5 g each, are supplemented with 1.0 mL aliquots of morantel tartrate standard solutions containing 1.0, 2.0, and 4.0 μg/mL as morantel base to determine accuracy and precision.)

Extraction of hydrolysate.—Pour hydrolysate into 60 mL centrifuge tube, and add 6–7 g KOH pellets. Stopper, cool the sample, and dissolve pellets by mixing on test tube mixer. Return tube to ice bath. Wash test tube used for hydrolysis with 25 mL toluene and transfer solvent to centrifuge tube containing basified hydrolysate. Stopper and hand-shake gently to extract. Centrifuge mixture 5 min at >1500 rpm to clarify phases. Recover toluene layer by using blow-out pipet equipped with Propipet bulb, and transfer this extract to 60 mL centrifuge tube. (Note: Avoid transferring any interface.) Re-extract hydrolysate with additional 25 mL toluene, centrifuge to clarify extract, and combine extracts. Discard aqueous layer. Add 5.0 mL 0.2N HCl to combined toluene extracts, mix on test tube mixer, and centrifuge. Aspirate toluene layer and discard. Pipet 4.0 mL aqueous-acid layer into 50 mL centrifuge tube, and add one drop of thymol blue indicator. Solution should be pink. If not, pH is too high for quantitative transfer of drug residue from toluene layer. Add 0.5 mL 0.2M boric acid and 1N NaOH until color of solution changes from pink (acid) to blue (pH ca 9.4). Add 1 mL 0.1M sodium tetraborate solution.

Derivatization.—Add 5 mL 0.2% 4-fluoro-3-nitrobenzotrifluoride solution, stopper, and mix by gentle hand-shaking. Vent and stopper loosely to relieve pressure. Place tube in 55°C water bath for 16 h (overnight). Cool solution to room temperature, and add 10.0 mL *n*-hexane and 25 mL water. Mix on test tube mixer and centrifuge to clarify.

Thin Layer Chromatography

Line TLC chamber with blotting paper, add toluene–ethyl acetate (85 + 15), and let stand in chamber overnight. Replace old solvent with new within an hour or so before chamber is used for TLC. Apply 0.100 mL portion of *n*-hexane extract of each sample in band, 1 inch long × ca 5 mm wide, to separate 5 × 20 cm plates such that center of 5 mm band is ca 12 mm above bottom of TLC plate. Also, prepare control plate by taking second 0.100 mL portion of one hexane sample extract, streaking this on separate plate, and overlaying this with 0.10 mL thin layer chromatography marker solution. (Note: Only one control plate is required for each chromatography jar; 5 × 20 cm plates and suitably slotted chromatography jars are recommended for this step. Keep TLC chamber out of drafts while it is being equilibrated or while plates are being developed.) Before chromatographic development, place bottom edge (ca 5 mm deep) of sample and control thin layer plates into beaker of ethyl acetate so solvent will rise through applied sample zones to form them into a narrow band 25 mm above bottom edge of plate. Remove plate from beaker and place in hood until it is

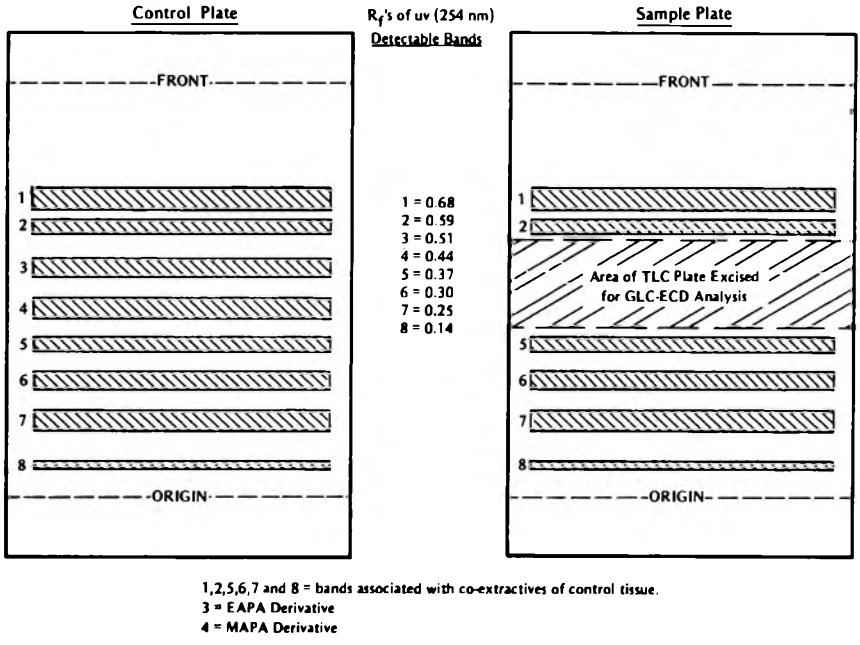

Figure 2. Thin layer chromatographic separation of MAPA and EAPA derivatives and of 4-fluoro-3-nitrobenzotrifluoride by-products of alkaline liver hydrolysate.

certain that ethyl acetate has evaporated (ca 20 min).

Scrape off silica gel layer 5 mm from top of each plate. Place prepared plates, with adsorbent layers facing each other, in chromatographic chamber lined with blotting paper and saturated with toluene–ethyl acetate (85 + 15), and develop each until solvent front reaches top of plate. Air-dry developed plates in a fume hood to remove toluene.

Examine plates under short wavelength ultraviolet light (254 nm) and locate on control plate the zone containing *N,N'-bis*-(2-nitro-4-trifluoromethylphenyl) derivatives of *N*-methyl-1,3-propanediamine and *N*-ethyl-1,3-propanediamine (R_f values ca 0.4 and 0.5, respectively). See Figure 2.

Line up tissue-related bands on sample and control plates. Mark ca 28 mm × 5 cm zone of silica gel on sample plate encompassing area containing each derivative. (Notes: Zones to be scraped should allow for irregularities in the chromatographic migration of sample bands by scraping along the periphery of bands 2 and 5 in Figure 2. Extracts of the derivative may be stored at room temperature for replication of the TLC step.) To facilitate its removal, score adsorbent in sample area, every 2 or 3 mm with sharp scalpel. Scrape silica gel zone from sample plate with scalpel or spatula and collect powder on glassine paper. Pour this powder into 15 mL centrifuge tube, add 1.0 mL of ethyl acetate, stopper, and mix on test tube mixer 30 s. Clarify suspension by centrifugation. Examine supernate by gas-liquid chromatography.

Gas-Liquid Chromatography

Inject into gas-liquid chromatograph equal μL aliquots of samples and GLC Calibration Solutions. Peak height of "EAPA" component of these standards should approximate 50% full scale deflection (Figure 3).

Standard curve.—Compute peak height (mm) ratio of *N*-methyl- to *N*-ethyl derivatives in each GLC Calibration Solution. Fit data to best straight line. Calculate slope and intercept for standard curve, using peak height ratio as y coordinate and equivalent ppm morantel level of standards as x coordinate.

Calculations.—From standard slope and intercept values and observed peak height ratio of *bis*-(2-nitro-4-trifluoromethylphenyl) derivatives of *N*-methyl-1,3-propanediamine (CP-17,347) and *N*-ethyl-1,3-propanediamine (CP-17,462) in sample, determine ppm morantel in tissue sample by following equation:

ppm Morantel in tissue = peak height ratio of

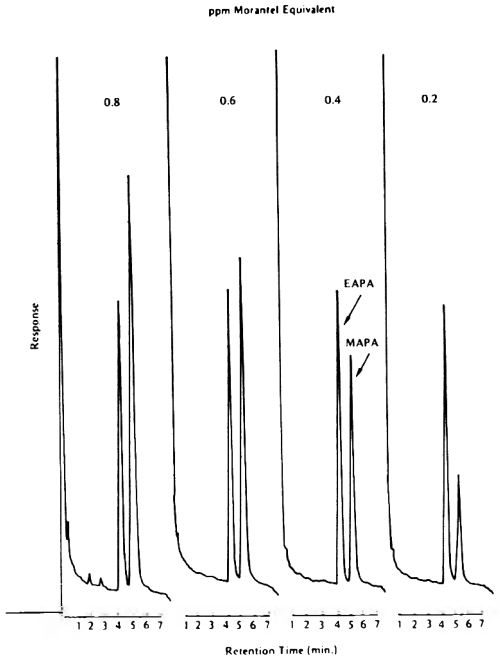

Figure 3. GLC chromatograms of calibration standards of MAPA (CP-17,347) and EAPA (CP-17,462) derivatives.

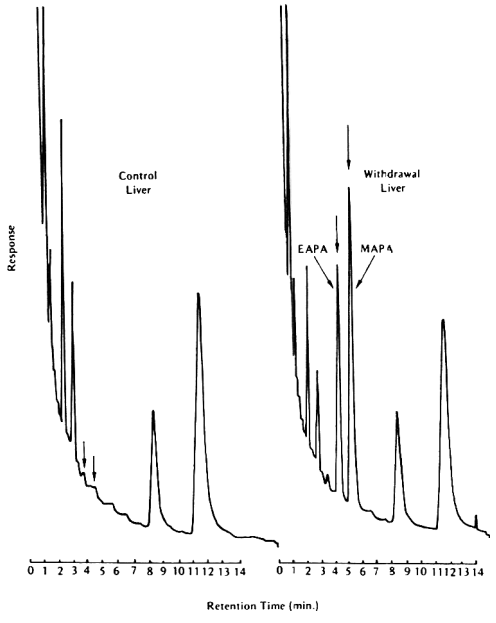

Figure 4. GLC chromatograms of control and withdrawal bovine liver specimens.

CP-17,347 to CP-17,462 in sample-intercept (std)/slope (std)

Results and Discussion

Preliminary studies established conditions for analysis of morantel-related residues. These studies included an assessment of alkaline hydrolysis of the tetrahydropyrimidine ring to *N*-methyl-1,3-propanediamine (MAPA), and its conversion to an *N,N'-bis*-(2-nitro-4-trifluoromethylphenyl) derivative (CP-17,347). The use of morantel labeled with carbon-14 at position 4 of the tetrahydropyrimidine ring guided this work. Information derived from earlier work with radiolabeled pyrantel was also used (9).

These studies revealed that an overnight digestion in 4M KOH is required to convert morantel to MAPA when the drug is supplemented in bovine liver at 0.4 ppm, and a molar concentration of at least 6M KOH is needed to partition MAPA into toluene. Derivatization, which follows the procedure of Crosby and Bowers (10), requires a 100-fold excess of reagent. The overall yield up to the TLC step is 67 ± 1.8% (n = 5), and results from efficiencies of 80–90% in the hydrolysis, extraction, and derivatization steps. For this reason, a closely related diamine, *N*-ethyl-1,3-propanediamine (EAPA), is added to tissue as an internal standard at the molar equivalent of 0.4 ppm morantel.

The physico-chemical properties of the 2-nitro-4-trifluoromethylphenyl derivative of MAPA and EAPA (CP-17,347 and CP-17,462, respectively) are well suited for isolating the diamines by extraction and thin layer chromatography, and quantitation by electron capture gas-liquid chromatography. Picogram sensitivity and near-baseline resolution of these derivatives are found under the recommended GLC conditions. Correlation coefficients of 0.999 have been found for replicates of the calibration curve.

The calibration curve is constructed by relating in a linear manner the relative peak height response of the derivatives to a weight ratio of each. The concentrations of the standards are selected to optimize the working range of the GLC instrument and to determine residue levels ranging from 50 to 200% of the regulatory level. Calibration solutions containing MAPA and EAPA derivatives in the molar ratios of 0.5, 1.0, 1.5, and 2.0 are equivalent to 0.2, 0.4, 0.6, and 0.8 ppm morantel when the internal standard is added at the molar equivalent of 0.4 ppm morantel.

The procedure described here was evaluated in 3 successive trials by assaying control, forti-

Table 2. Morantel levels (ppm) determined in control, fortified, and withdrawal liver samples

Trial	Control liver	Fortified control liver			Withdrawal liver
		0.2 ppm	0.4 ppm	0.8 ppm	
1	0.01	0.23	0.42	0.78	0.52
	0.00	0.21	0.36	—	0.57
	0.00	0.20	0.45	0.75	0.52
	0.00	0.20	0.35	0.72	0.54
2	0.02	0.22	0.42	0.88	0.60
	0.02	0.24	0.42	0.80	0.66
	0.13	0.23	0.41	0.86	0.58
	0.02	0.23	0.40	0.78	0.59
3	0.00	—	—	0.79	0.48
	0.00	—	—	0.78	0.50
		—	—	0.77	0.55
		—	—	0.78	0.59
Av.	~0.02	0.22	0.40	0.79	0.56
SD	—	0.015	0.033	0.045	0.050
CV, %	—	6.8	8.3	5.7	8.9

fied, and withdrawal liver samples to assess noninterference, accuracy, precision, and variation within a homogenized specimen containing physiologically incurred residues. This specimen (3 kg) was obtained from 2 calves that were sacrificed 4-6 days after receiving a bolus of morantel tartrate at the recommended use level.

As seen in Table 2, mean values of 0.22 ±0.015, 0.40 ± 0.033, and 0.79 ± 0.045 ppm are found for replicate analyses of control calf liver supplemented with morantel at 0.2, 0.4, and 0.8 ppm, respectively. No significant interference (about 0.02 ppm) was detected in control liver. A withdrawal liver specimen containing physiologically incurred morantel residues contained 0.56 ± 0.050 ppm. The coefficient of variation in these analyses was 8.9% (Table 2). Chromatograms depicting the analysis of control tissue and of a bovine liver sample containing 0.8 ppm morantel-related residues are given in Figure 4.

The results indicate that by using an internal standard the true recovery of the morantel derivative does not affect the accuracy and precision of the method.

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FERTILIZERS

Comparison of Azomethine H Microcolorimetric and AOAC Titrimetric Methods for Boron in Fertilizer: Collaborative Study

JAMES R. MELTON
Texas A&M University, Texas Agricultural Experiment Station, College Station, TX 77843

Collaborators: D. M. Coggin; M. Erickson; G. B. Hunter; N. Jones; J. A. Kummer; J. P. Minyard; M. F. Pelletier; R. N. Price; B. L. Reynolds; R. K. Sensmeier; J. L. Smith; R. Sweeney; R. M. Vickery; R. T. Wetherbee

Sixteen fertilizer samples were sent to 11 collaborating laboratories for boron determinations. Each sample was analyzed once by the azomethine H microcolorimetric method and once by the AOAC titrimetric method (2.114-2.116). There were no statistical differences in matched pairs by the 2 methods at the 98% confidence level even though quantities ranged from 0.01 to 15%. The azomethine H microcolorimetric method has been adopted official first action.

Several analytical chemists have expressed the need for additional AOAC methods for determining boron in fertilizers. Even though atomic absorption (1) and argon plasma emission (2, 3) methods have been published, some laboratories do not have the necessary equipment to use these methods. Thus, collaborative studies involving these methods have been postponed. Meanwhile, some laboratory personnel have expressed confidence in the azomethine H microcolorimetric method reported by Thorpe (4). Therefore, a collaborative study was initiated to compare the microcolorimetric method with the official final action AOAC method (**2.114-2.116** (5)).

Collaborative Study

Eleven collaborating laboratories were sent 16 samples to analyze by the azomethine H microcolorimetric method (4) and by AOAC **2.114-2.116** (5). Rough estimates of boron quantities were given because samples were selected to include a wide concentration range. Each collaborator was sent a copy of the manuscript submitted by Thorpe (4) describing the azomethine H microcolorimetric method and a copy of AOAC **2.114-2.116** (5).

Each sample was to be analyzed once by each method. Practice runs could be made, but only one result (no averages) per sample for each method could be reported (32 total results). All significant values to the right of the decimal were reported. Only results obtained by following exact instructions for each method were to be submitted.

The study was designed to follow Youden's procedure for closely matched pairs (6). Samples designated P, O, N, M, L, K, J, and I were blind duplicates of A, B, C, D, E, F, G, and H, respectively. The 4 matched pairs consisted of the following blind duplicates: A/P-D/M; B/O-C/N; E/L-F/K; G/J-H/I.

Matched pair calculations were made by Youden's method (6). Confidence intervals of 98% (2-tail t values at the probability of 0.02) were applied to the sums of paired results to identify outlier laboratories. Outliers are designated in the tables. If one result of a pair was missing or excluded, the pair was rejected.

Within-laboratory deviation (repeatability), between-laboratory deviation (bias), and overall standard deviation are designated by the symbols, S_r, S_b, and S_d, respectively; S_d is defined as $\sqrt{S_r^2 + S_b^2}$. Those 3 deviations were determined, and the S_d value was used to calculate the coefficient of variation (CV).

Acid- and Water-Soluble Boron Official First Action

Apparatus and Reagents

(a) *Spectrophotometer.*—Beckman Model 24/25, or equiv.

(b) *Precision pipet.*—100 μL Sherwood Lancer (Sherwood Medical Industries, Inc., St. Louis, MO 63103), or equiv.

(c) *Dispenser pipet.*—Automatic (Repipet, Lab-

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The recommendations of the Associate Referee for official first action were approved by the General Referee and Committee A and were adopted by the Association. See *J. Assoc. Off. Anal. Chem.* (1982), this issue.

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Table 1. Means [a] for percentage boron on blind duplicates for fertilizer Samples A–P

AOAC				Colorimetric			
A	0.7010	P	0.7011	A	0.7538	P	0.7643
D	2.02	M	1.93	D	2.09	M	2.08
B	13.02	O	13.01	B	13.21	O	13.49
C	14.81	N	14.61	C	14.93	N	14.85
E	0.0397	L	0.0385	E	0.0447	L	0.0438
F	0.0323	K	0.0317	F	0.0352	K	0.0351
G	0.0137	J	0.0093	G	0.0175	J	0.0191
H	0.0190	I	0.0229	H	0.0193	I	0.0193

[a] Outliers excluded.

industries, Berkeley, CA 94710), or equiv., 5 mL capacity.

(d) *Boron std solns.*—(*1*) *Stock soln.*—100 μg/mL. Dissolve 0.5716 g boric acid in H_2O and dil. to 1 L with H_2O. Mix well and transfer to plastic bottle. (*2*) *Working solns.*—0, 5, 10, 15, 20, 25, 30 and 45 μg/mL. Pipet 0, 5, 10, 15, 20, 25, 30, and 45 mL stock soln into sep. 100 mL vol. flasks, dil. to vol. with 1% HCl, mix well, and transfer to plastic bottles. Solns are stable.

(e) *Azomethine H color reagent.*—Dissolve 0.9 g azomethine H (Pierce Chemical Co., Rockford, IL 61105) and 2.0 g ascorbic acid in 100 mL H_2O. Store in refrigerator and discard after 14 days.

(f) *Buffer-masking soln.*—Dissolve 140 g ammonium acetate, 10 g potassium acetate, 4 g nitrilotriacetic acid, disodium salt 99 + % (Aldrich Chemical Co., Inc., Milwaukee, WI 53233), 10 g (ethylenedinitrilo)tetraacetic acid, and 350 mL 10% acetic acid (v/v) in H_2O and dil. to 1 L with H_2O. Soln is stable.

(g) *Color developing reagent.*—Place 35 mL azomethine H soln and 75 mL buffer-masking soln into 250 mL vol. flask and dil. to vol. with H_2O. Prep. fresh daily.

Preparation of Sample Solutions

(a) *Acid-soluble boron.*—Weigh 2.00 g sample into 100 mL vol. flask, add 30 mL H_2O and 10 mL HCl, stopper, and shake 15 min. Dil. to vol. with H_2O, mix well, and filter immediately into plastic bottle. Dil. as necessary, so final soln for color measurement falls within std curve.

(b) *Water-soluble boron.*—Weigh 2.00 g sample into 250 mL beaker, add 50 mL H_2O, and boil ca 10 min. Filter hot thru Whatman No. 40 paper, or equiv., into 100 mL vol. flask. Wash ppt 6 times with hot, boiled H_2O until vol. in flask is ca 95 mL. Cool, add 1.0 mL HCl, dil. to vol. with H_2O, and mix. Transfer to plastic bottle immediately; dil. as necessary so final soln for color measurement falls within std curve.

Determination

Pipet 100 μL aliquots of 0, 5, 10, 15, 20, 25, 30, and 45 μg/mL std and 100 μL aliquots of solns into sep. 10 mL erlenmeyers. Add 5.0 mL color developing reagent by automatic pipet dispenser (5 mL pipet is suitable but slower) and let stand 1 h at room temp. Transfer to 1 cm cell and read *A* at 420 nm against H_2O. Correct for reagent blank (0 mg/mL std). Construct std curve by plotting *A* against μg/mL stds and read concns (μg/mL) of sample solns from std curve.

Calculation

Boron, % = (μg/mL from std curve) × diln factor × (100/g sample) × 10^{-6}

Results and Discussion

There were no practical differences between means of blind duplicates (Table 1). This indicates that variations of individual measurements made on the same day are minimal for either AOAC **2.114-2.116** or the microcolorimetric method. Therefore, within-day standard deviation was not calculated separately from total within-laboratory deviation.

S_r, S_b, and S_d were nearly equal for 3 of the 4 pairs of fertilizer samples studied (Tables 2 and 3). However, the azomethine H microcolorimetric method was more precise for the pair containing 0.01–0.02% boron. At these low levels of boron, coefficients of variation were quite large for both methods. (Both methods may give slightly high results; the Associate Referee found 0.01% boron in Samples G–J by atomic absorption and argon plasma emission, and Collaborator 6 also found 0.01% boron in these 4 samples by argon plasma emission.)

Means for the colorimetric method compared favorably with those for AOAC **2.114-2.116.** A *t*-test revealed there were no differences in pairs at the 98% confidence level. Thus, no statistical

Table 2. Collaborative results for percentage boron by AOAC 2.114–2.116

	Pair 1		Pair 2		Pair 3		Pair 4	
Coll.	A	D	B	C	E	F	G	H
1	0.7200	1.95	12.68	14.66	0.0300	0.0300	0.0100	0.0100
2	0.6900	2.03	13.25	14.94	0.0380	0.0310	0.0088	0.0320
3	0.2290[a]	0.595[a]	12.75	14.86	0.6450[a]	0.0276[a]	0.0090	0.0179
4	0.6911	1.90	12.86	14.69	0.0477	0.0424	0.0252	0.0174
5	0.7000	2.05	13.15	14.78	0.0400	0.0360	0.0100	0.0120
6	0.7100	2.12	13.21	14.63	0.0390	0.0300	0.0110	0.0100
7	0.6570[a]	1.50[a]	11.60[a]	12.80[a]	0.0348	0.0297	0.0156	0.0401
8	0.7500	2.01	13.34	15.05	0.0540[a]	0.0360[a]	0.0330[a]	0.0550[a]
9	0.6770	1.95	12.90	14.90	0.0420	0.0320	0.0120	0.0140
10	0.6700	2.12	17.04[a]	19.98[a]	0.0460	0.0300	0.1080[a]	0.0840[a]
11	0.4600[a]	2.27[a]	10.48[a]	12.25[a]	0.0400	0.0300	0.0220	0.0180
	P	M	O	N	L	K	J	I
1	0.6700	1.87	12.69	13.84	0.0300	0.0300	0.0100	0.0100
2	0.6900	2.00	13.19	14.89	0.0400	0.0320	0.0096	0.0340
3	0.6860[a]	0.147[a]	12.29	14.57	0.0394	0.0380	0.0110	0.0098
4	0.7205	2.03	13.15	14.87	0.0392	0.0327	0.0544[a]	0.0141[a]
5	0.7200	1.70	13.71	14.89	0.0410	0.0340	0.0100	0.0130
6	0.7000	1.95	13.45	14.69	0.0350	0.0300	0.0040	0.0110
7	0.6790	1.82	12.10	14.00	0.0458	0.0335	0.0160	0.0394
8	0.7300	2.00	13.25	14.89	0.0400	0.0260	0.0050	0.0450
9	0.6810	2.10	13.30	14.90	0.0430	0.0310	0.0100	0.0140
10	0.7500[a]	2.22[a]	11.00[a]	11.75[a]	0.0320	0.0300	0.0080	0.0300
11	0.6150[a]	0.88[a]	10.35[a]	9.62[a]	0.0220[a]	0.0240[a]	—	0.0150
Mean	0.6999	1.98	13.02	14.71	0.0392	0.0320	0.0115	0.0206
Pairs	16		17		19		18	
S_r	0.0816		0.224		0.0032		0.0092	
S_b	−0.0073[b]		0.298		0.0029		0.0013	
S_d	0.0813		0.373		0.0044		0.0092	
CV, %	6.08		2.69		12.4		57.3	

[a] Outlier pair omitted.
[b] $S_r > S_d$ by chance; S_b obscured.

difference is indicated in the sets of results obtained by the 2 methods.

A few comments were made by the collaborators. For example, Collaborator 4 suggested that a table of weights and dilutions might be helpful for the azomethine H method. Collaborators 4 and 9 reported that some samples were slightly coarse (Collaborator 4 reground all samples). Also, according to Collaborator 4, it was necessary to heat Samples C and N in 30 mL water and 10 mL HCl to attain complete solution, and the buffer-masking solution for the azomethine H method was diluted to 1 L—as instructed by the Associate Referee—even though final volume directions needed clarifying. Collaborator 5 extended boiling time to 20 min at pH 3.5–4.5 for AOAC **2.114-2.116** to remove CO_2 interference. According to Collaborator 8, the colorimetric method was much more rapid and more economical in reagents than AOAC **2.114-2.116.** Some difficulties were encountered by Collaborator 9 in dilutions for Samples A–D and M–P due to high levels of boron. For the microcolorimetric method, Collaborator 11 substituted sodium acetate for potassium acetate, and 11.2 g of the sodium salt of (ethylenedinitrilo)tetraacetic acid was substituted for 10 g of the recommended non-sodium form.

Recommendations

It is recommended that the azomethine H microcolorimetric method be adopted official first action, as clarified for preparing the buffer-masking solution. The last line should read ". . . acetic acid (v/v) in H_2O and dil. to 1 L with H_2O. Soln is stable."

Also it is recommended that 2 changes be made in AOAC **2.116** as follows: Change "When reading of pH 6.30 is steady, read 0.025N NaOH buret . . ." to read "When reading of pH 6.30 is steady, read vol. of buret contg 0.025N NaOH . . .". Change "With practice, somewhat slow approach to equilibrium . . . end point.)" to read "A somewhat slow approach to equilibrium, which is characteristic of glass electrodes, can be

Table 3. Collaborative results for percentage boron by azomethine H microcolorimetric method

	Pair 1		Pair 2		Pair 3		Pair 4	
Coll.	A	D	B	C	E	F	G	H
1	0.7510	2.18	13.20	14.90	0.0460	0.0370	0.0220	0.0230
2	0.7200	2.01	13.30	14.80	0.0410	0.0310	0.0160	0.0170
3	0.8250	2.10	12.88	14.91	0.1340[a]	0.0860[a]	0.0230	0.0305
4	0.7523	2.05	12.93	15.02	0.0450	0.0375	0.0138	0.0145
5	0.7900	2.17	13.39	14.71	0.0420	0.0320	0.0120	0.0120
6	0.7300	2.11	12.90[a]	13.50[a]	0.0410	0.0360	0.0160	0.0210
7	0.7060	2.05	12.30	14.70	0.0321[a]	0.00749[a]	0.0211	0.0196
8	0.7240	1.898	13.40	14.87	0.0500	0.0370	0.0140	0.0170
9	0.7860	2.22	14.20	15.50	0.0480	0.0360	0.0140	0.0150
10	1.0200[a]	2.40[a]	16.50[a]	18.80[a]	0.1040[a]	0.1000[a]	0.0490[a]	0.0690[a]
11	0.6700[a]	2.84[a]	13.26	14.99	0.0260[a]	0.0270[a]	0.0230	0.0230
	P	M	O	N	L	K	J	I
1	0.7480	1.93	13.20	14.60	0.0430	0.0410	0.0300	0.0250
2	0.7000	2.06	13.20	14.80	0.0410	0.0320	0.0150	0.0160
3	0.8270	2.12	13.25	15.10	0.0860[a]	0.0880[a]	0.0327	0.0308
4	0.7879	2.10	13.15	14.34	0.0527	0.0438	0.0560[a]	0.0231[a]
5	0.7700	2.14	13.74	14.71	0.0400	0.0350	0.0110	0.0140
6	0.7400	2.23	13.10	14.80	0.0460	0.0400	0.0210	0.0210
7	0.7460	1.86	13.90	14.50	0.0367	0.0193	0.0181	0.0206
8	0.7500[a]	1.80[a]	13.68	15.63	0.0450	0.0350	0.0170	0.0170
9	0.7960	2.17	14.20	15.20	0.0460	0.0350	0.0120	0.0160
10	1.0000[a]	2.53[a]	16.45[a]	18.50[a]	0.1680[a]	0.0900[a]	0.0650[a]	0.0690[a]
11	0.809[a]	0.60[a]	10.79[a]	9.59[a]	0.0230[a]	0.0160[a]	0.0150	0.0130
Mean	0.7588	2.08	13.34	14.90	0.0442	0.0352	0.0181	0.0193
Pairs	17		18		15		19	
S_r	0.0697		0.314		0.0026		0.0019	
S_b	0.0413		0.247		0.0041		0.0053	
S_d	0.0810		0.400		0.0049		0.0056	
CV, %	5.70		2.83		12.4		29.9	

[a] Outlier pair omitted.

anticipated with practice so as not to overrun end point.)"

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M. Erickson, State Dept of Agriculture, Denver, CO

G. B. Hunter, Tennessee Valley Authority, Muscle Shoals, AL

N. Jones, State Dept of Agriculture, East Lansing, MI

J. A. Kummer, State Dept of Agriculture and Consumer Services, Tallahassee, FL

M. F. Pelletier and R. T. Wetherbee, University of Vermont, Burlington, VT

R. N. Price, University of Kentucky, Lexington, KY

B. L. Reynolds and R. M. Vickery, State Agricultural Experiment Station, Geneva, NY

R. K. Sensmeier, Purdue University, West Lafayette, IN

J. L. Smith, State Dept of Agriculture, Raleigh, NC

R. Sweeney, University of Missouri, Columbia, MO

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FOOD ADDITIVES

Gas-Liquid Chromatographic Determination of Adipate Content of Acetylated Di-starch Adipate

GORDON A. MITCHELL, MICHEL J. VANDERBIST, and FLORENT F. MEERT
CPC Europe Research and Development Center, Havenstraat 84, B-1800 Vilvorde, Belgium

A gas-liquid chromatographic method is described for rapid, quantitative determination of adipate content of acetylated di-starch adipate. The adipate group is very labile and, under mild alkaline conditions at ambient temperature, is easily hydrolyzed from the starch. Free adipic acid is formed by acidification of the solution with HCl, and then extracted with ethyl acetate. Ethyl acetate is removed under vacuum distillation, and a silyl derivative of the adipic acid is formed. Glutaric acid internal standard is introduced into the original starch sample before hydrolysis. An aliquot of the silylated solution is injected into a gas chromatograph fitted with a column having silicone oil as the active phase. A flame ionization detector is also incorporated. Results correlate well with the amount of adipylating reagent used. No adipic acid is detectable when a hydrolyzed, extracted sample of acetylated di-starch adipate is subjected to a second extraction. Recovery levels of adipic acid, from starches fortified with 100-500 ppm, are in the range of 97-102.5%.

Acetylated di-starch adipate is a modified starch legally permitted for food applications. It is used extensively in fruit pie fillings where it promotes increased viscosity and imparts a cohesive texture. In addition, the modification confers high stability on the starch under acid conditions.

Until now, no reliable method has been available for the quantitative determination of adipyl substitution, although a recent article (1) reported a gas-liquid chromatographic (GLC) method for determination of adipic acid in foods. Simple methods for determining the degree of substitution of starch esters, based on alkaline hydrolysis and back-titration of excess alkali, will give only the degree of total ester substitution. In Europe, the maximum substitution levels for acetyl and adipyl groups are, respectively, 2.5 and <0.12%. In the absence of a reliable quantitative method for adipyl determination, 0.12% effectively represents the amount of adipic anhydride, based on starch, that may be used in the reaction. This level is recommended by the Food and Agriculture Organization of the United Nations (2).

A method has now been developed for reliable quantitative determination of the degree of adipyl substitution in acetylated di-starch adipate.

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METHOD

Apparatus and Reagents

(a) *Gas chromatograph.*—Hewlett-Packard Model 7620A gas chromatograph equipped with flame ionization detector and Model 3370A integrator. Column parameters: stainless steel, 6 ft × 1/8 in. id, packed with 5% OV-17 on 80–100 mesh Chromasorb GAW-DMCS (Alltech Europe, Inc., B 9731 Eke, Belgium); precondition column 24 h at 350°C with nitrogen carrier gas at 40 mL/min. Operating gas flow rates (mL/min): nitrogen carrier 30, hydrogen 40, air 400. Temperatures (°C): injection 280, detector 250, column 140. Retention times (min): glutaric acid 2.83, adipic acid 4.50.

(b) *N,N-Bis-trimethylsilyl-trifluoroacetamide (BSTFA).*—Macherey-Nagel, D 5160 Düren, GFR.

(c) *Reference acids.*—Glutaric acid (Merck, Schuchardt, GFR); adipic acid (UCB, Brussels, Belgium).

Calibration

Weigh 1.0 g waxy corn starch into each of four 250 mL Erlenmeyer flasks. To each flask, add 50 mL water and 1.0 mL of an aqueous solution containing 1.0 mg glutaric acid/mL. Add, to one flask, 0.25 mL of an aqueous solution containing 1.0 mg adipic acid/mL; to the other three, add 0.50 mL, 0.75 mL, and 1.0 mL. Each flask then contains 1.0 mg glutaric acid and, respectively, 0.25, 0.50, 0.75, and 1.0 mg adipic acid. Agitate flasks manually to disperse the starch fully and add 50 mL 4N NaOH. Continue agitation another 5 min, place each flask in water bath at ambient temperature, and carefully add 20 mL 12N HCl to each. When each flask is cool, quantitatively transfer contents to 250 mL separatory funnel. Extract with 100 mL reagent

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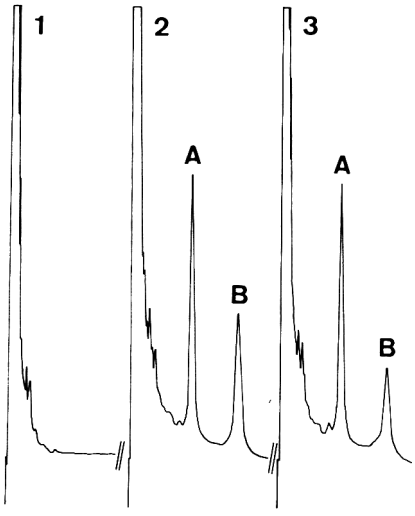

Figure 1. GLC chromatograms showing: 1, extracted unmodified starch; 2, extracted unmodified starch with glutaric acid (A) and adipic acid (B) added; 3, extracted acetylated di-starch adipate with glutaric acid (A) added as internal standard and adipic acid (B) present in the modified starch.

grade ethyl acetate. Drain bottom aqueous layer into beaker and collect upper organic layer in 500 mL Erlenmeyer flask containing 20 g anhydrous Na_2SO_4. Transfer aqueous portion back to separatory funnel and repeat ethyl acetate extraction twice more. Shake flasks periodically during 10 min and then filter contents through Whatman No. 1 paper into 1 L round-bottom flasks. Rinse flasks and insoluble residues in filters twice with 50 mL ethyl acetate. Under vacuum, at temperature not exceeding 80°C, evaporate total organic extraction and washings of each flask until completely dry.

Successively add 2 mL pyridine, 1 mL *N,N*-bis-trimethylsilyl-trifluoroacetamide, and, carefully, 1 mL trifluoroacetic acid to dry contents. Close round-bottom flasks with stoppers and rinse internal surfaces thoroughly with mixtures. Let flasks stand 1 h; then transfer ca 2 mL from each to small glass vials and immediately seal. Inject 4 μL into gas chromatograph.

Calculations.—Establish retention times for each acid and determine peak height for glutaric acid and for each level of adipic acid represented. A plot of peak height ratio of adipic acid to glutaric acid against amount of adipic acid is linear. This calibration curve may be used, but it is simpler to use a response factor (*RF*):

$$RF = (H \times W')/(W \times H')$$

where H and H' = peak heights of adipic acid and glutaric acid, respectively; and W and W' = weights of adipic acid and glutaric acid. *RF* should be verified weekly.

Table 1. Recovery by the proposed method of adipic acid added to acetylated di-starch adipate samples

Amt in unspiked sample, μg	Amt added, μg	Amt found, μg	Amt recd, μg	Rec., %
275	100	375	100	100
275	200	480	205	102.5
275	300	570	295	98.3
275	400	665	390	97.5
275	500	760	485	97.0

Sample Preparation

Weigh 1.0 g acetylated di-starch adipate into 250 mL Erlenmeyer flask, and add 50 mL water and 1.0 mL of an aqueous solution containing 1.0 mg glutaric acid/mL. Proceed as in *Calibration*, beginning "Agitate flasks manually . . .".

Calculation.—Record peak heights for adipic acid and glutaric acid (internal standard). Calculate amount of adipic acid contained in modified starch as follows:

$$W = (H \times W')/(RF \times H' \times S)$$

where W and W' = weights of adipic (μg) and internal standard (mg), respectively; H and H' = peak heights of adipic and internal standard; *RF* = response factor of adipic acid; and S = sample weight (g) taken for assay.

Results and Discussion

Typical chromatograms obtained from extractions of various starches are shown in Figure 1. The method was evaluated by recovery studies of known amounts of adipic acid added to acetylated di-starch adipate. In the range of 100–500 ppm adipic acid additions, recovery was 97–102.5% (see Table 1). The calibration method has been verified by derivatizing the pure acids without extraction; an identical response factor was found.

Five modified starch samples with adipic acid reagent levels of 0.1–0.3% of the starch were analyzed for reacted adipate. Table 2 shows good correlation between bound adipic acid and original reagent level. Reaction efficiencies varied from 28.5 to 36%. Two separate reactions with the same reagent level (0.2% on starch) gave identical adipic acid contents. The rate of hydrolysis of the adipate group was examined and no difference resulted from 4 h reflux compared with the short, ambient temperature treatment

Table 2. Assay of adipic acid in acetylated di-starch adipates made with various reagent levels

Adipic acid added at manufacturing stage, μg/g	Found, μg/g	Reaction efficiency, %
1000	285	28.5
1200	370	30.8
2000	715	35.8
2000	720	36.0
3000	910	30.3

used in the proposed method. Also, no adipic acid was detectable when a hydrolyzed, extracted acetylated di-starch adipate sample was subjected to a second extraction.

Ethyl acetate extraction is as efficient, and less hazardous in use, as solvent extraction with ethyl ether. Silylation, as opposed to methylation with diazomethane, was the more acceptable because diazomethane is a known carcinogen. A study of the silylation reaction showed completion after 1 h. The column active phase, OV-17, is widely available and is popularly used in sugar analyses.

As a test of reproducibility, 2 samples of acetylated di-starch adipate each were assayed 3 times by the described method. For each assay, 5 portions of the final solution were injected. The relative standard deviation (RSD) was 3.4% for one sample and 3.5% for the other. The detection limit of adipic acid by this method is 0.10 μg/g.

Conclusion

Acetylated di-starch adipate is an important food starch produced by several manufacturers. Until now no legislative limit has been applied to the actual reacted adipate content because of the absence of a reliable method for adipate determination. In addition, the lack of a method has handicapped the optimization of reaction conditions. The aim of the study was to produce a method which is not only reliable, but which is easy to perform and uses materials and apparatus common to food laboratories. This has been achieved and the method provides a basis for regulatory bodies and manufacturers to agree on legislative limits for actual adipate content of modified starch.

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MICROBIOLOGICAL METHODS

Microbial Hazards Associated with Bean Sprouting

WALLACE H. ANDREWS, PHILIP B. MISLIVEC, CLYDE R. WILSON, VERNEAL R. BRUCE, PAUL L. POELMA, REGINA GIBSON, MARY W. TRUCKSESS,[1] and KATHRYN YOUNG[1]
Food and Drug Administration, Division of Microbiology, Washington, DC 20204

The behavior of microorganisms was studied in mung beans and alfalfa seeds before and after germination in modified, commercially available bean-sprouting kits. The microorganisms were enumerated by the aerobic plate count (APC) and by total yeast and mold count procedures. *Salmonella* species were artificially inoculated into selected samples and were enumerated by the most probable number (MPN) method. After germination of the beans or seeds into mature sprouts, significant increases were noted in APCs and in MPN values of *Salmonella* species. Although counts of yeasts and molds did not increase significantly after germination, these samples showed an increase in toxic *Aspergillus flavus* and potentially toxic *Alternaria* species. The presence of toxic *Penicillium cyclopium* molds also increased substantially in 5 samples of a single brand of mung beans. Analysis of selected sprout samples, however, showed no presence of aflatoxin.

Germination of beans for human consumption has become a convenient method, both in the domestic kitchen and in large scale commercial operations, of ensuring the availability of fresh vegetables throughout the year. Diverse methods of bean sprouting have been popularized (1–14), each advocating its own schedule of soaking, washing, and rinsing the beans before and during germination. Most of these methods provide for the germination of the beans in a warm and moist environment, a condition also conducive to the rapid proliferation of microorganisms. Even low numbers of pathogenic bacteria and molds, which might be present on the dry beans, may have the potential of multiplying during germination to levels that could produce disease in humans.

In a recent survey (15) of health foods, *Salmonella* spp. were detected in samples of alfalfa and sunflower seeds, both of which are intended for consumption after germination. Although *Salmonella* spp. contamination is unacceptable at any level, an increase in the populations of *Salmonella* spp. or any other pathogens during the germination would increase the potential microbial hazard in sprouts. Moreover, levels of microorganisms in sprouts are particularly significant because sprouts are normally eaten raw in salads, soups, and sandwiches or are cooked only lightly to retain their crispness.

Both documented and potential hazards have been associated with bean sprouts. A 1973 outbreak (16) of *Bacillus cereus* food poisoning was traced to the consumption of vegetable sprouts grown in a commercially available seed-sprouting kit. In a later investigation of those kits, Portnoy et al. (17) reported that the *B. cereus* in the unsprouted seeds had proliferated to levels of 10^4–10^7 organisms/g in the germinated sprouts. In another outbreak in 1973 (16) of unknown etiology, alfalfa sprouts were incriminated as the vehicle of contamination. In examining alfalfa and bean sprouts at the retail level, Patterson and Woodburn (18) concluded that fecal coliforms appeared to be part of the normal flora and that *Klebsiella pneumoniae*, an opportunistic pathogen (19), constituted a major portion of the fecal coliform group. The present study was conducted to determine the extent, if any, to which selected microbial groups multiply during the germination of beans and seeds.

Experimental

General Approach

In the initial phase of this study the genera and species of molds indigenous to selected samples of mung beans and alfalfa seeds were determined. The extent to which germinating beans supported the proliferation of microorganisms was enumerated by the aerobic plate count (APC) and total yeast and mold count procedures. Dependent upon the species of molds present and the extent of their proliferation, sprout samples were examined for selected mycotoxins. We decided that if the germination procedure provided conditions suitable for the proliferation of the general aerobic microflora, as determined in the previous phases of this study, then we

[1] Division of Chemistry and Physics.

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would determine the extent of proliferation of specific pathogens, such as *Salmonella* spp., during the germination procedure.

Collection of Samples for APC, Mold, and Mycotoxin Analyses

For each germination experiment, a minimum of 1362 g each of mung beans and alfalfa seeds was obtained from individual retail packages weighing ca 454 g and representing a single code number. Samples of alfalfa seeds were generally free of extraneous matter, but stone fragments, dirt particles, damaged beans, and beans other than mung beans had to be removed from the mung bean samples. The individual retail packages of screened mung beans were mixed manually, as were the alfalfa seeds, in 46 × 33 × 13 cm (approx.) plastic trays to make the bulk samples as homogeneous as possible. After thorough mixing, 5 replicate 140 g (mung bean) and 130 g (alfalfa seed) samples were removed from the respective bulk samples. Each replicate sample was subdivided into the following portions: 50 g for APC and mold counts, 50 g for aflatoxin analysis, 20 g for direct plating to determine mold flora of seeds and beans, 20 g for germination of mung beans, and 10 g for germination of alfalfa seeds. Only selected replicate samples were analyzed for aflatoxins.

To ensure that the rate of germination of a particular lot or code of beans was satisfactory and that conditions for germination were favorable, the actual rate of germination of the mung beans used in an experiment was determined. A 20 g portion of mung beans was removed from the screened bulk sample and treated as a replicate sample. The rate of germination for a particular lot of mung beans was determined by dividing the number of sprouted beans by the total number of beans in the 20 g sample. Because the length of the sprouts varied at harvest, any sprouted bean, regardless of sprout length, was considered as having sprouted. Because alfalfa seeds were so small and contained a large number of seeds in a 10 g portion, determination of their germination rate was impractical.

Collection and Inoculation of Samples for Salmonella spp. Analysis

Two 454 g retail packages each of mung beans and alfalfa seeds of the same lot number were separately combined into samples weighing 908 g each and used for the *Salmonella* spp. analyses. Samples were screened and mixed as described above. Because samples naturally contaminated with *Salmonella* spp. were not available, the mung beans were inoculated with a pool of *S. anatum* and *S. montevideo* and the alfalfa seeds with *S. eimsbuettel* and *S. poona*. Individual serotypes of these pools were grown in brain heart infusion broth incubated at 35 ± 2°C for 18–20 h, harvested by centrifugation at 3090 × g, and washed twice in Butterfield's phosphate buffer, pH 6.8–7.2 (20). Five replicate 50 g portions of the beans or seeds were inoculated with one dilution of the washed *Salmonella* spp. pool, each thoroughly mixed by manual shaking in a plastic stomacher bag (A. J. Seward and Co. Ltd, London, UK) and combined to form a 250 g sample. This sample was again mixed manually and allowed to stand undisturbed at room temperature for ca 3 days. At that time 3 replicate samples of mung beans, 70 g each, and 3 replicate samples of alfalfa seeds, 60 g each, were weighed from each 250 g sample. Each 70 g replicate mung bean sample was then subdivided into a 50 g and a 20 g portion for determination of the *Salmonella* spp. level in the dry beans and after their germination, respectively. Similarly, each 60 g replicate alfalfa seed sample was subdivided into a 50 g and a 10 g portion for determination of *Salmonella* spp. level in the dry seeds and after their germination, respectively.

Germination Procedure

Before being soaked, samples were rinsed thoroughly and placed in a 3 L glass beaker covered with cheesecloth secured with rubber bands. Autoclaved distilled water which had tempered to room temperature was used to wash, soak, and rinse all samples. The water was added to the beakers, and the samples were washed by swirling the contents and discarding the supernate. This procedure was repeated twice for a total of 3 washings per sample. The washed beans or seeds were each placed in a 46 × 23 × 13 cm (approx.) plastic tray to which 2 L water was added. Mung beans were soaked for 16 ± 1 h, and alfalfa seeds were soaked for 12 ± 1 h; all soaking was done at room temperature. After the soaking, the water was decanted and the soaked beans or seeds were washed twice as described previously.

The soaked beans or seeds were germinated in Biosta Miracle Sprouter bean-sprouting kits (Miracle Exclusives, Inc., New York, NY), containing 3 corrugated trays, each equipped with a drain plug. The manufacturer recommends that water be poured through the top tray, passed in succession through the 2 lower trays, collected in a bottom tray, and discarded. A cover is to be

used to retain moisture within the kit. A choice had to be made to use the kit either exactly as called for in the manufacturer's instructions or with recommended modifications (1-14). The decision to use the kits with modifications was based on the following discrepancies in the manufacturer's instructions: No provision was made for initial washing of the seeds or beans; no specification was given for soaking seeds or beans; mung beans were to be washed only once daily, whereas alfalfa seeds were to be washed once only on the first and fourth days of germination; and kits were to remain level during germination, a condition which could cause small pools of water to form immediately after rinsing and would probably accelerate rotting of the seeds and beans (7).

The germinating tray was modified by making 3 small equally distant openings in the lower periphery of each tray for aeration (2, 3, 5, 9, 12, 14) and complete drainage (2, 3, 5, 8, 9, 11, 12) of the germinating beans. Although the sprouting kit was designed to accommodate 3 crops or samples, only 1 of the 3 germinating trays, along with the water collecting tray and the cover, was used for each replicate sample. The simultaneous use of all 3 trays per kit would have caused samples in each of the 3 trays to receive a different amount and quality of rinse water. Moreover, sprout samples in the lower germinating trays would have been cross-contaminated by those in the tray(s) above.

During germination, alfalfa seeds were rinsed twice daily at 6 ± 1 h intervals; mung beans were rinsed 3 times daily at ca 2 h intervals. For each rinsing, 800 mL of water was poured into the germinating tray in the level position and allowed to pass through to the collecting tray. After most of the water had passed through the germination tray, the kit was tilted so that any remaining water could pass through one of the small peripheral holes. After complete drainage, the rinse water was discarded. Except where noted, mung bean sprouts were harvested on the third day and alfalfa sprouts were harvested on the fourth day of germination.

All utensils, e.g., spoons and forceps, used in handling the germinating beans and seeds were either sanitized or sterilized; all glassware and trays used for mixing or soaking the beans were autoclaved. Because bean sprouting requires much handling and germinating beans require exposure to air, it can only be claimed that the beans and seeds were germinated under reasonably clean, but not aseptic, laboratory conditions.

Bacteriological Analysis

Samples of dry beans or seeds, weighing 50.0 ± 0.1 g, were placed directly into a sterile Waring blender jar or into a sterile beaker and then poured into a sterile blender jar. On the day of harvest, 50.0 ± 0.1 g of the sprouts were weighed from each replicate tray and refrigerated at 3-6°C until analysis, usually within 6 h. To each 50 g sample of dry beans, dry seeds, or sprouts, 450 mL of Butterfield's phosphate buffer was added, and samples were blended for 2 min at high speed (ca 22 000 rpm) on a commercial Waring blender base. Appropriate dilutions of standard methods plate count agar were used in triplicate for the APC determination. The same dilution bottles were used to inoculate appropriate media for the determination of total yeast and mold counts. To minimize analyst variability of the APC and mold count values, individual tasks (making the dilutions, pouring the agar, and counting the colonies) were generally performed by a single analyst. Plates for the APC determination were incubated 48 ± 2 h at 35 ± 2°C and counted manually with a Quebec colony counter.

Levels of *Salmonella* spp. in the dry beans, seeds, and sprouts were determined by the most probable number (MPN) method, with appropriate dilutions made in triplicate into lactose broth. Triplicate 1 g amounts represented the lowest dilution of sample inoculated. Inoculated lactose pre-enrichment broths were incubated 24 ±2 h at 35 ± 2°C, after which 1 mL amounts were subcultured to 10 mL volumes of tetrathionate broth with added brilliant green dye (10 mg/L).

The selective enrichment broths were incubated for 24 ± 2 h and streaked to plates of bismuth sulfite (BS) agar, which had been made the previous day. The streaked BS plates were examined after 24 ± 2 h of incubation, and colonies suspected to be *Salmonella* spp. were picked to triple sugar iron (TSI) agar. Negative BS plates were incubated an additional 24 ± 2 h and reexamined. The inoculated TSI agar slants were incubated for 18-26 h and the suspect *Salmonella* spp. slant cultures were confirmed as the somatic group(s) that had been inoculated into the dry bean samples.

Mycological Analysis

Appropriate dilutions were plated in triplicate on potato dextrose agar (PDA) with added chlortetracycline hydrochloride (40 ppm). Plates were incubated upright for 5 days at 22-

25°C, at which time the total yeast and mold count values were determined. Plates were then kept at room temperature for an additional 10–14 days to determine the presence and prevalence of toxigenic and nontoxigenic mold species.

The yeast and mold flora were determined by direct plating of the unsprouted mung beans and alfalfa seeds. From each replicate sample of mung beans, 50 randomly selected, intact, visibly mold-free beans were aseptically plated on malt salt agar (MSA) containing 20 g malt extract, 75 g sodium chloride, 40 mg chlortetracycline hydrochloride, and 15 g agar/L. The antibiotic, added just before the plates were poured, is an effective inhibitor of bacterial growth. Sodium chloride, effective in lowering the water activity of the agar substrate, was added at this high concentration to suppress the general bacterial population and limit the growth of undesired fast-growing species of *Mucorales*. Sodium chloride retards but does not completely inhibit the growth of most other fast-growing molds, thus allowing for the detection of normally slow-growing mold species that might otherwise go undetected. The xerophytic nature of the MSA combination also prevents bean germination and rapid sprouting, which possibly could result in cross-contamination as well as disorientation of Petri dish lids and stacks.

To determine whether the fungal flora encountered was due primarily to surface contamination or to actual internal invasion of the beans, an additional set of mung beans was surface-disinfected. From each replicate mung bean sample, 50 additional intact, unblemished beans were surface-disinfected 1 min in 5% sodium hypochlorite, rinsed 3 times in sterile water, and plated as described above.

Because alfalfa seeds are small, they were not surface-disinfected. The sodium hypochlorite could conceivably penetrate the seeds, rendering them "sterile" internally as well as externally. Alfalfa seeds were plated directly on MSA and on PDA with added chlortetracycline hydrochloride (40 ppm) so that a comparison could be made of the relative efficiency of the 2 media for growing mold flora. Because of the small size of these seeds, disorientation of Petri dishes and stacks caused by their germination and sprout formation were not considered a problem. Although it was possible for a germinating sprout to cross-contaminate other germinating seeds, this potential was considered minimal.

All plated mung beans and alfalfa seeds were incubated 10–21 days at room temperature. *Aspergillus* and *Penicillium* species were identified according to Raper and Fennell (21), Raper and Thom (22), and Mislivec (23). Molds of other genera were identified according to Barnett (24) and Gilman (25) but the isolates were not usually speciated.

Aflatoxin Analysis

Bean samples were refrigerated and sprout samples were frozen until initiation of analysis. Selected samples were analyzed for aflatoxins B_1, B_2, G_1, and G_2 by the AOAC official first action CB method (26) with the following minor modifications: Both bean and sprout samples were extracted by blending for 5 min in a Waring blender; sprout samples were extracted twice; total extract volumes of sprout samples were used for analysis; and the mobile phase used for thin layer chromatography of sprouts was anhydrous ethyl ether. The method could detect as little as 1.0 μg aflatoxin B_1/kg beans or seeds and 0.1 μg aflatoxin B_1/kg sprouts.

Results and Discussion

The occurrence of mycoflora as detected by direct plating of mung beans is shown in Table 1. Of the 1500 mung beans examined in this study, mold emerged from 736 (98.1%) of the 750 non-surface-disinfected (NSD) beans. However, bulk samples used for particular germination experiments had been mixed manually so that the replicate samples withdrawn would be as homogeneous as possible. Thus, some degree of cross-contamination of the mycoflora indigenous to the individual beans may have been unavoidable. Of the 750 surface-disinfected (SD) mung beans, mold emerged from only 13 (1.8%). The presence of mold, therefore, was probably due primarily to surface contamination, and no internal invasion may have occurred. However, because of the relatively small size of these beans, the possibility exists that sodium hypochlorite may have penetrated the internal tissues during the disinfecting process, causing internal disinfection or even sterilization.

Seven genera, including at least 20 species, were detected in the NSD mung beans. The flora were dominated by species of the *Aspergillus glaucus* group and the toxigenic *Penicillium cyclopium*, which can produce penicillic acid, cyclopiazonic acid, penetrem A, and penetrem B (27). The other species encountered on 5% or more of the beans examined were *A. candidus*, the toxigenic *A. flavus*, which can produce aflatoxins (28), and *A. versicolor*, which can produce sterigmatocystin (29), plus several unidentified isolates of *Penicillium*. Isolates of *Alternaria* spp.,

Table 1. Occurrence of mold species in mung beans and alfalfa seeds [a]

Mold species	Mung beans [b]		Alfalfa seeds [c]	
			Non-surface-disinfected	
	Non-surface-disinfected	Surface-disinfected	Malt salt agar	Potato dextrose agar
Alternaria spp.	25	2	41	56
Aspergillus candidus	83	0	0	0
A. clavatus	1	0	0	0
A. flavus	53	2	2	2
A. glaucus	374	8	31	12
A. niger	5	0	0	0
A. ochraceus	11	0	4	0
A. restrictus	2	0	0	1
A. tamarii	1	0	0	0
A. terreus	0	0	2	3
A. versicolor	35	0	0	0
Cladosporium spp.	5	0	8	5
Dematiaceae	11	0	7	2
Moniliaceae	24	1	2	1
Mucor spp.	1	0	0	0
Paecilomyces varioti	2	0	0	0
Penicillium brevi-compactum	1	0	0	0
P. cyclopium	264	0	0	0
P. frequentans	1	0	0	0
P. islandicum	2	0	0	0
P. viridicatum	2	0	0	0
Penicillium spp.	131	1	11	1
Rhizopus nigricans	1	0	1	1
Slimy yeast	0	0	0	15
Yeast	0	0	3	13

[a] Number of postive beans or seeds. Mold and yeast emerged from 736 (98.1%) of 750 non-surface-disinfected beans and from 13 (1.8%) of 750 surface-disinfected beans.

[b] Plated on malt salt agar only.

[c] Mold and yeast emerged from 103 (20.6%) of 500 seeds plated on malt salt agar and from 103 (20.6%) of 500 seeds plated on potato dextrose agar.

most of which appeared to be *A. alternata*, regularly encountered in other bean types in previous studies (30, 31), were detected on only 25 beans. Except for nonsporulating isolates of the Moniliaceae, detection of all other genera and species was sporadic, occurring in less than 2% of all beans examined. *Aspergillus flavus*, the only toxigenic mold species in the SD mung beans, was found in only 2 of the 13 moldy beans. Yeasts were not detected in NSD or SD mung beans.

Of the 1000 NSD alfalfa seeds examined, mold emerged from only 103 (20.6%) of the 500 seeds plated on MSA and from 103 (20.6%) of the 500 seeds plated on PDA. This relatively low occurrence of mold as compared with that of the NSD mung beans (98.1%) indicated a low level of surface contamination. The small size of these seeds precluded surface disinfection; therefore, no data were obtained on possible internal flora. Only 5 mold genera, including 9 species, were detected on both the PDA- and MSA-plated seeds (Table 1). Except for the sporadic occurrence of *Aspergillus ochraceus* on MSA and the single occurrence of *A. restrictus* on PDA, the same genera and species were encountered on both media. Qualitatively, the 2 media may therefore be of equal value in detecting foodborne molds in the absence of fast-growing "spreaders" of the Mucorales. *Alternaria* spp. dominated the flora on both media. Although species of all isolates were not identified, most appeared to be *A. alternata*. Regarding other genera and species, only the *A. glaucus* group was detected on at least 5% of the seeds plated on MSA. The other mold species were detected sporadically, i.e., on 2% or less of all seeds examined. The toxigenic *A. flavus* was detected only twice on each medium. Although not detected on mung beans, 2 yeast types occurred at low levels on PDA. Based on Lodder's descriptions (32), the slimy yeast was a species of *Cryptococcus*, though not *C. neoformans*, and the other yeast type was *Saccharomyces* spp.

The APC and the total yeast and mold count values in the dry beans and germinated sprouts

Table 2. Increase in aerobic plate count and in yeast and mold count values during germination of mung beans and alfalfa seeds

Bean/seed	Brand	Germination rate (%)		Geometric mean/g[c]			
				Bean/seed		Sprout	
		Claimed[a]	Actual[b]	Aerobic plate count	Yeast and mold count	Aerobic plate count	Yeast and mold count
Mung	A	93	99	2.1×10^5	2.8×10^2	1.1×10^8	2.4×10^3
	B	ND[d]	99	1.3×10^5	7.8×10^2	9.9×10^7	4.5×10^3
	C[e]	ND	97	1.0×10^4	2.6×10^2	5.5×10^7	1.4×10^3
Alfalfa	A	ND	ND	8.8×10^5	1.2×10^3	7.7×10^8	6.0×10^4
	D	90	ND	9.1×10^5	1.1×10^2	8.7×10^8	5.4×10^4

[a] As claimed by producer.
[b] As determined in laboratory.
[c] Based on 5 replicate determinations.
[d] Not determined.
[e] Harvested on second day of germination.

were compared (Table 2). In only 1 instance (Brand A of mung beans) was it possible to make a direct comparison between the germination rate as claimed by the producer (93%) and the actual germination rate as determined in our laboratory (99%). This finding was not unexpected since, as discussed earlier, the bulk samples of mung beans had been screened to remove visibly damaged beans before withdrawing the replicate samples to be used in any given experiment. The germination rate, which was never lower than 97%, indicated that the beans were being germinated under favorable, if not ideal, conditions. For 2 of the 3 brands of mung beans and for both brands of alfalfa seeds, the geometric means of the APC values increased by approximately 3 log units during germination and by more than 2 log units for Brand B of mung beans.

The increase in yeast and mold counts during germination was not as pronounced as that of the APC values for bacterial growth. This relatively lower increase in the mold counts, which may be due in part to the linear growth of molds as contrasted with the logarithmic growth of bacteria, may be important, depending on the mycoflora involved. With Brands A and B of mung beans, the mold counts consisted primarily of *Alternaria* spp., *A. glaucus* spp., and *Penicillium* spp., with *A. flavus* and yeast present at generally lower levels. However, with the higher sprout counts, *A. glaucus* and the *Penicillium* spp. virtually disappeared. The predominance of *A. flavus*, *Alternaria* spp., and the yeast indicated active growth of these microorganisms. The counts obtained from both the beans and the sprouts of Brand C mung beans consisted only of the toxic *P. cyclopium* and indicated active growth and possible mycotoxin production. The toxigenicity of *A. flavus*, *P. cyclopium*, and some species of *Alternaria* (33) suggests the possibility of mycotoxin production during the germination of mung beans.

Yeast and mold count increases for the 2 brands of alfalfa seeds examined were somewhat greater than those for the mung beans during the germination procedure (Table 2). Flora of the seeds were dominated by *Alternaria* spp., *Aspergillus terreus*, and *Cladosporium* spp.; *A. flavus*, *A. glaucus*, and a *Cryptococcus*-like yeast species were present at low levels. After sprout maturation, the higher counts consisted primarily of increases of *Alternaria* spp. and yeast, with only slight increases in the *A. flavus* counts. *A. glaucus*, *A. terreus*, and *Cladosporium* spp were absent from the alfalfa sprouts. As with the mung beans, the possibility of the production of toxins by *Alternaria* spp. and of aflatoxins during the germination of alfalfa seeds would seem to exist.

Selected samples of beans and sprouts were analyzed for aflatoxins. Brand A (beans and sprouts) and Brand B (sprouts only) of mung beans and both brands of alfalfa (seeds and sprouts) were examined and found negative for aflatoxins B_1, B_2, G_1, and G_2. Nonetheless, since environmental conditions of high moisture at room temperature are ideal for growth and toxin production of the toxigenic molds detected, the potential production of the toxins of *Alternaria* spp. and possibly *P. cyclopium* cannot be overlooked.

Because both the APC and mold count values indicated the possibility of microbiological

Table 3. Increase in levels of *Salmonella* spp. during germination of mung beans and alfalfa seeds

Bean/seed type	*Salmonella* serotypes inoculated	*Salmonella* spp. most probable number (geometric mean)/g[a]	
		Bean/seed	Sprout
Mung[b]	*S. anatum* and *S. montevideo*	2.6×10^0	1.7×10^5
		3.3×10^2	5.6×10^6
Alfalfa	*S. eimsbuettel* and *S. poona*	6.9×10^{-1}	1.4×10^3
		1.5×10^{2c}	7.5×10^{5c}

[a] Based on 3 replicate determinations, except where noted.
[b] Harvested on second day of germination.
[c] Single determination.

multiplication during germination, the behavior of *Salmonella* spp. in germinating beans was investigated (Table 3). Two levels of pooled *Salmonella* spp. serotypes inoculated into the mung beans and alfalfa seeds were examined. In all instances, the *Salmonella* spp. levels increased significantly.

Any one or a combination of 3 parameters (enzymatic, nutritional, and environmental) could account for the proliferation of *Salmonella* spp. and other microorganisms during germination. The presence of toxicants naturally occurring in plant foodstuffs has been detailed by Liener (34). One of the more common of these toxicants is the trypsin inhibitor (TI) which, in soy beans, may provide a defense mechanism by attacking the trypsin-like enzymes of invading insects and bacteria (35). This same toxicant has been found in golden mung beans and alfalfa seeds (36), whereas reduction of TI activity has been reported in soaked and germinated navy beans (37). Investigating changes in TI in germinating mung beans, Gupta and Wagle (38) found that TI decreased during the first 9 h, increased through the third day, then sharply declined. One explanation for this decrease may be that during germination, the TI leaches to the surface and is washed away during the rinsing procedure. Thus, if the levels of TI and the susceptibility of the beans to microbial invasion are directly correlated, proliferation of microorganisms could be expected as levels of TI and other enzymatic defense mechanisms diminish.

Changes in the nutritional content of germinating beans may also affect microbial levels in mature sprouts. In a study of the effect of germination on the nutritive content of mung beans, Kylen and McCready (39) found low glucose (0.4%) and fructose (0.04%) contents which increased 10-fold after the beans sprouted. El-Shimi et al. (40) noted increases in sucrose and fructose and decreases in amylose, amylopectin, and raffinose levels in germinating broad beans. Similarly, Gupta and Wagle (38) observed that during the 96 h germination of mung beans, the sucrose content rose while levels of raffinose and stachyose decreased. Finally, Hamilton and Vanderstoep (41) reported that essential amino acids were significantly higher in alfalfa sprouts than in the seeds from which the sprouts were derived. Thus, the availability of carbohydrates, amino acids, and other substrates during establishment of metabolic pathways could determine the nature and extent of growth of selected microorganisms in germinating beans and seeds.

Favorable environmental conditions may also contribute to the proliferation of microorganisms. Beans are germinated in a high moisture environment, and the increase in moisture content of the mature sprouts has been documented (39, 41, 42). Coupled with a germination temperature generally at or above room temperature, this high moisture environment would be favorable for the proliferation of many mold species and mesophilic bacteria. Because some handling of the germinating beans and seeds is unavoidable, the extent of microbial contamination in the mature sprouts depends to a certain degree on the cleanliness of conditions under which germination occurs.

This study has demonstrated the microbial hazards associated with the germination of mung beans and alfalfa seeds under controlled laboratory conditions. The assumption is made that beans germinated under conditions any less stringent, such as those found in many domestic kitchens, would lead to an exacerbation of the microbial hazard potential. The results from this study were not meant to be exhaustive, but rather to alert the consumer to the microbial hazards associated with the sprouting of 2 specific bean or seed types. It is hoped that the behavior of other microorganisms, particularly *B. cereus* and *Escherichia coli*, in germinating beans and seeds will be undertaken by other investigators.

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PESTICIDE FORMULATIONS

Gas-Liquid Chromatographic Determination of Pyrethrins and Piperonyl Butoxide: Collaborative Study

VERNON J. MEINEN and DEAN C. KASSERA
McLaughlin Gormley King Co., 8810 Tenth Ave N, Minneapolis, MN 55427

Collaborators: P. Anderson; J. B. Audino; B. R. Bennett; D. J. Carlson; W. L. Chang; P. J. Conigliaro; P. Earls; T. P. Garbrecht, Sr; G. M. Gentry; B. Guzman; E. H. Hayes; W. E. Hodgins; D. Jorengby; P. D. Korger; J. E. Launer; R. Martin; P. Nielson; G. O. Tannahill; L. Torma; C. Vaught; T. Vonnahme

A gas-liquid chromatographic method is described for the quantitative determination of pyrethrins and piperonyl butoxide in various formulations. The samples are diluted in acetone and analyzed by gas chromatography, with a flame ionization detector and a column packed with OV-101 or OV-1 on Chromosorb W (HP). The method was collaboratively studied with technical piperonyl butoxide and 3 pyrethrin-piperonyl butoxide formulations. The coefficients of variation for piperonyl butoxide ranged from 1.36 to 3.41% for the 5 samples. For pyrethrins in 3 formulations, the coefficients of variation were 2.20, 4.73, and 2.22%. An examination of the *t*-values indicated little or no contribution to systematic error, with the exception of one formulation. The method has been adopted official first action.

Many aerosol and liquid household insecticides contain a combination of pyrethrins and piperonyl butoxide (PBO) as the active ingredient. The current AOAC titrimetric method (1) for pyrethrins is nonspecific and does not apply to dilute liquids containing synergized pyrethrins. The same is true for PBO: Current colorimetric AOAC methods (2, 3) are nonspecific and subject to many interferences. Because of these limitations there is a need for a more specific method for analysis of these compounds.

Numerous references in the literature report chemistry and analysis of pyrethrins and PBO. Basic chemistry and analytical information regarding pyrethrins can be found in books edited by Casida (4), Nelson (5), and Zweig (6). Zweig (6) also gives information regarding PBO.

Cail and Secreast (7) reported a colorimetric method to measure PBO residues in flour. Olive (8) reported on thin layer chromatography for a semiquantitative determination of pyrethrins and PBO.

Other literature references pertain to the gas-liquid chromatographic (GLC) analysis of pyrethrins and PBO: Barrette and Payfer (9) and Bevenue et al. (10) report GLC analysis of PBO-pyrethrin formulations, and Bruce (11) analyzes for PBO with a modified electron capture detector. Miller and Tweet (12) report a GLC method for determining PBO in formulations.

Generally, the GLC column is a nonpolar material such as SE-30 and flame ionization detection is cited. Our experience with these conditions has been favorable, and we tested OV-101 in a collaborative study.

Collaborative Study

Each collaborator received 5 samples (see Table 1) to be analyzed by the proposed GLC method. Two samples (A and B) were technical PBO, prepared to follow Youden's procedure (13) for closely matched pairs. Sample C was an oil-based liquid, and D was a water-based liquid. Sample E was a water-based insecticide aerosol. Single determinations were requested on each of the 5 samples. All samples were laboratory-prepared. Collaborators were also supplied with GLC column packing, dicyclohexyl phthalate, and standards of PBO and pyrethrins.

Pyrethrins and Piperonyl Butoxide Official First Action

Principle

Sample is dild with acetone contg dicyclohexyl phthalate internal std and detd by GLC with flame ionization detector. Method is applicable to tech. piperonyl butoxide [80% butylcarbityl

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Table 1. Description of collaborative samples for GLC determination of piperonyl butoxide and pyrethrins

Sample	Description	Pyrethrins, %	Piperonyl butoxide, %
A	tech. material	—	84.4
B	tech. material	—	82.2
C	liq. insecticide	0.500	0.844
D	liq. insecticide	0.100	0.169
E	insect. aerosol	0.300	0.506

6-propylpiperonyl ether and 20% related compounds] and most formulations contg pyrethrins and piperonyl butoxide except shampoo products. Occasionally, an oil diluent will interfere with GLC detn. Method may not be applicable to samples contg <0.1% pyrethrins. Variation in active constituents of pyrethrin ext may cause minor deviations from expected results.

Apparatus and Reagents

(a) *Gas chromatograph.*—Equipped with flame ionization detector and 122 cm × 4 mm id glass column packed with 5% OV-101 or 5% OV-1 (Analabs, Inc.) on 80–100 mesh Chromosorb W(HP). Operating conditions: column 210°, injection port 250°, detector 250°; gas flows (mL/min)—N carrier gas 50, air 350–400, hydrogen 40–50; sensitivity 10^{-10} AUFS. Adjust attenuation to maintain 50–75% FSD for 1.0–1.5 μg piperonyl butoxide. Before use, condition column 2–3 h at 275° with N flow 50 mL/min. If necessary, vary column temp. or gas flow to attain retention times of ca 13–15 min for internal std. Theoretical plates/ft must be >400, based on dicyclohexyl phthalate peak.

Calc. theoretical plates/ft (N) as follows: $N = 16 \times (L^2/M^2 \times F)$, where L = retention of GLC peak (mm); M = peak baseline (mm) produced by drawing tangents to points of inflection of peak; and F = length of column (ft).

(b) *Internal std soln.*—8.0 mg dicyclohexyl phthalate (Chem Serv, Inc., West Chester, PA 19380)/mL acetone.

(c) *Std soln.*—(*1*) *Soln A.*—0.5 mg piperonyl butoxide/mL. Accurately weigh ca 0.25 g piperonyl butoxide (available from McLaughlin Gormley King Co., 8810 Tenth Ave N, Minneapolis, MN 55427) into 50 mL vol. flask and dil. to vol. with acetone. Pipet 10 mL this soln into 100 mL vol. flask, add 5 mL internal std soln by pipet, and dil. to vol. with acetone. Use this soln for detn of tech. piperonyl butoxide. (*2*) *Soln B.*—Accurately weigh ca 0.25 g piperonyl butoxide into 50 mL vol. flask. Add weighed amt of pyrethrins such that ratio of active ingredients closely resembles that which is expected in sample. Dil. to vol. with acetone. Pipet 10 mL of this soln into 100 mL vol. flask, add 5 mL internal std soln by pipet, and dil. to vol. with acetone. Use this soln for detn of pyrethrins and piperonyl butoxide in formulations.

Preparation of Sample

(a) *Technical piperonyl butoxide.*—Accurately weigh ca 0.25 g sample into 50 mL vol. flask and dil. to vol. with acetone. Pipet 10 mL this soln into 100 mL vol. flask, add 5 mL internal std soln by pipet, and dil. to vol. with acetone.

(b) *Pyrethrins–piperonyl butoxide formulations.*—(*1*) *Liqs.*—Accurately weigh sample contg ca 0.05 g piperonyl butoxide into 100 mL vol. flask, add 5 mL internal std soln by pipet, and dil. to vol. with acetone. (*2*) *Aerosol formulations.*—Caution: Open aerosol behind safety shield and in hood. Weigh aerosol can to nearest 0.1 g (G). Puncture as *small a hole as possible* in top of can with sharp punch and hammer to allow propellant to release very slowly. (Best results can be obtained by allowing punctured can to stand overnight.) After hiss of escaping propellant is no longer evident, cut open top of can with hand can opener. Leave ca 1 cm attached to can and bend top open. Carefully warm can in beaker of warm tap H_2O several minutes to ensure complete removal of propellant. Transfer aerosol nonvolatiles to vol. flask with aid of acetone. Rinse can thoroly, adding rinses to vol. flask. If aerosol is 8 oz, use 2 L (V) vol. flask. This vol. is necessary to ensure complete miscibility of oil phase of aerosol contents. Dil. to vol. with acetone and mix thoroly. Dry empty can and weigh (T). Transfer aliquot (A) (must be ≤90 mL), equiv. to 50 mg piperonyl butoxide, to 100 mL vol. flask, add 5 mL internal std soln by pipet, and dil. to vol. with acetone.

$$\text{Wt sample} = (G - T) \times (A/V)$$

Gas Chromatography

Inject 2–3 μL aliquots of std soln until internal std ratios vary ≤2% for successive injections. Det. baseline by drawing straight line to min. on either side of peak of interest. For pyrethrins, use combined ht of cinerin I and pyrethrin I peaks for internal std ratio. Repeat injection procedure with sample soln, followed by injection of std soln. If std peak ratios differ ≥±2.0%, repeat series of injections. Injection vol. should not vary >±10%. Calc. peak ht ratios (sample

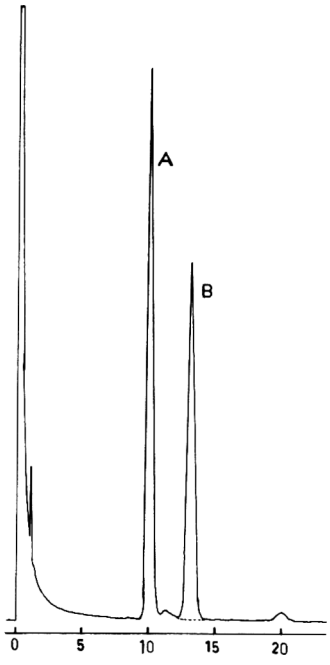

Figure 1. Typical gas chromatogram of technical piperonyl butoxide (A); dicyclohexyl phthalate (B).

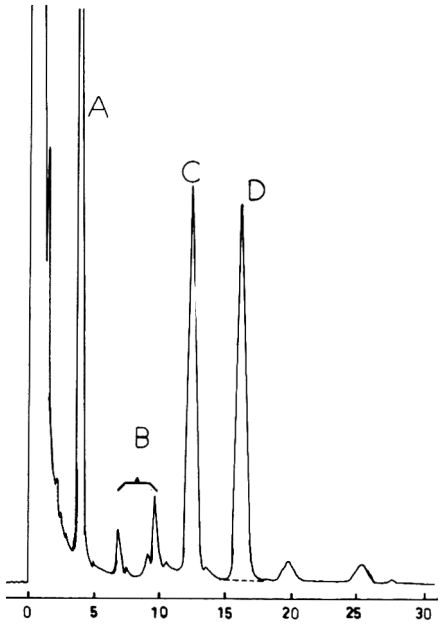

Figure 2. Typical gas chromatogram of liquid insecticide containing 1.67% MGK 264 (A), 0.5% pyrethrins (B) (cinerin I and pyrethrin I, respectively), 1.67% piperonyl butoxide (C), internal standard dicyclohexyl phthalate (D).

peak ht/internal std peak ht) of std injections before and after sample injections and average std ratio preceding and following sample injections. Calc. av. peak ht ratios for sample injections. After elution of piperonyl butoxide, allow ca 7 min for elution of extraneous peaks.

$$\% \text{ Piperonyl butoxide or pyrethrins} = (W_s \times P_s \times R_x)/(W_x \times R_s)$$

where W_s = g std in final diln; W_x = g sample in final diln; P_s = % purity of std; R_s = ratio of std; and R_x = ratio of sample.

Discussion

Several liquid phases are satisfactory for GLC resolution of pyrethrins and PBO. Polar phases were not considered because of relatively long retention times. We decided to use OV-101 for this study because of our experiences as well as several literature references regarding nonpolar phases. Other nonpolar phases such as OV-1 and SE-30 give similar results. Acceptable resolution and good reproducibility of ±2% for pyrethrins and ±1% for PBO can easily be obtained with the OV-101 phase.

Dicyclohexyl phthalate was chosen as the internal standard because it elutes at a retention time where no interfering GLC peaks are found. Figures 1 and 2 show the chromatograms of technical PBO and a mixture of PBO and pyrethrins. The dotted line indicates the sample baseline.

Detector response is linear for pyrethrins in the range from 0.15 to 1.1 μg and for PBO from 0.3 to 2.0 μg. The upper limit of linearity was not established.

Individual ingredients of an oil-based synergized pyrethrin formulation were injected to evaluate for interfering peaks. One small peak elutes as a shoulder on the pyrethrin I peak. It does not interfere with a peak height measurement, but will cause problems with peak area determination. There are no other interfering peaks. It has also been our experience that adequate injection reproducibility of pyrethrin peak area measurement is difficult to attain. Because of these 2 problems, the method calls for peak height measurement for quantitation.

The method is not suitable for shampoo products. Occasionally, an oil diluent will cause GLC interference. The method is not reliable for analysis of pyrethrins at less than 0.10%.

Table 2. Collaborative results for GLC determination of piperonyl butoxide in technical material

Coll.	Sample A	Sample B	Diff. (D) A − B	Total (T) A + B	Di − D	Ti − T
1	84.2	80.8	3.4	165.0	+0.99	−1.6
2	83.8	81.3	2.5	165.1	+0.99	−1.3
3	85.0	81.8	3.2	166.8	+0.79	+0.4
4	84.5	81.6	2.9	166.1	+0.49	−0.3
5	83.4	76.3[a]	—	—	—	—
6	82.6	80.1	2.5	162.7	+0.09	−3.7
7	85.5	88.8[a]	—	—	—	—
8	84.9	84.0	0.9	168.9	−1.51	+2.5
9	84.1	81.5	2.6	165.6	+0.19	−0.8
10	84.4	81.8	2.6	166.2	+0.19	−0.4
11	80.4[a]	79.6	—	—	—	—
12	87.5	83.9	3.6	171.4	1.19	+4.3
13	86.5	85.8	0.7	172.3	−1.71	+5.9
14	85.3	81.4	3.9	166.7	+1.49	+0.3
15	86.0	84.5	1.5	170.5	−0.91	+4.1
16	84.4	82.4	2.0	166.8	−0.41	+0.2
17[b]	82.3	83.1	—	—	—	—
18	85.5	81.7	3.8	167.2	+1.39	+0.8
19	84.2	82.9	1.3	167.1	−1.11	0.7
20	85.0	81.1	3.9	166.1	+1.49	−0.4
21	83.1	83.5	−0.4	166.6	−2.81	+0.2
Av.	84.7	82.2	2.41	167.1		
SD	1.15	1.35				
CV, %	1.36	1.64				
S_d						1.71
S_r					0.89	
S_b						1.03
t-Value						0.51

[a] Excluded from statistical evaluation with 95% confidence on the basis of the Dixon test (14).
[b] Discarded because of low theoretical plate count for GLC column.

Collaborative Results and Recommendation

Table 2 gives the collaborative results for the matched pair samples of technical PBO. Using the Dixon criterion (14), 3 outliers were excluded from the original data. Also, all data from Collaborator 17 were excluded because of the low theoretical plate count for the GLC column. Sample A averaged 84.7% (range 82.3–87.5) with a coefficient of variation (CV) of 1.36%. Sample B averaged 82.2% (79.6–85.8) with a CV of 1.64%. The expected result for Sample A was 84.4% and for Sample B, 82.4%.

From the differences between samples, the standard deviation of random error, S_r, is 0.89, and the overall standard deviation S_d is 1.71. The *F*-ratio (13) of 3.70 meets the minimum of 3.37 required for the presence of systematic errors at the 99% confidence level. The estimate of standard deviations, S_b, for distribution of systematic errors is 1.03. From the *t*-value (13), it can be concluded with 5% risk that the difference between the average percent found and the expected value is not a result of appreciable systematic error. A 2-sample chart of Samples A and B (Figure 3) shows the expected oval pattern of data points. The chart also shows data from Collaborators 5, 7, and 11 (excluded by Dixon test) far removed from the main group of plotted points.

The collaborative results for Samples C, D, and E are given in Tables 3, 4, and 5, respectively. For Samples C and E, the CVs for PBO and pyrethrins range from 1.54 to 2.22%. The average

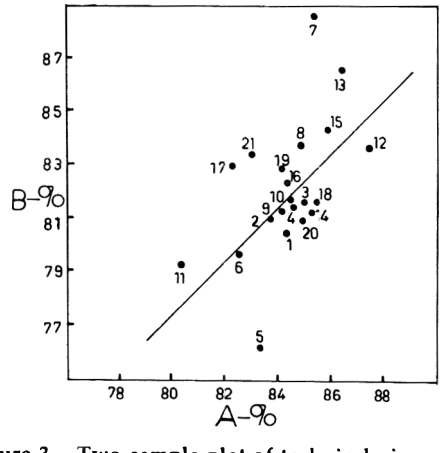

Figure 3. Two-sample plot of technical piperonyl butoxide matched pair Samples A and B.

Table 3. Collaborative results for GLC determination of piperonyl butoxide and pyrethrins in Sample C [a]

	Pyrethrins		Piperonyl butoxide	
Coll.	Found, %	Recd, %	Found, %	Recd, %
1	0.480	96.0	0.830	98.3
2	0.490	98.0	0.850	100.7
3	0.500	100.0	0.840	99.5
4	0.500	100.0	0.840	99.5
5	0.520	104.0	0.819	97.0
6	0.484	96.8	0.867	102.7
7	0.499	97.8	0.850	100.7
8	0.500	100.0	0.810[b]	96.0
9	0.517	101.4	0.852	100.9
10	0.490	98.0	0.830	98.3
11	0.495	99.0	0.893[b]	105.8
12	0.500	100.0	0.845	100.1
13	0.516	103.2	0.981[b]	116.2
14	0.488	97.6	0.852	100.9
15	0.506	101.2	0.863	102.3
16	0.520	104.0	0.860	101.9
17	—[c]	—	0.840[d]	99.5
18	0.500	100.0	0.840	99.5
19	0.503	100.6	0.843	99.9
20	0.500	100.0	0.830	93.3
21	0.495	99.0	0.853	101.1
Av.	0.499	99.8	0.845	100.1
SD	0.011		0.013	
CV, %	2.20		1.54	
t-Value	0.41		0.32	

[a] Liquid, oil-based insecticide containing 0.500% pyrethrins, 0.844% piperonyl butoxide, and 1.67% *N*-octyl bicycloheptene dicarboximide (MGK 264 Synergist).

[b] Excluded from statistical evaluation with 95% confidence on basis of the Dixon test (14).

[c] No results reported.

[d] Results discarded because of low theoretical plate count for GLC column.

percent recoveries range from 99.8 to 100.7. The *t*-values for these 2 samples indicate no appreciable systematic error. However, the CV of 4.73% for pyrethrins and 3.41% for PBO in Sample D are somewhat higher than expected. This is probably due to lower levels of pyrethrins and PBO in the sample.

The pyrethrin (average 93% recovered) *t*-value of 6.94 indicates a problem other than random error. In an effort to explain the high *t*-value, we analyzed Sample D several times (about 6 months after preparation) and found 0.092% pyrethrins. A freshly prepared sample gave the expected higher results. This indicates an apparent slight degradation of the pyrethrins in Sample D. We feel that the high *t*-value for pyrethrins in Sample D is a result of an actual change in pyrethrin content and not of the proposed method.

The standard deviation for the matched pair samples was somewhat higher than we expected. The chromatography appeared to be excellent with good resolution of the GLC peaks. The theoretical plate count of most GLC columns had in excess of 400 plates per foot. The method used in the study called for an initial weight of 50 mg PBO for sample and standard. We believe this small weight may have been responsible for the higher than expected standard deviations for Samples A and B. The method has been modified by increasing the initial weight to 0.25 g followed by an appropriate dilution.

The ranking test for laboratories (13) indicates that none of the collaborators shows a pronounced systematic error. Collaborator 1 is below the lower limit of 37. His results for Samples A and B are close to average as shown by the 2-sample chart. The pyrethrin results from Samples C, D, and E were located on the low end with 2 of them being Dixon outliers. The results from Collaborator 1, with the exception of the Dixon outliers, were retained for the statistical analysis. All other collaborators ranked between 55 and 117. The lower and upper limits are 37 and 131, respectively.

Collaborators 9, 19, and 20 used peak area measurement in addition to peak height. Area results generally show good agreement with Samples A and B, but not with C, D, and E. Three collaborators used 6 ft GLC columns. This

Table 4. Collaborative results for GLC determination of piperonyl butoxide and pyrethrins in Sample D [a]

	Pyrethrins		Piperonyl butoxide [a]	
Coll.	Found, %	Recd, %	Found, %	Recd, %
1	0.080[b]	80.0	0.160	94.7
2	0.096	96.0	0.167	98.8
3	0.102	102.0	0.165	97.6
4	0.100	100.0	0.170	100.6
5	0.096	96.0	0.162	95.9
6	0.093	93.0	0.166	98.2
7	0.092	92.0	0.166	98.2
8	0.090	90.0	0.160	94.7
9	0.087	87.0	0.149[b]	88.2
10	0.090	90.0	0.160	94.7
11	0.090	90.0	0.177	104.7
12	0.093	93.0	0.166	98.2
13	0.085	85.0	0.180	106.5
14	0.094	94.0	0.168	99.4
15	0.093	93.0	0.173	102.4
16	0.100	100.0	0.160	94.7
17[c]	—	—	—	—
18	0.090	90.0	0.150[b]	88.8
19	0.091	91.0	0.170	100.6
20	0.094	94.0	0.162	95.9
21	0.088	88.0	0.168	99.4
Av.	0.093	93.0	0.167	98.8
SD	0.0044		0.0057	
CV, %	4.73		3.41	
t-Value	6.94		1.49	

[a] Water-based, liquid insecticide containing 0.100% pyrethrins, 0.169% piperonyl butoxide, and 0.333% MGK 264 Synergist.

[b] Excluded from statistical evaluation with 95% confidence on the basis of the Dixon test (14).

[c] No results reported; sample lost.

was considered a minor modification to the method. Several collaborators suggested a change in detector sensitivity to increase pyrethrin peak heights. Collaborator 7 suggested that MGK 264 should be present in the standard solution because of potential for interference. As stated previously, with peak height measurement, there appears to be no interference. With peak area, there will be some interference with the pyrethrin I peak. Collaborator 12 suggested a note of caution to the analyst to allow for clearing of extraneous peaks from the GLC column before the next injection. A cautionary statement has been added to the method. Collaborator 19 was of the opinion that, for AOAC official methods, there should be a separate method for each ingredient. He felt that this allows for better accuracy because placement and concentration of internal standard and active ingredient can be matched more appropriately. However, this collaborative study shows that the method meets AOAC requirements.

It is recommended that the proposed gas-liquid chromatographic method be adopted official first action for the determination of pyrethrins and piperonyl butoxide in formulations.

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P. Anderson, Dow Chemical Co., Midland MI

J. B. Audino, California Dept of Food and Agriculture, Sacramento, CA

B. R. Bennett, State Chemist Laboratory, West Lafayette, IN

D. J. Carlson, McLaughlin Gormley King Co., Minneapolis, MN

W. L. Chang, University of Hawaii, Honolulu, HI

P. J. Conigliaro, S. C. Johnson & Son, Inc., Racine, WI

P. Earls, Texas A&M University, College Station, TX

T. P. Garbrecht, Sr, O&M Scott & Sons, Marysville, OH

G. M. Gentry, Florida Dept of Agriculture and Consumer Services, Tallahassee, FL

B. Guzman, Penick Corp., Orange, NJ

Table 5. Collaborative results for GLC determination of piperonyl butoxide and pyrethrins in Sample E [a]

	Pyrethrins		Piperonyl butoxide	
Coll.	Found, %	Recd, %	Found %	Recd, %
1	0.280[b]	93.3	0.490	96.8
2	0.306	102.0	0.518	102.4
3	0.301	100.3	0.503	99.4
4	0.310	103.3	0.510	100.8
5	0.320[b]	106.7	0.496	98.0
6	0.302	100.7	0.506	100.0
7	0.310	103.3	0.520	102.8
8	0.290	96.7	0.470[b]	92.9
9	0.300	100.0	0.509	100.6
10	0.310	103.0	0.510	100.8
11	0.299	99.7	0.519	102.6
12	0.300	100.7	0.516	102.0
13	0.295	98.3	0.543[c]	107.3
14	0.300	100.0	0.517	102.0
15	0.316	105.3	0.536[b]	105.9
16[c]	—	—	—	—
17[d]	0.277	92.3	0.450	88.9
18	0.300	100.0	0.510	100.8
19	0.292	97.3	0.500	100.0
20	0.300	100.0	0.510	100.8
21	0.409[b]	136.0	0.714[b]	141.1
Av.	0.302	100.7	0.509	100.6
SD	0.0067		0.0085	
CV, %	2.22		1.67	
t-Value	1.19		1.37	

[a] Water-based insecticide aerosol containing 0.300% pyrethrins, 0.506% piperonyl butoxide, and 1.00% MGK 264 Synergist.

[b] Excluded from statistical analysis with 95% confidence on the basis of the Dixon test (14).

[c] No results reported because of loss of aerosol contents.

[d] Results discarded because of low theoretical plate count for GLC column.

E. H. Hayes, Environmental Protection Agency, Beltsville, MD

W. E. Hodgins, State Chemical Laboratory, Mississippi State, MS

D. Jorengby, 3M Co., St. Paul, MN

P. D. Korger, Wisconsin Dept of Agriculture, Madison, WI

J. E. Launer, Oregon Dept of Agriculture, Salem, OR

R. Martin, Prentiss Drug & Chemical Co., Inc., New York, NY

P. Nielson, Kemikaliekontrollen, Lyngby, Denmark

G. O. Tannahill, Agriculture Canada, Ottawa, Ontario, Canada

L. Torma, Montana Dept of Agriculture, Bozeman, MT

C. Vaught, Conwood Corp., Memphis, TN

T. Vonnahme, State Chemical Laboratory, Vermillion, SD

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SUGARS AND SUGAR PRODUCTS

High Pressure Liquid Chromatographic Determination of Mono- and Disaccharides in Presweetened Cereals: Collaborative Study

LUCIAN C. ZYGMUNT
The Quaker Oats Co., John Stuart Research Laboratories, Barrington, IL 60010

Collaborators: E. Anderson; B. Behrens; R. Bowers; M. Bussey; G. Cohen; M. Colon; C. Deis; P. S. Given; A. Granade; C. Harms; J. C. Heroff; D. Hines; G. W. Hung; W. J. Hurst; J. Keller; F. B. Laroche; W. Luth; D. McKay; T. Mertle; M. Navarre; R. Rivera; R. Scopp; F. Scott; R. Sherman; K. Sloman; C. Sodano; K. D. Trick; B. R. Vandine; N. G. Webb

A collaborative study was conducted using a modified AOAC method (sugars in chocolate) for the determination of fructose, glucose, sucrose, and maltose in presweetened cereals by high pressure liquid chromatography (HPLC). Eight samples consisting of 6 products were analyzed in duplicate by the HPLC method and the AOAC Lane-Eynon method. The AOAC method was modified to use water-alcohol (1 + 1) and Sep-Pak C_{18} cartridges for sample cleanup. The HPLC results indicate precision comparable to the Lane-Eynon method and the chocolate method. The modified HPLC method has been adopted official first action.

The primary goal of this collaborative study was to validate a method for the simultaneous determination of fructose, glucose, sucrose, and maltose in presweetened cereals by high pressure liquid chromatography (HPLC). The method is an extension of the present AOAC liquid chromatographic method for sugars in chocolate (1), which was modified for cereals. Current AOAC copper reduction methods (2, 3) for cereal products lack specificity and require skilled analysts to obtain adequate precision.

Six presweetened cereals, each representing more than 1% of the children's cereal market, were analyzed by the modified HPLC method and the AOAC Lane-Eynon (2) method. Total sugar values obtained by the HPLC method were compared with the copper reduction method; such a comparison would allow past copper reduction data to be meaningfully related to current HPLC work.

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The recommendation of the Associate Referee was approved by the General Referee and Committee D and was adopted by the Association. See *J. Assoc. Off. Anal. Chem.* (1982), this issue.

Collaborative Study

Of the 6 products listed in Table 1, 2 cereal samples were distributed twice each (blind duplicates) among the collaborators and one cereal was of known formulation. The products were selected on the basis of sizable market share, label claim of sugars content, and varying sucrose content. The objective was to obtain a reasonable distribution of total sugars content with a broad range of sucrose percentage.

Each collaborator was sent 9 glass amber jars of ground cereal, including a practice sample of Kellogg's Sugar Frosted Flakes, and twenty Sep-Pak C_{18} cartridges. Each sample was to be analyzed in duplicate by at least one of 3 methods: the proposed HPLC method, the Lane-Eynon method (1), and the AOAC Munson-Walker (3) method. Detailed instructions and reporting forms were provided for each method. Unfortunately, only 3 collaborators chose to perform the Munson-Walker test for total and reducing sugars, effectively eliminating this method from statistical comparison. Collaborators were asked to measure even the minor sugar constituents by HPLC to ascertain method sensitivity. Laboratories were randomly selected to do either the HPLC or chemical test first; moreover, in most laboratories different

Table 1. Sucrose and other sugars

	Label claim	
Children's cereal	g/oz	% ± approx. 2
General Foods Alphabits	11	39
General Foods Super Sugar Crisp	14	49
General Mills Trix	10	35
Kellogg's Froot Loops	13	46
Kellogg's Sugar Smacks	16	56
Quaker Oats Cap'n Crunch	12	42

0004-5756/82/6502-0256-09$01.00

analysts analyzed the samples by either the official copper reduction method or the proposed HPLC method.

Fructose, Glucose, Sucrose, and Maltose in Presweetened Cereal High Pressure Liquid Chromatographic Method Official First Action

Apparatus

(a) *Chromatography equipment.*—See **13.A01(a)** thru (**d**), but change capacity factor in **13.A01(b)** to 1.5; include automatic injectors (Waters Associates, Inc., WISP 710B, or equiv.) and use specific injection vol. in 10–50 μL range in **13.A01(c)**.

(b) *Filter cartridge.*—Sep-Pak C_{18} (Waters Associates, Inc.), or equiv.

(c) *Guard column packing.*—Optional. C_{18} Corasil, 100 × 2 (id) mm (Waters Associates, Inc.), or equiv. as long as overall HPLC system meets column criteria of **13.A01(b)**.

Reagents

(a) *Sugar std solns.*—Dry individual sugar stds (fructose, glucose, sucrose, and maltose; available from Sigma Chemical Co.) 12 h at 60° under vac. Dissolve in H_2O or, optionally, alcohol–H_2O (1 + 1) to obtain concns of 3 mg/mL each for fructose, glucose, and maltose and 15 mg/mL for sucrose. After HPLC injection, compare peak response of sample and std, and adjust concns of std soln proportionately to obtain std response within 10% of sample responses.

(b) *Mobile phase.*–Acetonitrile (HPLC grade) and H_2O (charcoal-filtered) (80 + 20). Filter thru Whatman GF/F 0.7 μm glass fiber filter and degas in ultrasonic bath before use. Optionally, filter CH_3CN and H_2O separately thru 0.45 μm PTFE and cellulose ester membranes, respectively. Vary CH_3CN–H_2O ratio and flow rate if necessary to meet column criteria.

Preparation of Sample

(a) *Fat extraction.*—Weigh 2.00–10.00 g finely ground cereal into ≥100 mL centrf. bottle. If sample does not warrant fat extn, proceed to step (**b**). Add 50 mL pet. ether and centrf. ca 10 min at 2000 rpm. Aspirate and discard pet. ether without siphoning off solid material. Repeat extn. Evap. residual pet. ether with gentle stream of N and break up solid material with glass rod.

(b) *Sugar extraction.*—Add 100 mL alcohol–H_2O (1 + 1) and weigh. Place in 80–85° H_2O bath 25 min and stir occasionally. Cool to room temp. and add alcohol to original wt. Centrf. 10 min at ca 2000 rpm. If very cloudy, recentrf. portion of ext 5 min at ca 3500 rpm and filter thru 0.45–0.7 μm Swinney syringe filter. If guard column is used, omit step (**c**) and save filtered ext for HPLC analysis.

(c) *Cleanup.*—Fill Sep-Pak C_{18} cartridge with mobile phase and force thru filter, leaving liquid above packing. Repeat with sample ext twice, collecting eluate from second pass for HPLC analysis. Filter thru 0.45–0.7 μm Swinney syringe filter if necessary.

Determination

Inject sample soln (10–50 μL) into column with flow rate of mobile phase at 1.5–2.5 mL/min. Inject same vol. of std soln that will give peak response ±10% of sample peak response. Two injections of sample and std soln are required for adequate precision.

Measure areas or peak hts of each sugar peak in sample and std, but measure only peak ht for components which are near detection limit and have adjacent interfering peak.

$$\% \text{ Component} = (R/R') \times (C'/W) \times V \times 100$$

where R and R' = area or peak ht of sample sugar and std sugar, resp.; V = mL alcohol–H_2O added to sample = 100; W = g sample; C' = concn sugar std in g/mL.

Results and Discussion

The value of an HPLC method for the determination of sugars in cereals is obvious upon consideration of possible ingredient sources of sugars: sucrose (sugar), brown sugar, molasses, corn syrup, honey, fruit, flours, grains, flavorings, and vitamin premixes. It is not unrealistic to have the same total sugar value stem from sucrose alone or from sucrose plus 3 other sugars. Figures 1 and 2 illustrate the information obtained by HPLC in this collaborative study. Both chromatograms were obtained by Collaborator 7 under the conditions listed in Tables 2 and 3 with Sugar Smacks run at 2 mL/min and Trix at 1.5 mL/min. These tables demonstrate the wide variety of chromatographic equipment and experimental parameters used by collaborators in the HPLC method.

The Trix example in Figure 2 illustrates a problem possible with the measurement of the glucose peak and the need to adhere to the col-

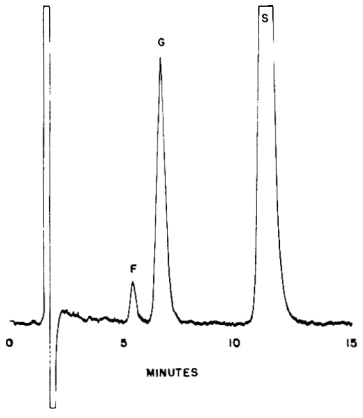

Figure 1. High pressure liquid chromatogram of Sugar Smacks: F, fructose (1.2%); G, glucose (12.1%); S, sucrose (44.7%).

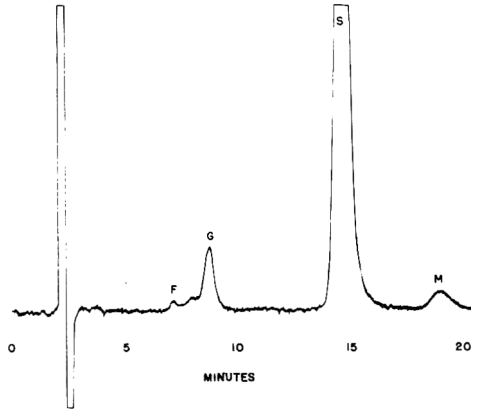

Figure 2. High pressure liquid chromatogram of Trix: F, fructose (0.3%); G, glucose (2.6%); S, sucrose (30.7%); M, maltose (1.6%).

umn criteria of the method. Two collaborators noted interference with the glucose quantitation. Besides Trix, both Cap'n Crunch and Alphabits have a component that elutes close to glucose. Table 4 in the G column under Cap'n Crunch demonstrates the interfering peak since this product is known to have a glucose content equal to that of fructose. This component may result from the cereal manufacturing process; in past years it was eluted midway between glucose and sucrose from the Waters Associates carbohydrate analysis column, posing no difficulty. However, with recent columns, this component can be eluted either just before or just after glucose; hence peak measurement has to be done carefully. The method has been amended to specify measurement of peak height for components which are near the detection limit and have an adjacent interfering peak.

Collaborators reported no difficulty in performing the HPLC method, but many complained about the time and need of the defatting procedure. The 6 cereals in the collaborative study were found by acid hydrolysis to contain 1.4–6.0% fat. Since no difficulty was experienced with the HPLC method in the analysis of these cereals without the defatting step, the method was amended to allow its omission.

Unfortunately, results from several collaborators in this study had to be rejected for failure to follow the HPLC method in 2 critical areas of chromatography: (*1*) The column criteria, capacity factor and resolution, were not met; and (*2*) calibration of HPLC system was inadequate, i.e., the same sugar standard was used for the analysis of all 8 cereal samples, different injection volumes of standard and sample were used, and standard solutions of single sugars were each injected separately.

One collaborator thought the participants should have been instructed to dry the cereals before weighing. The cereal samples in the

Table 2. HPLC equipment used by collaborators

Lab.	Injector	Pump	Detector	Data (integrating) system
1	Rheodyne 7010	Varian 4200	Varian 02-1528-02	Perkin-Elmer Sigma 1
2	Waters U6K	Waters 6000	Waters R400	none
11	Spectra-Physics 8000 (Valco)	SP8000	Waters R401	SP8000
12	Rheodyne 7125	LDC Constametric III	Waters R401	HP1000E computer and C1S software package
5	Beckman 210	Beckman 110A	Waters R400	Hewlett-Packard 3353B
7	Waters WISP 710B	Waters 6000A	Waters R400	Perkin-Elmer Sigma 10
8	Rheodyne 7010	Nester-Faust 1200	Waters R401	Varian CDS-111
9	Waters WISP 710A	Waters 6000A	Waters R401	none

Table 3. HPLC variable parameters[a] used by collaborators

Lab.	Sample range, g	Alcohol-water, mL	Injection, μL	RI attn[b]	CH_3CN $-H_2O$	Flow, mL/min	Peak measurement[b]	Column age	Type M or A[b]
1	4.997–10.337	100	10	2x	80 + 20	2.0	area-E	fairly new	M
2	3.00–7.03	100, 200	40	2x	80 + 20	2.0	area-M	new	M
11	3.46–7.22	100	20	4x	80 + 20	2.0	area-E	new	A
12	1.990–8.768	100	20	4x	85 + 15	2.0	area-E	old	M
5	3.50–7.00	100	20	4x	80 + 20	2.0	peak ht-M area-E	new	M
7	7.00	100	25	4x	80 + 20	1.4–2.0	area-E peak ht-M	new	M
8	3.31–6.95	100	20	4x	80 + 20	1.4–2.66	area-E	fairly new	M
9	3.50–7.00	100	25	4x	84 + 16	2.0	peak ht-M	fairly new	A

[a] All laboratories used Sep-Pak C_{18} cartridges.
[b] M = manual, A = automatic, E = electronic, attn = attenuation.

study contained 1.8–3.2% moisture and were shipped in sealed jars. Unless extraordinary conditions are encountered in which the cereal becomes wet, this step will have little effect on the result and will lengthen the procedure considerably, especially on occasions when a quick analysis is needed.

Three collaborators mentioned difficulty in integration of small peak areas of fructose, glucose, and maltose with their computing integrators. Two solved the problem by manually measuring peak heights.

One collaborator claimed that sugars in a standard prepared in water yielded lower peak heights than those prepared in alcohol–water. When column criteria are met, repeated injections of standards prepared in water and in alcohol–water failed to produce different peak heights in the Associate Referee's laboratory. It is apparent, however, that operation of electronic peak measuring systems can be made more difficult when the sample solution of alcohol–water yields a negative peak after the elution of water but the aqueous standard solution has no negative peak. To lessen the chance of error with chromatographic peak handling systems, the method now gives the analyst the option of using alcohol–water in place of water for preparing standards.

Two collaborators had opposite views as to the value of using an anion exchange resin in addition to the C_{18} packing for sample cleanup. No clear, consistent benefit could be seen in using an anion exchange resin for a wide variety of cereals analyzed in the Associate Referee's laboratory. Collaborators did agree on the absence of lactose in all the samples. Although the HPLC method can be used for lactose determination, the collaborative study was limited to presweetened children's cereals.

A benefit from the collaborative study was the concurrence of other laboratories with the Associate Referee's experience that a C_{18} guard column can effectively replace the use of the Sep-Pak C_{18} cartridge. The restriction on the use of the guard column is that the column criteria should still be met. A guard column of pellicular C_{18} packing more easily helps to meet these column criteria than would porous material of equivalent or larger size for the overall HPLC system. Also, a collaborator's suggestion to recentrifuge a portion of the extract at 3500 rpm for 5 min, if extracts are cloudy after the first centrifugation, has been incorporated into the method.

The Lane-Eynon and HPLC results of duplicate determinations for the blind duplicate samples (yielding 4 replicate analyses for Cap'n Crunch and Froot Loops) are presented in Table 4. The results of duplicate determinations for the other 4 cereals are presented in Tables 5–8. For the Lane-Eynon test, the coefficients of variation were 1.3–4.4% compared with 2.7–4.0% for total sugar by HPLC. Of course, the sucrose coefficients of variation, 1.7–4.3%, were close to those of the total sugar. The coefficients of variation for the minor sugar constituents were reasonable, 5.1–21.5%, except for maltose in Sugar Crisp, as long as the component exceeded 1%. This precision is certainly as good as that for the HPLC chocolate method (4). From the collaborators' chromatograms it was evident that the

Table 4. Collaborative data[a] for Lane-Eynon (LE, as sucrose) and HPLC of sugars (%) in blind duplicate samples

		Cap'n Crunch					Froot Loops				
		HPLC					HPLC				
HPLC Lab.	LE Lab.	F	G	S	Total	LE	F	G	S	Total	LE
1	1	ND	1.5	38.7	40.2	42.2	ND	ND	46.7	46.7	47.9
		0.4	3.2	38.2	41.8	41.5	ND	0.8	43.6	44.4	47.2
1	1	ND	ND	39.0	39.0	41.5	ND	ND	45.3	45.3	46.6
		0.4	1.9	38.8	41.1	40.9	ND	ND	46.1	46.1	47.6
2	2	ND	1.2	41.1	42.3	43.8	ND	ND	37.6[b]	37.6[b]	46.9
		ND	0.9	40.6	41.5	43.7	ND	ND	36.4[b]	36.4[b]	46.8
2	2	TR	0.6	40.3	40.9	43.8	ND	ND	45.8	45.8	46.9
		TR	0.8	40.4	41.2	43.9	ND	ND	45.5	45.5	46.6
11	3	0.5	ND	40.5	41.0	43.3	ND	ND	45.3	45.3	47.5
		0.4	0.9	40.1	41.4	40.7	ND	ND	44.9	44.9	47.7
11	3	0.4	0.6	41.5	42.5	43.2	ND	ND	45.8	45.8	47.1
		0.6	0.4	38.3	39.3	43.9	ND	ND	45.1	45.1	48.6
12	4	ND	2.1	40.9	43.0	44.1	ND	ND	48.1	48.1	40.9[b]
		0.4	2.6	39.5	42.5	45.8	ND	1.5[b]	45.7	47.2	40.3[b]
12		ND	1.4	40.5	41.9	43.9	ND	ND	47.9	47.9	47.7[b]
		0.4	2.9	39.5	42.8	43.4	ND	1.2[b]	47.4	48.6	48.5[b]
5	5	TR	TR	42.2[c]	42.2[c]	40.9	ND	ND	46.0[c]	46.0[c]	46.3
		0.4	1.4	36.0[c]	37.8[c]	41.2	TR	0.4	39.6[c]	40.0[c]	46.4
5	5	TR	TR	39.4[c]	39.4[c]	41.5	ND	ND	48.0[c]	48.0[c]	47.4
		0.4	1.4	35.2[c]	37.0[c]	41.5	TR	0.4	44.3[c]	44.7[c]	47.3
—	6					42.0					47.4
—						42.7					47.4
—	6					42.3					47.2
						41.8					47.9
7	7	0.5	0.5	39.9	40.9	41.5	ND	ND	46.0	46.0	45.8
		0.5	0.5	38.4	39.4	41.6	ND	ND	45.5	45.5	47.0
7	7	0.4	0.4	39.6	40.4	39.9	ND	ND	45.3	45.3	46.4
		0.5	0.6	39.2	40.3	40.8	ND	ND	45.1	45.1	45.5
8	8	0.4	0.8	40.3	41.5	41.2	ND	ND	44.7	44.7	47.0
		0.5	0.7	39.6	40.8	40.9	ND	ND	45.1	45.1	47.0
8	8	ND	ND	39.3	39.3	40.7	ND	ND	45.0	45.0	46.6
		0.5	ND	39.7	40.2	40.3	ND	ND	44.6	44.6	46.6
9	9	ND	ND	39.8	39.8	41.5	ND	ND	46.4	46.4	47.4
		ND	ND	41.1	41.1	41.6	ND	ND	42.8	42.8	47.4
9	9	ND	ND	40.9	40.9	40.5	ND	ND	45.7	45.7	47.3
		ND	ND	40.6	40.6	40.5	ND	ND	42.6	42.6	47.3
—	10					41.8					47.5
						41.3					47.6
—	10					42.8					46.9
						42.7					47.4
—	13					37.4[c]					43.2[c]
						34.5[c]					40.8[c]
Av.		≤0.4	0.8	39.9	41.0	42.1	ND	ND	45.5	45.6	47.1
CV, %		99.7	108	2.3	2.7	3.3	—	—	1.3	3.1	1.3

[a] Abbreviations: ND = not detectable; TR = trace
[b] Outlying result excluded by Dixon's test, using sum of values or individual results.
[c] Results of outlying laboratory excluded by ranking test.

precision near the detection limit could be improved by removing pulsations and minor baseline fluctuations from the chromatography. Better pumps, especially better maintained pumps (e.g., changing check valves), and refractive index detectors that are clean and optically aligned for optimum sensitivity would improve precision.

As Table 9 shows, the mean values for total sugar by the HPLC method are within 97% of the average values of the Lane-Eynon method with the exception of Sugar Crisp. Statistically, the means of the 2 methods were not significantly different at the 99% level for Trix, but were significant for the remaining cereals. These results are not surprising because the 2 methods do not measure the same components in the same way. With the HPLC method, the nearly ideal case of

Table 5. Collaborative data[a] for Lane-Eynon (LE, as sucrose) and HPLC of sugars (%) in Super Sugar Crisp

		HPLC					
HPLC Lab.	LE Lab.	F	G	S	M	Total	LE
1	1	1.7	3.5	38.5	ND	43.7	50.7
		1.6	4.3[b]	36.2	1.8	43.9	50.5
2	2	0.7[b]	4.7[b]	39.9	2.4	47.7	52.4
		1.2	5.2[b]	40.3	1.7	48.4	51.8
11	3	1.5	3.2	38.4	4.0	47.1	50.7
		1.7	3.5	39.3	1.2	45.7	50.7
12	4	1.5	3.5	39.2	2.5	46.7	39.7[b]
		1.5	3.6	38.5	2.0	45.6	38.7[b]
5	5	1.5	2.8	37.2[c]	1.7	43.2[c]	48.8
		1.5	3.2	34.7[c]	1.3	40.7[c]	49.4
—	6						51.9
							51.6
7	7	1.5	3.5	38.3	1.5	44.8	49.7
		1.4	3.3	37.3	1.5	43.5	49.1
8	8	1.2	4.5[b]	37.6	4.9	48.2	49.6
		1.3	3.0	38.3	3.2	45.8	49.1
9	9	1.6	3.7	39.4	ND	44.7	50.2
		1.6	3.3	39.0	ND	43.9	49.8
—	10						50.4
							51.4
—	13						43.7[c]
Av.		1.5	3.3	38.6	1.9	45.7	50.4
CV, %		10.7	7.9	2.8	74.6	3.8	2.2

[a–c] See footnotes, Table 4.

Table 6. Collaborative data[a] for Lane-Eynon (LE, as sucrose) and HPLC of sugars (%) in Trix

		HPLC					
HPLC Lab.	LE Lab.	F	G	S	M	Total	LE
1	1	ND	2.2	31.4	1.3	34.9	35.8
		ND	2.0	29.0	1.8	32.8	35.4
2	2	1.2[d]	3.0	33.1	1.8	37.9	36.6
		1.5[d]	2.6	32.8	2.0	37.4	36.5
11	3	ND	1.7	30.6	1.0	33.3	36.7
		0.2	2.7	29.9	1.7	34.5	37.2
12	4	ND	3.8[b]	31.7	ND[b]	35.5	38.9
		ND	4.3[b]	29.6	2.0	35.9	38.8
5	5	TR	2.2	28.6[c]	TR[b]	30.8[c]	34.9
		0.4	3.5	28.8[c]	1.4	34.1[c]	34.8
—	6						36.4
							37.0
7	7	0.3	2.6	29.5	1.4	33.8	34.3
		0.3	2.6	29.3	1.3	33.5	32.8
8	8	ND	3.0	30.0	1.2	34.2	35.4
		ND	2.3	30.6	1.4	34.3	35.3
9	9	ND	2.3	31.2	ND[b]	33.5	34.4
		ND	3.6	31.4	ND[b]	35.0	34.4
—	10						34.0
							35.8
—	13						31.2[c]
Av.		≤0.3	2.6	30.7	1.5	34.6	35.8
CV, %		—	20.7	4.2	21.5	3.5	4.4

[a–c] See footnotes, Table 4.
[d] Measured baseline noise; omitted from total sugar value.

Table 7. Collaborative data[a] for Lane-Eynon (LE, as sucrose) and HPLC of sugars (%) in Alphabits

HPLC Lab.	LE Lab.	HPLC F	HPLC G	HPLC S	HPLC M	HPLC Total	LE
1	1	ND	ND	41.2	ND	41.2	42.3
		ND	0.9	39.4	ND	40.3	42.6
2	2	TR	0.3	40.9	ND	41.2	42.5
		TR	1.0	38.9	ND	39.9	42.7
11	3	0.6	0.6	41.9[b]	ND	43.1	42.8
		0.3	0.5	41.6[b]	ND	42.4	43.2
12	4	ND	1.2	40.6	ND	41.8	43.4
		ND	2.5[b]	40.0	ND	42.5	43.2
5	5	ND	TR	39.2[c]	ND	39.2[c]	42.0
		0.2	0.9	39.8[c]	ND	40.9[c]	42.1
—	6						42.0
							43.8
7	7	0.3	0.6	40.8	TR	41.7	41.9
		0.3	0.6	39.6	TR	40.5	42.0
8	8	ND	1.0	39.7	ND	40.7	41.5
		ND	1.3	39.8	0.3[b]	41.4	41.4
9	9	ND	ND	40.9	ND	40.9	42.5
		ND	ND	39.8	ND	39.8	42.5
—	10						42.6
							42.9
—	13						34.5[c]
Av.		≤0.3	0.6	40.1	ND	41.2	42.5
CV, %		—	77.2	1.7	—	2.4	1.5

[a–c] See footnotes, Table 4.

Table 8. Collaborative data[a] for Lane-Eynon (LE, as sucrose) and HPLC of sugars (%) in Sugar Smacks

HPLC Lab.	LE Lab.	HPLC F	HPLC G	HPLC S	HPLC M	HPLC Total	LE
1	1	1.0	12.8	48.1	ND	61.9	60.8
		1.2	11.9	42.6	ND	55.7	59.6
2	2	1.2	11.6	46.3	0.7[d]	59.1	61.1
		1.1	13.6	46.9	0.6[d]	61.6	61.0
11	3	1.8[b]	11.9	44.5	ND	58.2	61.2
		1.3	12.2	43.7	ND	57.2	59.6
12	4	1.1	12.4	46.3	ND	59.8	53.5[b]
		1.1	11.7	45.6	ND	58.4	53.5[b]
5	5	1.1	11.1	41.3[c]	ND	53.5[c]	59.2
		1.1	11.8	42.5[c]	ND	55.4[c]	59.1
—	6						62.7
							62.3
7	7	1.3	12.2	43.3	ND	56.8	59.4
		1.3	12.2	43.5	ND	57.0	60.3
8	8	1.2	13.2	44.8	ND	59.2	58.0
		1.3	12.1	45.7	ND	59.1	57.6
9	9	ND[b]	11.7	43.0	ND	54.7	60.0
		1.3	11.8	41.3	ND	54.4	59.8
—	10						61.6
							60.9
	13						47.0[c]
Av.		1.2	12.1	44.7	ND	58.1	60.2
CV, %		8.8	5.1	4.3	—	4.0	2.3

[a–c] See footnotes, Table 4.
[d] Measured baseline noise; omitted from total sugar values.

Table 9. Comparison of Lane-Eynon average values and HPLC average values

Cereal	LE as sucrose, %	HPLC total, %	HPLC/LE
Alphabits	42.5	41.2	0.969
Cap'n Crunch	42.1	41.0	0.974
Froot Loops	47.1	45.6	0.968
Sugar Smacks	60.2	58.1	0.965
Super Sugar Crisp	50.4	45.7	0.907
Trix	35.8	34.6	0.966

individual sugars being measured one at a time is achieved. With the copper reduction method, the water-soluble material of the sample is used to titrate Fehling's solution (Soxhlet reagent). Moreover, the calibration of the Lane-Eynon method is done with one sugar type, whereas in the HPLC method, calibration is performed essentially with a replica of the sugars in the sample. Thus it is reasonable to conclude that the collaborative study showed good agreement between the 2 methods. In addition, the average HPLC total sugar of 41.0% for Cap'n Crunch cereal agreed well with the known formulation of 41.1% total sugar.

The statistical evaluation was performed following the AOAC statistical manual (5) and the advice of the Committee D statistician. A one-way analysis of variance as summarized in Tables 10 and 11 was used to compare the average values for each cereal product. Laboratory 13 for Lane-Eynon results and Laboratory 5 for HPLC of sucrose and total sugars were determined to be outlying laboratories with consistently low results by the ranking test. In light of the significant rank totals, Laboratory 5's use of AX/Corasil in the guard column is suspect. Laboratory 13 provided only single determinations for the Lane-Eynon method. The Dixon test using the sum of results was used to exclude (a) the Lane-Eynon results of Laboratory 4 for Froot Loops, Sugar Crisp, and Sugar Smacks; (b) the sucrose HPLC result of Laboratory 11 for Alphabits; (c) the glucose HPLC result of Laboratory 2 for Sugar Crisp and of Laboratory 12 for Trix. For individual results, the Dixon test was used to reject (a) the zero maltose value of Laboratories 5, 9, and 12 for Trix; (b) the 2 low values of Laboratory 12 for sucrose and total sugar for Froot Loops; (c) the high glucose value of Laboratory 12 for Froot Loops, of Laboratory 1 for Sugar Crisp, and of Laboratory 12 for Alphabits; (d) the low fructose value of Laboratory 2 for Sugar Crisp and the high and low fructose value of Laboratories 11 and 9, respectively, for Sugar Smacks.

For the AOAC Lane-Eynon method, there were significant differences between laboratories at the 99% level for Cap'n Crunch, Froot Loops, Sugar Crisp, Sugar Smacks, and Trix. At the 95% level, there was a significant difference also for Alphabits. The HPLC method fared better. For total sugar there was no significant difference between laboratories in the analysis of Alphabits and Sugar Smacks, but at the 95% level 3 of the 4 remaining products showed differences between laboratories. Also there was a significant difference for the fructose determination in Sugar Crisp. However, the sucrose and glucose results for Alphabits, Sugar Crisp, Sugar Smacks, and Trix, the maltose results for Sugar Crisp and Trix,

Table 10. Statistical analysis of collaborative data for blind duplicate samples

Statistic	Cap'n Crunch F	Cap'n Crunch G	Cap'n Crunch S	Cap'n Crunch Total	Froot Loops S	Froot Loops Total
Lane-Eynon						
Mean				42.1		47.1
Reproducibility				1.4		0.6
CV, %				3.3		1.3
Repeatability				0.7		0.4
CV, %				1.7		0.9
HPLC						
Mean	≤0.4	"0.8"	39.9	41.0	45.5	45.6
Reproducibility	0.2	0.9	0.9	1.1	1.3	1.4
CV, %	99.7	108.1	2.3	2.7	2.9	3.1
Repeatability	0.2	0.6	0.7	0.9	1.1	0.9
CV, %	71.1	74.4	1.8	2.1	2.4	2.1

Table 11. Statistical analysis of collaborative data

Statistic	Sugar Crisp					Trix				Alphabits			Sugar Smacks		
	F	G	S	M	Total	G	S	M	Total	G	S	Total	F	G	S	Total
Lane-Eynon																
Mean					50.4				35.8			42.5				60.2
Reproducibility					1.1				1.6			0.6				1.4
CV, %					2.2				4.4			1.5				2.3
Repeatability					0.4				0.6			0.4				0.6
CV, %					0.8				1.6			1.0				0.9
HPLC																
Mean	1.5	3.3	38.6	1.9	45.7	2.6	30.7	1.5	34.6	0.6	40.1	41.2	1.2	12.1	44.7	58.1
Reproducibility	0.2	0.3	1.1	1.4	1.8	0.5	1.3	0.3	1.2	0.5	0.7	1.0	0.1	0.6	1.9	2.3
CV, %	10.7	7.9	2.8	74.6	3.8	20.7	4.2	21.5	3.5	77.2	1.7	2.4	8.8	5.1	4.3	4.0
Repeatability	0.1	0.2	0.8	1.0	0.9	0.6	0.9	0.3	0.8	0.4	0.9	0.7	0.1	0.7	1.6	1.9
CV, %	4.8	6.4	2.0	51.9	2.0	23.2	2.9	18.9	2.2	67.0	2.3	1.7	6.0	5.5	3.6	3.2

and the fructose results for Sugar Smacks showed no significant difference between laboratories.

Recommendation

The Associate Referee recommends that this method be adopted official first action.

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William Luth and Ray Scopp, Universal Foods Corp., Milwaukee, WI

Raul Rivera, U.S. Dept of Agriculture, Weslaco, TX

Fraser Scott and Keith D. Trick, Health Protection Branch, Ottawa, Ontario, Canada

Norma G. Webb, Florida Dept of Agriculture, Tallahassee, FL

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FEEDS

Ceramic Fiber for Replacement of Asbestos as Filter Aid in Crude Fiber Determination: Collaborative Study

DAVID O. HOLST
University of Missouri, Food Science and Nutrition, Columbia, MO 65211

Collaborators: C. J. Cashman; J. DeCann; L. Komenda; S. Lewis; J. J. Psotka; J. R. Rigler; D. M. Salah; R. L. Smallidge; G. Tinsley; R. Vick

A commercially available ceramic fiber has been tested collaboratively for replacement of asbestos as a filter aid. Eleven laboratories participated, using 10 samples with crude fiber content from 1 to 40%, one sample high in starch, and 2 samples high in protein. The following data were obtained for asbestos and the proposed ceramic fiber, respectively, in AOAC method 7.061-7.065: s_d = 0.52 and 0.62; s_r = 0.22 and 0.28; s_b = 0.47 and 0.55; average range between independent analyses = 0.18 and 0.24% crude fiber; and average maximum spread among laboratories = 1.65 and 2.42% crude fiber. The blank value for ceramic fiber varied considerably among laboratories, necessitating more precise control over size or weight of ceramic filter pad. A ceramic filter pad or material of 1.5-2.0 g dry weight will give a uniform blank of approximately 2 mg. Ceramic fiber is suitable for replacing asbestos and is not known to be carcinogenic. Replacement of asbestos in 7.061-7.065 with ceramic fiber has been adopted official first action.

The official final action AOAC method **7.061-7.065** (1) specifies the use of asbestos, a carcinogenic (2, 3) material that is no longer available commercially in the United States. Many substances have been tried as a filter aid; the ceramic fiber reported here, if the blank is controlled, is a satisfactory replacement for asbestos. No attempt was made at reclaiming the used ceramic fiber.

Collaborative Study

Ten samples—meat and bone meal, soybean meal 48% solvent-extracted, soft white wheat, hard red wheat, alfalfa meal, 2 corn brans, an oat fraction, soy fiber, and nutrisoy fiber—were sent to 13 laboratories for analysis. Results were received from 11 laboratories who determined crude fiber by substituting ceramic fiber for asbestos fiber in method **7.061-7.065.** In addition, 5 laboratories performed the analyses by method **7.061-7.065,** using asbestos, and 5 laboratories reported results for the asbestos-free method (**7.066-7.068**).

Crude Fiber

Revise **7.062(c)** to read:

(c) *Prepared ceramic fiber.*—Place **60** g ceramic fiber (Cat. No. 1740M, Lab Safety Supply Co., PO Box 1368, Janesville, WI 53545) in blender, add 800 mL H_2O, and blend 1 min at low speed.

Det. blank by treating ca 2 g (dry wt) of prepd ceramic fiber with acid and alkali as in detn. Correct crude fiber results for any blank, which should be negligible (ca **2** mg).

Revise **7.065** as follows: Replace "Add ca 1 g prepd asbestos . . ." with "Add ca 1.5-2.0 g dry wt prepd ceramic fiber. . ."

Results and Discussion

Table 1 gives the data for all samples by each method. The following statistical treatment of the data was made to determine how well the ceramic fiber filter aid agreed with the official AOAC (asbestos) method in the collaborating laboratories.

The paired sample design of Youden and Steiner (4) was used to show the relationship of random and systematic errors. Figure 1 represents plots of the independent values for paired Samples 3 and 6 (AACC soft white wheat and hard red wheat) for each of the collaborators. In these plots, an arbitrary circle with a radius of approximately 4% of the crude fiber content was drawn, centered at the origin. Any point that fell within the respective circle indicated that the 2 paired assays were within ±4% of the relative value for crude fiber. The closer the points are clustered around the origin, the more accurate

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The report of the Associate Referee was approved by the General Referee and Committee A and was adopted by the Association. See *J. Assoc. Off. Anal. Chem.* (1982), this issue.

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Table 1. Collaborative results for determination of crude fiber (%) [a]

Lab.	Sample 1	2	3[b]	4	5	6[b]	7	8	9	10
					AOAC **7.061–7.065** with Asbestos					
1	2.40	4.10	9.63	27.13	14.94	10.19	20.32	1.39	12.10	39.19
	2.43	4.12	9.75	27.12	15.01	10.14	20.17	1.28	12.16	39.03
2	2.12	4.12	9.72	27.39	14.74	10.13	19.46	1.09	11.69	38.19
	2.20	3.98	9.68	27.29	14.63	9.91	19.43	1.07	12.02	37.53
3	2.27	4.11	9.60	27.92	14.78	10.05	20.00	1.18	12.51	39.56
	2.18	4.05	9.63	28.03	14.79	10.00	20.05	1.15	12.58	39.63
7	2.05	3.73	10.66	28.82	15.83	11.02	21.14	1.19	13.31	41.78
	2.12	4.54	9.99	29.24	15.80	10.40	20.74	1.07	13.13	42.91
9	2.69	4.27	10.06	27.55	14.61	10.01	19.79	1.04	12.10	38.71
	2.72	4.44	9.20	27.44	14.63	10.03	19.60	1.03	11.80	38.59
					AOAC **7.061–7.064** with Ceramic Fiber					
1	2.21	4.08	9.85	28.25	15.53	10.88	20.37	1.20	12.12	39.93
	2.20	3.93	10.04	28.34	15.48	10.91	20.41	1.20	12.17	40.03
2	1.81	4.04	9.86	27.88	15.23	11.00	19.68	1.32	11.91	38.87
	2.02	4.19	10.24	27.93	15.02	11.16	19.84	1.32	11.94	39.06
3	2.01	4.33	9.88	26.78	14.72	10.66	19.78	1.18	12.07	38.75
	1.93	4.31	9.82	26.62	14.76	10.73	20.04	1.13	12.05	39.12
4	2.18	3.63	9.02	—	14.79	10.35	19.43	1.15	11.46	38.31
	1.86	4.05	10.00	26.64	14.65	10.50	19.82	1.07	11.39	38.19
5	1.95	4.03	9.69	26.06	14.69	10.62	19.53	1.00	12.45	39.07
	2.05	4.25	9.32	26.07	15.26	10.40	19.56	1.03	12.38	39.65
6	1.60	3.88	9.22	26.10	15.25	10.28	19.30	1.11	—	36.50
	1.45	3.62	8.97	26.24	14.63	10.98	20.41	1.03	10.93	37.03
7	1.85	3.80	10.35	30.86	15.09	11.70	20.50	0.79	13.04	42.29
	2.04	3.87	10.44	29.90	15.69	11.68	21.37	0.94	13.45	41.69
8	2.29	4.14	10.20	27.28	15.44	11.17	20.66	1.30	14.58	—
	2.42	4.30	10.42	28.69	16.03	11.07	20.15	1.35	14.25	—
9	2.56	4.37	9.55	26.38	14.34	10.66	19.96	1.29	12.79	39.39
	2.41	4.31	9.50	26.48	14.20	10.53	20.10	1.36	12.74	39.66
10	2.25	3.28	10.05	28.11	15.15	11.89	20.10	1.10	12.36	38.28
	2.33	3.69	10.32	28.25	15.15	10.07	19.85	—	11.93	39.28
11	2.55	4.39	10.34	29.60	14.94	11.42	20.83	1.14	12.20	—
	2.31	4.20	10.02	29.25	14.79	11.23	20.71	1.11	12.05	—
					AOAC **7.066–7.068** Asbestos-Free					
3	1.71	3.48	9.67	26.81	13.78	9.82	18.44	1.20	—	36.71
	1.60	3.56	9.55	26.83	13.78	9.93	18.47	1.12	—	35.79
5	1.82	3.87	9.40	26.20	11.94	8.84	18.52	0.94	—	36.24
	1.90	3.86	9.44	—	10.78	9.49	—	1.09	—	37.52
6	1.38	3.17	8.45	26.05	—	9.58	17.81	1.14	—	34.73
	1.90	3.47	8.26	24.06	11.89	8.99	16.52	0.98	—	—
8	2.17	2.95	10.01	25.81	14.50	9.53	17.92	1.29	—	35.42
	2.09	3.27	9.00	—	13.36	10.78	19.01	1.20	—	35.04
11	2.03	3.68	10.29	26.64	14.26	10.92	18.56	1.10	12.17	37.31
	2.09	3.60	10.48	26.80	14.36	10.20	18.48	1.10	12.13	38.22

[a] All fiber values on dry matter basis.
[b] Paired samples (AACC Wheat Brands).

and precise are the results. If the pattern made by the points tends to lie on the 45° line, a systematic error is indicated. If each of the 4 quadrants contain about the same number of points, then the scatter of points represents a random distribution.

The divergent lines in the scatter chart (Figure 2) represent ±4% of the crude fiber value drawn on either side of the 45° line, and show that the points lie within the divergent lines.

The mean and standard deviation for each sample for each method is given in Table 2.

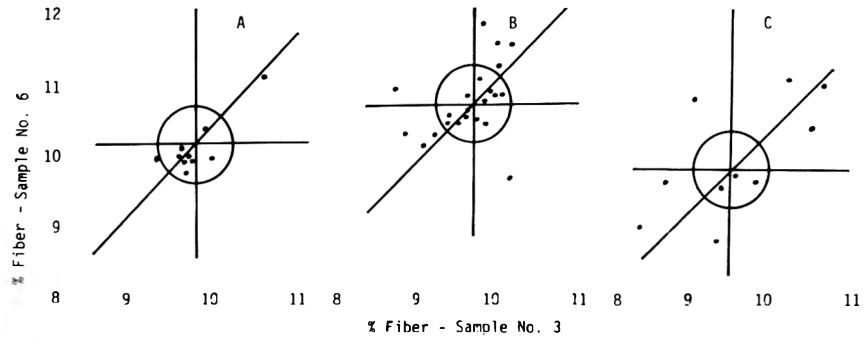

Figure 1. Plots of determinations of % crude fiber in paired samples Nos. 3 and 6 by 3 methods: A, AOAC 7.061 (5 labs); B, proposed method (11 labs); C, AOAC 7.066 (5 labs).

Table 2. Comparison of data (% crude fiber) for 3 methods for AOAC 7.061, proposed, and AOAC 7.066

	Mean[a]			Std dev.		
Sample	**7.061–7.065**	Proposed	**7.066–7.068**	**7.061–7.065**	Proposed	**7.066–7.068**
1	2.32	2.10	1.89	0.24	0.29	0.25
2	4.15	4.03	3.59	0.23	0.29	0.43
3	9.79	9.87	9.56	0.38	0.45	0.78
4	27.79	27.70	26.25	0.72	1.41	0.92
5	14.98	15.04	13.34	0.46	0.44	1.35
6	10.19	10.90	9.91	0.32	0.48	0.72
7	20.07	20.11	18.38	0.55	0.51	0.89
8	1.15	1.15	1.16	0.12	0.15	0.17
9	12.34	12.39	12.02	0.54	0.87	0.23
10	39.51	39.06	36.58	1.64	1.35	1.38
Mean	14.23	14.24	13.27			
Pooled std dev.				0.52	0.62	0.71
Rel. std dev., %				3.65	4.35	5.35

[a] For **7.061** and **7.066**, each value is average of 10 determinations. For proposed method, each value is average of 22 determinations.

Table 3. Precision of intralaboratory analyses (% crude fiber)

	Average range[a]		
Lab.	**7.061–7.065**	Proposed	**7.066–7.068**
1	0.078	0.070	—
2	0.173	0.156	—
3	0.057	0.113	0.190
4	—	0.214	—
5	—	0.220	0.368
6	—	0.392	0.506
7	0.404	0.396	—
8	—	0.414	0.535
9	0.183	0.116	—
10	—	0.322	—
11	—	0.230	0.230
Av.	0.179	0.240	0.366

[a] Each value is average of range between independent analyses for 10 samples.

Table 3 indicates the repeatability of independent analyses within a laboratory, showing an average range for each laboratory, and an average range for each method. Table 4 shows the reproducibility, or the maximum spread, among laboratories for each sample and shows the average spread for each method. Table 5 is a statistical comparison of the data for all methods. The s_r, or precision standard deviation, is calculated by the formula $s_r = \sqrt{\Sigma d^2/2N}$, where d^2 is the square of the difference between independent analyses (4). s_b (b = bias), or standard deviation of the systematic error, is calculated by the formula $s_b^2 = s_d^2 - s_r^2$ (4). Data for the AOAC asbestos-free method, **7.066-7.068**, were inadequate for comparison, but the ceramic fiber was comparable to the asbestos fiber. So far as is known, the ceramic fiber is not carcinogenic.

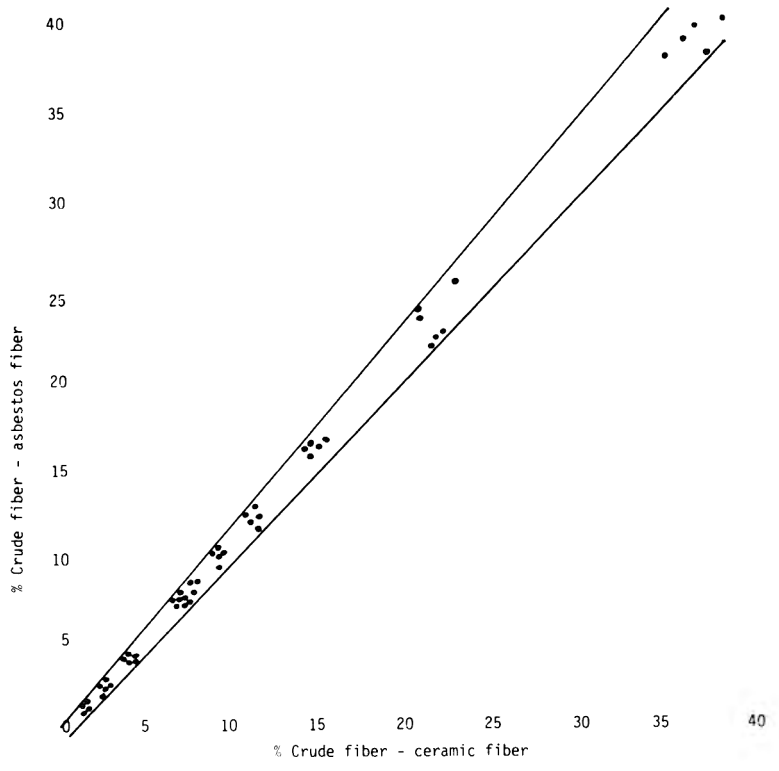

Figure 2. Scatter chart of crude fiber determinations by AOAC 7.061 and by the proposed method.

Recommendation

On the basis of the collaborative results, it is concluded that the accuracy and precision of method **7.061-7.065** are equivalent with ceramic fiber or asbestos filter aid. The Associate Referee recommends that ceramic fiber be adopted official first action for the determination of crude fiber in feeds.

Acknowledgments

The Associate Referee thanks the following collaborators for participating in this study:

Chester J. Cashman, Agway, Inc., Ithaca, NY

Jane DeCann, Cornell University, Geneva, NY

Linda Komenda, Vigortone Agriculture Products, Cedar Rapids, IA

Table 4. Precision of interlaboratory analyses (% crude fiber)

	7.061–7.065			Proposed method			**7.066–7.068**		
Sample	High	Low	Max. spread[a]	High	Low	Max. spread[a]	High	Low	Max. spread[a]
1	2.72	2.05	0.67	2.56	1.45	1.11	2.17	1.38	0.79
2	4.54	3.73	0.81	4.39	3.28	1.11	4.58	2.95	1.63
3	10.66	9.20	1.46	10.44	8.97	1.47	10.64	8.26	2.38
4	29.24	27.12	2.12	30.86	26.06	4.80	27.07	24.06	3.01
5	15.83	14.61	1.22	16.03	14.20	1.83	14.79	10.78	4.01
6	11.02	9.91	1.11	11.89	10.07	1.82	10.92	8.84	2.08
7	21.14	19.43	1.71	21.37	19.30	2.07	20.02	16.52	3.50
8	1.39	1.03	0.36	1.36	0.79	0.57	1.56	0.94	0.62
9	13.31	11.69	1.62	14.58	10.93	3.65	12.17	11.75	0.42
10	42.91	37.53	5.38	42.29	36.50	5.79	38.86	34.73	4.13
Method av.			1.646			2.422			2.257

[a] For **7.061** and **7.066,** each value is spread between extremes of 10 determinations; for proposed method each value is spread between 22 determinations.

Table 5. Statistical comparison of 3 methods for determination of crude fiber (%)

Statistic	7.061–7.065	Proposed method	7.066–7.068
Mean	14.23	14.24	13.27
Av. range	0.18	0.24	0.37
Av. max. spread	1.65	2.42	2.26
Pooled std dev.	0.52	0.62	0.71
s_r	0.22	0.28	0.45
s_b	0.47	0.55	0.55

Stacy Lewis, Cargill Research Dept, Minnetonka, MN

James J. Psotka, Quaker Oats Co., Cedar Rapids, IA

John R. Rigler, Raltech Scientific Services, St. Louis, MO

Dale M. Salah, U.S. Department of Agriculture, Commodity Testing Laboratory, Washington, DC

Robert L. Smallidge, Purdue University, West Lafayette, IN

Gary Tinsley, Missouri Farmer Association, Central Laboratory, Columbia, MO

Ramond Vick, Sanitation Laboratories, Cedar Rapids, IA

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AOAC Regional Section Meetings

AOAC Midwest Regional Section Meeting, June 2–3, 1982, Ames, IA. For more information, contact H. Michael Stahr, Iowa State University, 515/294-1950

■

AOAC Pacific Northwest Regional Section Meeting, June 16–17, 1982, Olympia, WA. For more information, contact H. Michael Wehr, Oregon Department of Agriculture, 503/378-3793

■

AOAC Northeast Regional Section Meeting, June 22–23, 1982, Syracuse, NY. For more information, contact Audrey Gardner, NY State Agriculture Experiment Station, 315/787-2281

EXTRANEOUS MATERIALS

Screening Procedure for Uric Acid as Indicator of Infestation in Spices

SUSAN M. BROWN, SUSAN ABBOTT, and PHILIP A. GUARINO
McCormick & Co., Inc., Corporate Analytical Services, 11104 McCormick Rd, Hunt Valley, MD 21031

Uric acid levels in selected spice products were determined by using a glucose analyzer, modified and calibrated for uric acid. In the method, uricase is used to decompose the uric acid; the reaction is monitored with an oxygen-sensitive electrode. Uric acid levels correlated with infestation as determined by AOAC methods.

Spices, like other stored agricultural products, are subject to infestation. To assure high quality, it is necessary to monitor insect levels and provide adequate cleaning of the material when required. The currently accepted procedures for the determination of the sanitary quality are flotation procedures, as specified by AOAC (1). The flotation procedures are time consuming and the reports are influenced by the preparation and handling of the material unrelated to infestation. The need for more rapid and objective monitoring procedures becomes more evident as cleaning technology is improved and less analysis time is allowable with the system.

Uric acid, an end product of the nitrogen cycle of some insects, has been advocated as a good criterion for the chemical determination of infestation. Studies have shown a close correlation between the uric acid content of flour and infestation as evidenced by insect fragment counts (2).

Investigation of colorimetric, staining, and extraction procedures for determination of uric acid have offered little promise for spices because of interferences from the sample matrix (3). Conversely, enzymatic techniques have shown a minimum of interferences (4, 5).

The glucose analyzer, modified according to the manufacturer's specifications for the determination of uric acid (6), offers a rapid and inexpensive means of monitoring uric acid levels in an aqueous system. Quantitation is based on the rate of oxygen up-take in the enzymatic breakdown of uric acid as shown in the following equation:

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$$\text{Uric Acid} + H_2O + O_2 \xrightarrow{\text{Uricase}} \text{Allantoin} + CO_2 + H_2O_2$$

The following investigation deals with the preparation of samples and the results obtained using the analyzer in determining the uric acid content of selected spice samples, and the comparison of these results with insect fragment counts, as reported by specified AOAC procedures (1). The addition of known quantities of live insects to the products and the resulting uric acid levels was also investigated.

METHOD

Apparatus and Reagents

(a) *Glucose analyzer.*—(Beckman Instruments, Inc., 2500 Harbor Blvd, Fullerton, CA 92634). Modified for uric acid determination (6).

(b) *Stock uric acid solution.*—50 mg uric acid (J. T. Baker Chemical Co., Phillipsburg, NJ, No. X-347-5)/mL. Add 60 mg Li_2CO_3 to 50 mL water in 100 mL volumetric flask. Warm flask 1 h at 32°C. Add 50 mg uric acid. Rewarm flask until all uric acid is in solution. Cool flask to room temperature. Dilute to volume with water and refrigerate. Prepared solution is stable 1 week.

(c) *Working uric acid solution.*—Dilute 10 mL stock solution to 100 mL with borate buffer solution.

(d) *Borate buffer solution.*—0.1M sodium borate, pH 8.3–8.5 (Fisher Scientific Co., Fair Lawn, NJ, No. S-248).

(e) *Uricase reagent.*—Beckman Instruments, Inc., No. 671340.

Procedure

Weigh sample into 1 L blender jar (40.00 g anise seed; 10.00 g chillies; 30.00 g cumin seed; 40.00 g black pepper). Add 200 mL borate buffer solution. Blend 5 min at medium speed. Transfer liquid to centrifuge tube and centrifuge 30 min at 2000 rpm. Filter centrifugate through

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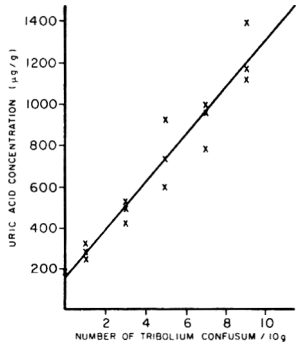

Figure 1. Correlation of uric acid level with known amounts of added insects.

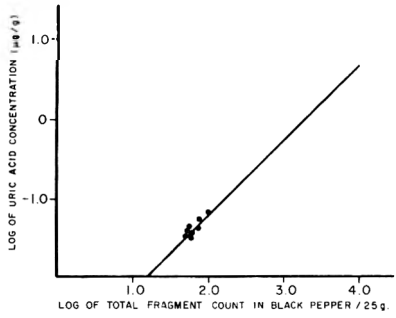

Figure 2. Correlation of uric acid level with insect fragment levels obtained by AOAC flotation method for black pepper.

Whatman No. 1 paper. Adjust pH to 8.4 ± 0.1, noting dilution factors. Warm filtrate 15 min at 32°C. Aerate sample by mixing vigorously on vortex mixer 30 s. Re-incubate 30 s. Pipet 50 µL into analyzer previously calibrated according to manufacturer's instructions, using working uric acid standard solution.

Results and Discussion

The objective of this study was to formulate a method which would provide a rapid determination of uric acid in spice products that could be correlated with infestation.

Tribolium confusum were added to product at various levels and allowed to reside 20 days at room temperature in tins before analysis. The number of insects residing in a given quantity of product exhibited a linear correlation with the uric acid level (Figure 1).

These findings which are related to whole insects are of greater significance than those findings related to insect fragment counts commonly used to evaluate the sanitary condition of a spice product. Any physical manipulation of a sample can reduce a fragment to several smaller ones. Cleaning and grinding of products can significantly increase the total number of fragments, independent of any additional infestation.

Current specifications are based on the number of fragments with no consideration as to their size. Therefore, a 10 mm fragment is considered as a single fragment prior to grinding and after processing may result in several smaller particles, each of which are regarded as separate entities.

Because grinding would tend to falsely elevate the number of insect fragments, only whole spices were used, thereby yielding a better indication of actual infestation. Correlations were done on individual spice items because specific products are subject to infestation by different insects, thereby affecting the total amount of uric acid observed. This was particularly significant for black pepper (Figure 2).

Results obtained correlating uric acid levels with insect fragment counts in cumin seed are shown in Figure 3 (R = 0.92); similar correlations were obtained for black pepper, anise seed, and chillies. These findings indicate a linear relationship between uric acid levels and insect fragment counts obtained by using AOAC procedures and that this correlation is obtainable by using the outlined methodology.

The nature of insect fragment counts causes concern; however, this is the currently accepted means of evaluating products to determine the sanitary quality of the item. The intent of correlating uric acid values with AOAC flotation results is to document the trend which exists between the two. A direct one-to-one correla-

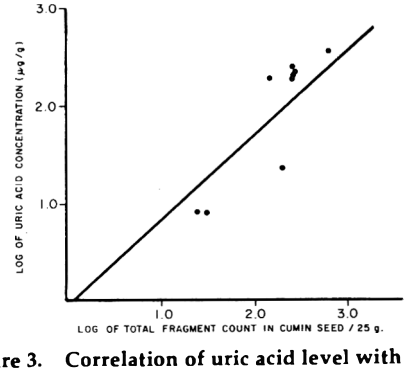

Figure 3. Correlation of uric acid level with insect fragment levels obtained by AOAC flotation method for cumin seed.

tion was never anticipated. Plotting the results as a log-log relationship gives a clearer indication of the trend than does the linear plot, because of range of results.

Recovery studies with the outlined methodology yield less than 100% recovery, 54–60% depending on the product. The decreased magnitude of recovery was expected, based on results reported in the literature (5). Total recovery can be achieved by extracting the products at a higher pH value and adjusting to the buffer range. This is time consuming, requires careful monitoring of dilution factors, and is outside the objective of this method.

Sample size is of concern. The hygroscopic nature of some spices does not allow a constant sample size. The suggested sample sizes were workable for the stated products.

The method is sensitive to abrupt changes in pH, alcohol contamination, and deactivation of the system by cyanide ions. Cyanide becomes a factor if the sample is heated excessively, which results in a breakdown of the uric acid with cyanide as an end product.

This method is judged to be a good working method for the rapid screening of spices for infestation. The instrument is linear over the range of 0–10 mg per 100 mL. Linearity shifts only 10% when range is extended to 20 mg per 100 mL. This allows detection of uric acid in such products as cumin seed over the range of 1.0 to 350 μg/g with no additional calibration.

Additional study of infestation by such insects as *Lasioderma serricorne* and in such spice products as celery seed and herbs is required before adoption of the outlined method as an industry-wide screening method.

Acknowledgments

Authors thank the technical personnel of Beckman Instruments who did feasibility testing of the instrument before purchase, and Strasburger and Siegel, Inc., Baltimore, MD, who performed the fragment counts in accordance with AOAC procedures.

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TECHNICAL COMMUNICATIONS

Safe Storage Vessel for Small Quantities of Diazomethane

CHARLIE J. BARNES, JAMES T. JONES,[1] and JEAN E. MATUSIK
Food and Drug Administration, Division of Chemistry and Physics, Washington, DC 20204

Two relatively safe, inexpensive, and easily fabricated vessels for the storage of small quantities of ethereal diazomethane are described.

Solutions of diazomethane (bp −23°C) in diethyl ether (bp 34°C) are relatively volatile. Diazomethane is highly toxic, a powerful irritant, and a known carcinogen in laboratory animals. Its contact with ground glass surfaces, sharp glass edges, heat, or metals can produce explosions.

Our animal drug residue laboratory frequently uses diazomethane in diethyl ether for the methylation of microgram quantities of sulfonamide drugs or other compounds of interest. Typically, we prepare a solution of approximately 3 g diazomethane in 200 mL diethyl ether, using the Diazald kit and Diazald reagent. Because of its toxicity and volatility, we have given considerable thought to the safe storage of this ethereal solution of diazomethane. For our needs, we assembled the vessel shown in Figure 1 from materials commonly found in the chemical laboratory. From this initial model, our machine shop fabricated another version of this vessel, which is shown in Figure 2.

Experimental

Apparatus

(a) *Vessel assembled from laboratory materials.*—Shown in Figure 1. The parts of the vessel are (A) spring, made from 0.3 mm od wire; coil dimensions, 9 cm long × 0.5 cm od; (B) plastic screw cap (Cat. No. 14-930-15J, Fisher Scientific Co., Pittsburgh, PA 15219) that fits a 25 mm od culture tube and is notched to accept spring (A); (C) aluminum seal (uncrimped) and (D) Teflon-clad septum (both under Cat. No. 224223, Wheaton Scientific, Millville, NJ 08332); (E) 50 mL serum bottle (Cat. No. 223745, Wheaton Scientific); (F) rubber bumper, made from rubber tubing; (G) hose clamp, ca 5 cm od; (H) wire hooks.

(b) *Vessel fabricated by machine shop.*—Shown in Figure 2. The parts of the vessel are (A) plastic cap with holes drilled on opposite edges to receive the wire ends of the springs; (B) aluminum seal (uncrimped) and (C) Teflon-clad septum (both under Cat. No. 224223, Wheaton Scientific); (D) springs, made from 0.3 mm od spring wire; coil dimensions, 5 cm long × 0.5 cm od; (E) 100 mL serum bottle (Cat. No. 223747, Wheaton Scientific); (F) aluminum cylinder, 20 mm high × 55 mm od × 52 mm id, hollowed to receive bottom of serum bottle.

(c) *Plastic food container.*—With tightly fitting snap-on lid.

Reagents

(a) *Diazomethane solution.*—Typically ca 3 g/200 mL diethyl ether, prepared from Diazald kit (Cat. No. Z10,024-0, Aldrich Chemical Co., Milwaukee, WI) and Diazald reagent (Cat. No. D2800-0, Aldrich Chemical Co.). *Caution:* Diazomethane is an extremely dangerous compound. The toxicity and explosiveness of diazomethane make it an undesirable reagent for use in most laboratories.

(b) *Cation exchange resin.*—AG® MP-50, 100–200 mesh, hydrogen form, analytical grade, macroporous (Cat. No. 1430841, Bio-Rad Laboratories, 32nd & Griffin Ave, Richmond, CA 94804), or equivalent, i.e., probably any cation exchange resin in the hydrogen form.

Assembly of Apparatus

Figure 1 (bottom) shows a closed diazomethane storage vessel. Here the septum (D) with the Teflon film on the underside and the uncrimped aluminum seal (C) cover the opening of the serum bottle (E). The spring (A) and the notched cap (B) are used to hold both the septum and aluminum seal tightly on the bottle opening. The notch in the cap helps to keep the spring from slipping off the cap. A hose clamp (G) and the rubber bumper (F), used for snugging the clamp, anchor the spring. A small hook (H) made from wire is used to attach the spring to the hose clamp.

Additional items needed to complete the storage system are a plastic food container with

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[1] Present address: Cushman, Darby & Cushman, Washington, DC 20006.

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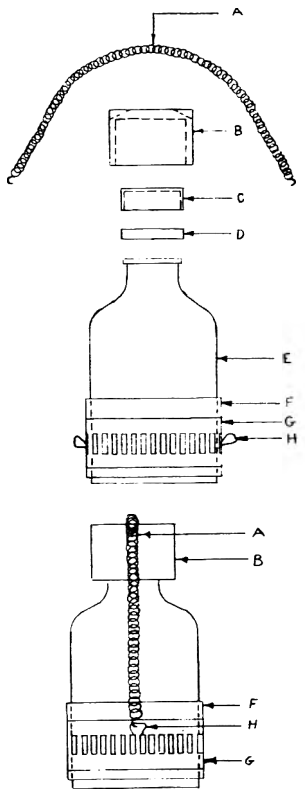

Figure 1. Vessel assembled from laboratory materials. See Apparatus for key to letters.

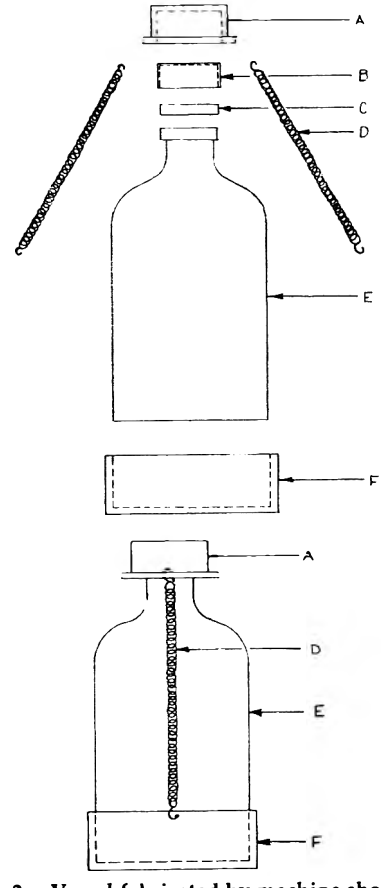

Figure 2. Vessel fabricated by machine shop. See Apparatus for key to letters.

a tightly fitting snap-on lid and a cation exchange resin. The diazomethane vessel is placed in the food container to which 50 g of resin has been added, the lid is snapped on, and this entire system is stored in a freezer at 0°C or below. The cation exchanger is used to destroy any diazomethane that might vent into the plastic container from the glass vessel if the freezer accidentally shuts down. To store a 200 mL preparation of diazomethane, 3 or 4 of these storage vessels, each in a separate plastic container with resin, are needed.

For access to the diazomethane, the storage vessel (Figure 1) is removed from the safety container and is immediately placed in an approved fume hood. The bottle, while resting on a firm surface, is held securely in one hand while the spring is removed from the bottle cap (B) with the other hand. The cap (B) and the seal (C) with septum can then be easily removed for access to the diazomethane. If the storage vessel is kept out of the freezer for more than a few minutes, it should be capped and partially immersed in crushed ice.

Discussion

Our laboratory has successfully used these glass storage vessels and the outer plastic safety containers for 3 years. Although we normally discard diazomethane solutions after 30 days, we have stored them in these containers for as long as 6 weeks without loss of the characteristic yellow color. The containers have no ground glass surfaces, no sharp glass edges, and minimum sources of contamination. Any excess pressure in the bottle is safely vented. We believe that these containers may be an adequate solution to the problem of storing toxic and potentially harmful ethereal diazomethane solutions.

Acknowledgment

We thank the Machine Shop, Bureau of Foods, Food and Drug Administration, for their fabrication of the storage vessel shown in Figure 2.

Time Required to Achieve Homogeneity in Swine Feed Mixtures

GEORGE J. IKEDA, EUGENE MILLER, MARK T. KING, GRACIA A. PERFETTI,[1] CHARLES R. WARNER,[1] NICHOLAS C. ADAMO,[2] and EUGENE M. SPORN
Food and Drug Administration, Division of Toxicology, Washington, DC 20204

FD&C Red No. 3 was mixed with 20 kg pig feed to give a concentration of 0.1%. A mixing time of 30 min was sufficient to achieve homogeneity for this mixture. For larger amounts or more flocculent types of additives, a longer time may be required. Ammoniated glycyrrhizin was mixed with 8 separate batches of pig feed at a concentration of 1%; 1 h was sufficient mixing time.

In toxicological studies, the test compound frequently must be mixed with animal feed. Good Laboratory Practices (GLP) Regulations (*Federal Register*, Dec. 22, 1978, Part II, p. 60007) specify that "once the uniformity of a feed mixture has been established for a given set of mixing conditions, it is not necessary to establish the uniformity of each subsequent batch that is mixed according to the same specifications." In the present study, we used a dye to trace the progress of mixing in a batch of pig feed. After determinations of an appropriate mixing time, we used these conditions to mix several batches of feed with a test substance, ammoniated glycyrrhizin. The concentration of ammoniated glycyrrhizin in 8 different batches of pig feed was then determined over a 6-month period.

Experimental

The mixer used was a 2 cu. ft liquid–solids blender (Patterson-Kelley Co., Inc., East Stroudsburg, PA). The mixing chamber was cleaned with detergent and hot water, and was then allowed to air dry before each use. The feed used throughout the studies was a standard pig ration (Standard Ration No. 3, National Agricultural Research Center, Beltsville, MD).

Feed Mixture with FD&C Red No. 3

Twenty kilograms of feed was weighed out and transferred to the mixing chamber; 20 g FD&C Red No. 3 (Warner-Jenkinson Co., St. Louis, MO; purity certified at 92%) was then accurately weighed and added to the feed. The mixing chamber was closed, and the feed mixture was tumbled for 15 min. The mixing was halted, the chamber was opened, and samples were obtained from the top right and top left of the chamber and from the exit chute on the bottom. The feed mixture was removed through the exit chute, and 1 sample was obtained from the middle of the chamber (after 10 kg feed had exited from the chute). The feed was then returned to the mixing chamber, tumbling was continued for another 15 min, and the sampling procedure was repeated. Tumbling was continued and samples were again obtained after 45 and 60 min of mixing.

Approximately 1 g of each sample was accurately weighed into a 600 mL beaker, and 300 mL 50% ethanol and 1 g Na_2CO_3 were added. The beaker was covered with a watch glass and heated on a steam bath for 15 min with occasional stirring. The mixture was allowed to cool and then was filtered through a medium-porosity 600 mL sintered glass funnel. The beaker and stirring rod were quantitatively rinsed into the funnel with three 30 mL portions of 50% ethanol. The funnel was then rinsed with 50 mL 0.5% NH_4OH. The funnel was removed from suction, the contents were rinsed back into the beaker with 50% ethanol, and 100 mg Na_2CO_3 was added. The beaker was again covered with the watch glass and the mixture was heated for 15 min on a steam bath with occasional stirring. The mixture was allowed to cool and was then filtered through a second medium-porosity sintered glass funnel. The filtrates from each sampling time were quantitatively transferred to separate 1 L volumetric flasks and diluted to volume with 0.5% NH_4OH. The contents of the volumetric flasks were mixed well and absorbance at 526 nm was measured with a Model B spectrophotometer (Beckman Instruments, Inc., Fullerton, CA). A standard curve was prepared by measuring absorbances of solutions of FD&C Red No. 3 (0.001–0.01 mg/mL) in 50% ethanol containing 0.1% NH_4OH.

Feed Mixtures with Glycyrrhizin

Ammoniated glycyrrhizin (Lot No. 791, McAndrews & Forbes Co., Camden, NJ) was mixed into the feed to give a concentration of 1%

[1] Division of Chemistry and Physics.
[2] Division of Color Technology.
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glycyrrhizin by weight. Two types of mixtures were prepared: pig diet with ammoniated glycyrrhizin and sodium chloride and pig diet with ammoniated glycyrrhizin plus an equal weight of cellulose (Alphacel, ICN Pharmaceuticals, Inc., Life Sciences Group, Cleveland, OH) in place of sodium chloride. After the batches were mixed for 1 h, 4 samples were taken from each as described earlier.

Approximately 0.3 g portions of each sample from the 8 batches of feed mixture were accurately weighed into 15 mL screw-cap centrifuge tubes and extracted with three 7 mL portions of absolute methanol. The tubes were centrifuged at low speed to settle the solids during the extraction procedure. An 8-sample study showed that recovery of glycyrrhizin was 98.6% (SD ± 1.1%). The extracts from each batch were combined in a 25 mL volumetric flask and diluted to volume with methanol. The extract was filtered through a 1.0 μm filter (Fluoropore, Millipore Corp., Bedford, MA) before injection into the liquid chromatograph.

The extract was analyzed by a high pressure liquid chromatographic procedure modified for our instrumentation, which was a Model 3500B gradient liquid chromatograph (Spectra-Physics Inc., Santa Clara, CA) with a Model 8200 detector (Spectra-Physics, Inc.) set at 254 nm. A Model 725 automatic injector (Micromeritics Instrument Corp., Norcross, GA) with a 10 μL sample loop and a 25 cm × 4.6 mm id reverse phase column packed with Zorbax C_8 (E.I. DuPont de Nemours & Co., Analytical Instruments Division, Wilmington, DE) were used. The mobile phase consisted of water–acetonitrile–glacial acetic acid (64 + 35 + 1). The flow rate was 1.6 mL/min, and the output was recorded on a Model MT-22 strip chart recorder (Westronics, Inc., Fort Worth, TX).

A stock solution of mono-ammonium glycyrrhizinate (MacAndrews & Forbes Co.) was prepared in 40% methanol–water to contain 2 mg/mL, and 1, 2, 3, and 4 mL aliquots were diluted to 50 mL with 40% methanol–water to prepare standard solutions containing 40, 80, 120, and 160 μg/mL, respectively. Aliquots (10 μL) of each standard were injected into the liquid chromatograph, and peak heights vs concentration of glycyrrhizin (in μg/mL) were used to construct a calibration curve. The concentration of glycyrrhizin in the feed extract was determined by injecting duplicate 10 μL aliquots of each extract into the liquid chromatograph and using peak heights for quantitation.

The amount of glycyrrhizin in the pig feed sample was calculated by the following equation:

$$\text{Glycyrrhizin, \%} = (C/W) \times 0.0025$$

where C = concentration of glycyrrhizin in sample extract in μg/mL, and W = weight of pig feed sample in g.

The glycyrrhizin content of Lot 791 of ammoniated glycyrrhizin was determined to be 24.1%. Therefore:

Ammoniated glycyrrhizin, % = % glycyrrhizin/0.241

Table 1. Distribution of ammoniated glycyrrhizin in 8 batches of pig feed after 1 h of mixing [a]

Batch	Glycyrrhizin content (% ± SD)
S1	0.973 ± 0.061
S2	1.011 ± 0.059
S3	1.005 ± 0.070
S4	0.969 ± 0.040
C1	1.042 ± 0.079
C2	0.993 ± 0.045
C3	1.012 ± 0.103
C4	1.029 ± 0.053

[a] Ammoniated glycyrrhizin was added at a level of 1%. Values are given as means of 4 samples (duplicate determinations) from each batch.

Results and Discussion

The concentration of FD&C Red No. 3 in pig feed (mg/g ± SD) was 1.269 ± 0.212, 1.154 ± 0.124, 1.210 ± 0.80, and 1.154 ± 0.120 at 15, 30, 45 and 60 min, respectively. The samples appeared to become homogeneous after the feed had been mixed for 30 min or longer. An unmixing phenomenon is possible with prolonged mixing and may have occurred during the 45 and 60 min intervals.

The results of mixing ammoniated glycyrrhizin with the pig feed are summarized in Table 1. Although the greatest deviation (10.3%) occurred with sample C3, the average standard deviation for all of the ammoniated glycyrrhizin–feed samples was 6.4%.

These results show that 30 min is probably adequate for mixing 20–50 g of an easily weighed and handled powder like FD&C Red No. 3 and other substances that have similar physical characteristics. Ammoniated glycyrrhizin, however, is a fluffy amorphous solid, and the volume of the 800 g that was added to 20 kg feed exceeded 2 L. Because of the large volume being added and the physical characteristics of ammoniated glycyrrhizin, the test batches of feed

were mixed for 1 h. This was apparently sufficient mixing time, as indicated by the homogeneity of the feed mixtures (SD ≤ 10.2%).

The pig feed used tends to be a coarse powder. Because of this, variation between replicate samples was greater than would normally be found and our standard deviations generally ranged from 5 to 10%. We found that by increasing our sample size to 2 g, the standard deviation was approximately 3.7%.

It appears that at least a 30-min mixing time should be used to mix small quantities of an additive (50 g or less) with powdered animal diet (20 kg) in this type of mixer. For larger amounts or more flocculent types of additives, the mixing time should probably be extended. We found that 1 h was sufficient for mixing ammoniated glycyrrhizin with pig feed. Individual investigators will need to determine whether or not their particular mixture is homogeneous, because our findings may not be universally applicable.

Acknowledgment

We thank Peter Vora, MacAndrews & Forbes Co., Camden, NJ, for providing the original liquid chromatographic procedure for determination of ammoniated glycyrrhizinate.

Technique for Preparing Spike Elements for Study Samples of Extraneous Materials

CLARENCE C. FREEMAN
Food and Drug Administration, 4298 Elysian Fields Ave, New Orleans, LA 70122

A microanalytical technique was developed to prepare spike elements for samples of extraneous materials to be used in collaborative studies. The technique eliminates the tedious individual transfer of spike elements from the preparatory medium to the study sample by affixing the elements to a gelatin film for mass transfer.

Rodent hair and insect fragments of known and recognizable character are added to study samples to compare methods or to test recovery by newly developed methods. The filth elements are prepared under the microscope and individually transferred to the study sample or to some intermediate medium before being added to the sample. The handling of up to 40 elements per subsample takes considerable time, patience, skill, and care to make certain that the added spike is exactly consistent in all subsamples and is acceptable in quantity and quality.

The mass transfer of the spike elements from the initial preparation medium to a thin transparent film of gelatin circumvents the individual handling and permits elements to be re-evaluated before addition to the study sample or after storage.

The film is prepared by slowly adding 5 g gelatin powder to 100 mL cool water with constant rapid stirring, then heating almost to boiling. The solution is removed from the heat and transferred in 10 mL aliquots to the bottom of 100 × 15 mm plastic Petri dishes and allowed to harden into a film. Hardening can be accelerated by placing the uncovered dishes overnight in a forced draft oven set not higher than 50°C. Once the film is formed, it can easily be removed simply by flexing the plastic dishes to release the edges, then lifting out with forceps. The film can be left intact or cut into strips about $\frac{1}{4} \times 1$ in. and stored for later use.

The filth spike elements are usually prepared by cutting *Tribolium* beetle elytra into uniform squares and rodent hairs into fragments of equal lengths (Brickey, P. M., Jr, Gecan, J. S., Thrasher, J. J., & Eisenberg, W. V. (1968) *J. Assoc. Off. Anal. Chem.* **51,** 872–876). This initial preparation is usually done on a microscope slide in an aqueous medium. For mass transfer of the elements from the preparation medium to the study sample, the film strip is held with forceps while one side is moistened. The moistened side is pressed down onto the spikes and then peeled upward. The filth elements adhere to the gelatin film and are removed from the preparation medium. In this state, they can be further evaluated and immediately added to the samples or stored for later use.

The rodent hair and insect elytral squares are released into the sample by the dissolution of the film during the hot aqueous phase of the methodology.

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FOR YOUR INFORMATION

AOAC Has Its 95th Annual Meeting

Despite dire predictions of poor attendance because of government cutbacks, AOAC's 95th Annual Meeting was well attended. Held October 19–22, 1981, at the Marriott Motor Hotel, Twin Bridges, Washington, DC, the meeting attracted about 1100 scientists.

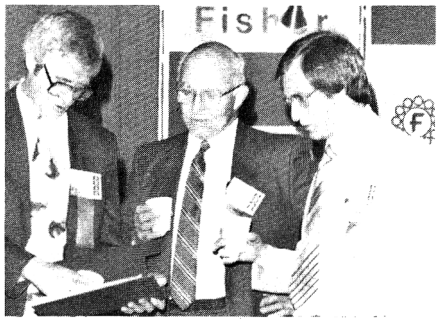
The General Session officially opened the meeting on Monday morning. Helen Reynolds gave her Presidential address, "Growing Pains," describing AOAC's evolution from a dependent to an independent association and the maturation which accompanies such a change. Incoming President James Minyard, Jr, presented the Presidential plaque to Ms Reynolds. Raymond J. Gajan, Food and Drug Administration (FDA), Washington, DC, Associate Referee for polarography (metals), received the 1981 award for the best Associate Referee Report of the Year. Ms Reynolds presented certificates to the 1981 Fellows: Louis L. Gershman, FDA, Boston, MA; Kenneth Helrich, Rutgers University–Cook College, New Brunswick, NJ; Arthur R. Johnson, FDA, Washington, DC; Valva C. Midkiff, University of Kentucky, Lexington, KY; Sidney Williams, FDA, Washington, DC (retired); and James P. Minyard, Jr, Mississippi State Chemical Laboratory, Mississippi State, MS. Two 1981 Fellows, Charles C. Clark, Drug Enforcement Administration, Miami, FL, and Robert D. Stubblefield, U.S. Department

Helen L. Reynolds

James P. Minyard

Sidney Williams, Valva C. Midkiff, Kenneth Helrich, Louis L. Gershman, Arthur R. Johnson, and James P. Minyard, Jr

Leonard Stoloff



Donald L. Grant, Darrell F. Wood, and Murray D. Sutton

of Agriculture, Peoria, IL, did not attend. The AOAC Wiley Scholarship winner, Rita A. Manning of the University of Texas, was announced.

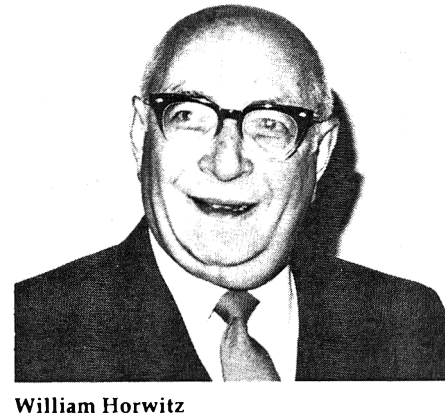
For planning and conducting the 1981 Spring Workshop and Exhibition, Donald L. Grant, Health and Welfare Canada, Murray D. Sutton, the National Research Council of Canada, and Darrell F. Wood, Agriculture Canada, received special awards, as did Frits J. Mulder, Duphar BV, The Netherlands, for service as the first Associate Referee outside North America, for development of chemical methods for vitamin D, and for world-wide efforts to acquaint scientists with AOAC and its methodology. The membership voted to confer Honorary Membership on William Horwitz for his many years of extraordinary service to AOAC.

The last item on the General Session agenda, an address, "The Total Picture," by 1981 Harvey W. Wiley Award winner, Leonard Stoloff, FDA, Washington, DC, proposed strategies for increasing support and participation in AOAC.

On Monday evening, at the Harvey W. Wiley Award banquet, the keynote speaker was Arthur Hull Hayes, Jr, Commissioner, FDA.

For the balance of Monday and the next 3 days and evenings, attendees had their choice of over 200 papers, 23 poster presentations, 5 symposia, 2 seminars on automated analysis conducted by Technicon Industrial Systems, a chromatography workshop conducted by Kontes, Inc., numerous reports and special

Frits Mulder



William Horwitz

meetings, and 42 exhibits, which filled the regular exhibit areas and spilled over into additional rooms.

The 5 symposia, several of which dealt with subjects recently in the news, were titled: Problems and Solutions in Trichothecene Methodology, Infant Formula Regulation and Infant Food Problems, Analytical Methodology for Lead in Foods, Computers in the Laboratory, and AOAC Methods Development—Challenge of the Next Decade.

A course, Laboratory Automation: Micro-, Mini-, or Midi-Computers, sponsored by the American Chemical Society, was held on the 2 days preceding the meeting.

An interpreter for the deaf, provided by AOAC, met with scientists with hearing impairments and arranged to translate the sessions they expected to attend.

1981 Associate Referee Report of the Year Award

Raymond J. Gajan, Food and Drug Administration, Washington, DC, Associate Referee for polarography (metals) won the 1981 award for the best Associate Referee Report of the Year. Mr. Gajan was nominated by Committee E for his report, "Determination of Lead and Cadmium in Foods by Anodic Stripping Voltammetry."

Every year each Committee is asked to nominate one scientist for this award. The nominees this year were: Committee A—Vernon J. Meinen, McLaughlin Gormley King Co., Minneapolis, MN; Committee B—Walter Holak, Food and Drug Administration (FDA), Brooklyn, NY; Committee C—Nrishinha P. Sen, Health and Welfare Canada; Committee D—Peter S. Vora, MacAndrews and Forbes Co., Camden, NJ; Committee F—Russell G. Dent, FDA, Washington, DC; and Committee G—David W. Fink, Merck, Sharp, and Dohme Research Laboratories, Rahway, NJ.

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 1981 *Journal of the Association of Official Analytical Chemists.* Please forgive us if we have overlooked anyone.

Thank you: R. G. Ackerman, H. Albert, R. H. Albert, T. G. Alexander, M. Alligrini, D.

Arthur Hull Hayes, Jr.

Raymond J. Gajan

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Spring is Almost Here and With It the 7th Annual AOAC Spring Workshop in New Orleans

AOAC will hold its 7th Annual Spring Workshop and Exposition April 13–15, 1982, at the Fairmont Hotel, New Orleans, LA. There will be 14 main sessions, 4 supplementary symposia and seminars, a wine and cheese party, and an exhibition.

Session topics and chairmen will be: *Animal Feeds* (including drugs and antibiotics, and a session on monensin) chaired by Alan Hanks, Agricultural Analytical Services Lab., Texas A & M University; 713/845-4111. *Fertilizers*, chaired by C. H. McBride, USS Agricultural Chemicals, Decatur, GA; 404/292- 2525. *Pesticide Formulations*, chaired by Edwin Jackson, Mississippi State Chemical Lab., Mississippi State, MS; 601/325-3324. *Pesticide Residues*, chaired by W. E. McCasland, Texas Dept of Agriculture, Benham, TX; 713/836-5641. *Analysis for Toxicological Research*, chaired by Malcolm C. Bowman, National Center for Toxicological Research, FDA, Jefferson, AR; 501/541-4000. *Analysis Related to Seafood Quality*, chaired by Beverly Smith, National Marine Fisheries Laboratory, Pascagoula, MS; 601/762-4591. *Mycotoxins*, chaired by Louise Lee, USDA/SRRC, New Orleans, LA; 504/589-7589. *Environmental Monitoring*, chaired by James P. Wood, Carbon Systems, Inc., Baton Rouge, LA; 504/343-3353. *Laboratory Automation*, chaired by Robert Beine, Division of Regulatory Services, University of Kentucky, Lexington, KY; 606/257-1656. *Laboratory Quality Assurance*, chaired by Patricia Smith, Woodson-Tenent Laboratories, Memphis, TN; 901/525-6333. *Veterinary Toxicology*, chaired by Steven S. Nicholson, Louisiana State University, Baton Rouge, LA; 504/388-4141. *Drug and Antibiotic Residues in Animal Tissue*, chaired by Raymond Ashworth, U.S. Dept. of Agriculture, Food Safety Inspection Service, Beltsville, MD; 301/344-2468. *Environmental Contamination in Food Such as Meat Products* (2 sessions), chaired by Raymond Ashworth and Robert Epstein, 301/344-2468. Symposia and seminars will be held on: *Food Toxicology*, sponsored by the Association of Food and Drug Officials; for information, contact Martha Rhodes, Florida Dept of Agriculture, Tallahassee, FL; 904/488-0670, or John Turner, Food and Drug Administration, Baltimore, MD; 301/962-3790. *Pesticide Enforcement Laboratory Procedures* (*Residue and Formulation*), a training program in the pesticide enforcement grant program, sponsored by EPA National Enforcement Investigations Center; for information, contact Dean Hill, EPA/NEIC, Denver, CO; 303/234-3751.

For display booth space at this meeting, contact Joseph Ford, U.S. Dept of Agriculture Pesticide Monitoring Laboratory, PO Box 989, Gulfport, MS 39501. Registration fee is $65.00. Hotel arrangements should be made directly with Fairmont Hotel, New Orleans, LA. For additional information, contact co-chairmen: Nicole F. Hardin, U.S. Food and Drug Administration, 4298 Elysian Fields Ave, New Orleans, LA 70122; telephone 504/589-2471, or Hershel Morris, Louisiana Department of Agriculture, PO Box 16390-A, University Station, Baton Rouge, LA 70893; telephone 504/388-2755.

Additional Private Sustaining Members Join AOAC

Two more companies have joined the growing list of financial supporters of independent methods validation. These two new AOAC

Private Sustaining Members are The Kroger Company, Cincinnati, OH, and Raltech Scientific Services, Madison, WI. Welcome to the team.

Meetings

April 13–15, 1982: 7th Annual AOAC Spring Training Workshop and Exposition, Fairmont Hotel, New Orleans, LA. (For more information, see article above.)

April 21–22, 1982: ASTM E-41 Meeting on Laboratory Apparatus, ASTM Headquarters, 1916 Race St, Philadelphia, PA. For information, contact Jim A. Dwyer at 215/299-5499.

May 4, 1982: Tenth Annual Pharmaceutical Symposium, Update 82, The Pharmaceutical Industry—The Decade in Review, in Toronto, Ontario, Canada. Sponsored by the Toronto Pharmaceutical Sub-Group of the Chemical Institute of Canada and the Montreal Pharmaceutical Discussion Group. For information, contact M. Puhacz, c/o Health Protection Branch, 2301 Midland Ave, Scarborough, Ontario, Canada, M1P 4R7; telephone 416/291-4231.

May 11–14, 1982: Fourth International Symposium on Quantitative Mass Spectrometry in Life Sciences, Rijksuniversiteit, Gent, Belgium. For information, contact Prof. A. De Leenheer, Symposium Chairman, Laboratoria voor Medische Biochemie en voor Klinische Analyse, De Pintelaan 135, B-9000 Gent, Belgium.

June 6–11, 1982: International Symposium on the Synthesis and Applications of Isotopically Labeled Compounds, Hyatt-Regency Hotel, Kansas City, MO. Topics will encompass synthesis, analysis, purification, and storage of isotopically labeled compounds, and their applications in biomedical, clinical, and environmental studies, in metabolism, pharmacokinetics, and toxicology. For information, contact Alexander Susàn, Scientific Secretary of the Symposium, c/o Midwest Research Institute, 425 Volker Blvd, Kansas City, MO 64110; telephone 816/753-7600, extension 268.

June 6–11, 1982: ASTM E-14 Meeting on Mass Spectrometry, at the Hilton Hawaiian, Honolulu, HI. For information, contact Ms Louise Neall, 215/299-5400.

June 20–23, 1982: International Conference on Chromatography and Mass Spectrometry in Biomedical Sciences, Grand Hotel del Mare, Bordighera, Italy. Organized by the Italian Group for Mass Spectrometry in Biochemistry and Medicine and the International Scientific Center. Topics: chromatography, mass spectrometry and chromatography-mass spectrometry, and their areas of application, including biochemistry, medicine, toxicology, drug research, forensic science, clinical chemistry, and pollution. For information, contact Dr. Alberto Frigerio, Gruppo Italiano di Spettrometria di Massa in Biochimica e Medicina, Via Eritrea, 62, 20157 Milano, Italy.

June 22–24, 1982: ASTM D-16 Meeting on Aromatic Hydrocarbons and Related Chemicals, Sheraton Center, Toronto, Canada. For information, contact Ms Alice Cavallaro, 215/299-5486.

June 23–25, 1982: ASTM E-15 Meeting on Industrial Chemicals, Sheraton Center, Toronto, Canada. For information, contact Ms Alice Cavallaro, 215/299-5486.

September 20–23, 1982: Symposium on Food Research and Data Analysis, Voksenasen Hotel, Oslo, Norway. Organized by the Norwegian Food Research Institute; sponsored by the International Union of Food Science and Technology (IUFoST). For scientists interested in the development and use of computer-aided analysis of multivariate food research data. Advanced knowledge in mathematics or computer science will not be necessary to understand the oral presentations. Fee: NKr 1800 (≈US$360). Final registration June 1, 1982. For information, contact Symposium Secretariat: Norwegian Food Research Institute, Bjorn Eldstuen, PO Box 50, N-1432 Aas-NLH, Norway.

September 23–24, 1982: 5th European Seminar on Quality Control in the Pharmaceutical and Cosmetic Industries—Administrative and Economic Problems, University of Geneva, Switzerland. Organized by the Swiss Association for the Promotion of Quality and the Economics of Quality Control (EOQC) Section for Quality Control in the Pharmaceutical and Cosmetic Industries. The Seminar will be divided into 3 parts: Quality Cost Systems—A Key to Economics of Quality Assurance, Introduction and Implementation of Electronic Data Processing into Quality Assurance, and Use of Electronic Data Processing in Quality Assurance Operations. An equipment exhibition will be held. For further details, contact EOQC Pharma Cosmetic Section, c/o

SAQ, PO Box 2613, CH-3001 Berne, Switzerland; Telex: 33528 atag ch; Tel.: 031 22 03 82.

October 12–14, 1982: Dioxin 82—3rd International Symposium/Workshop on Chlorinated Dioxins and Related Compounds, International Conference Centre, Salzburg, Austria. Sponsored by the International Association of Environmental Analytical Chemistry and the International Society of Toxicological and Environmental Chemists. Topics: incineration sources; analysis and standards; fate, distribution, and levels; laboratory safety and disposal practices; toxicology and risk assessment; legal and regulatory aspects. Symposium proceedings will be published. For information, contact Dr. E. Merian, Im Kirsgarten 22, CH—4106 Therwil, Switzerland.

October 25–28, 1982: 96th Annual AOAC Meeting, Shoreham Hotel, Washington, DC. For information, contact Kathleen Fominaya, AOAC, 1111 N 19th St, Arlington, VA 22209; telephone 703/522-3032.

July 17–23, 1983: SAC 83—International Conference and Exhibition on Analytical Chemistry, the University of Edinburgh, Scotland. To be covered: atomic spectroscopy, biochemical methods, chromatography, electroanalysis, electrophoresis, enzyme techniques, immunoassay, mass spectrometry, microanalysis, molecular spectroscopy, photoacoustic spectrometry, probe methods, radiochemistry, sample preparation, thermal methods, X-ray methods, automation, data processing, process control, microcomputers, microprocessors, and quality control. For information, contact P. E. Hutchinson, Secretary, Analytical Division, Royal Society of Chemistry, Burlington House, London, W1V OBN, U.K.

July 27–30, 1983: 3rd International Conference on the Instrumental Analysis of Foods and Beverages—Recent Developments, the Corfu Hilton, Corfu, Greece. Cosponsored by the Agricultural and Food Chemistry Division of the American Chemical Society, the Institute of Food Technologists, and the Society of Flavor Chemists, Inc., in association with the Department of Food Science of the University of Ioannina, Greece, and the Cereal Institute of Thessaloniki, Greece. For information, contact C. J. Mussinan, IFF R & D, 1515 Highway 36, Union Beach, NJ 07735; telephone 201/264-4500.

CORRECTION

J. Assoc. Off. Anal. Chem. (1982) **65,** 184–187, "Manual Headspace Gas-Solid Chromatographic Determination of Sub-Parts per Trillion Levels of Acrylonitrile in 3% Acetic Acid," by T. McNeal & C. V. Breder

Change title to "Manual Headspace Gas-Solid Chromatographic Determination of Sub-Parts per Billion Levels of Acrylonitrile in 3% Acetic Acid"

BOOK REVIEWS

Environmental Carcinogens. Selected Methods of Analysis. Volume II. Methods for the Measurement of Vinyl Chloride in Poly(Vinyl Chloride), Air, Water and Foodstuffs. Edited by H. Egan. IARC Scientific Publications Number 22. Published by International Agency for Research on Cancer, Lyon, France, 1978. Available from WHO Publications Center USA, 49 Sheraton Avenue, Albany, NY 12210. 142 pp. Price $45.00. ISBN 92-832-1122-7.

Analytical chemists are the target audience for this volume, the second in a series on the analysis of environmental carcinogens. The opening chapter puts into perspective the vinyl chloride problem and briefly reviews the available data on the carcinogenicity of and human exposure to vinyl chloride. The second chapter critically reviews methods that have been reported for the measurement and monitoring of vinyl chloride in a variety of matrices. Essentially all techniques for the measurement of volatile compounds are discussed with specific reference to vinyl chloride. Brief descriptions are given of each method with useful comments on problems associated with some of the methods.

The final section, comprising more than half the book, contains 8 fully detailed methods for determination of vinyl chloride with at least one method of analysis for each type of environmental matrix. There is, unfortunately, no comment on why these 8 methods were selected over other methods discussed in the review chapter. Techniques for the preparation of vinyl chloride standards in air, water, and solvents are described in detail.

Much of the information in the review chapter and in the methods section has relevance to the analysis of volatile compounds other than vinyl chloride. This book, therefore, is useful for reference purposes and to some extent as an analytical manual.

DAVID T. WILLIAMS

Health and Welfare Canada
Health Protection Branch
Ottawa, Ontario
Canada, K1Y OC4

Treatise on Analytical Chemistry, Part I Theory and Practice, Volumes 1 and 2, Second Edition. Edited by I. M. Kolthoff and Phillip J. Elving. Published by John Wiley & Sons, New York, NY. Volume 1: 881 pp, price $76.00, ISBN 0-471-03438-X. 1978. Volume 2: 815 pp, price $74.00, ISBN 0-471-05510-7. 1980.

To quote from the preface to the first edition of the *Treatise:* "The aims and objective of this treatise are to present a concise, critical, comprehensive, and systematic, but not exhaustive, treatment of all aspects of classical and modern analytical chemistry. The *Treatise* is designed to be a valuable source of information to all analytical chemists, to stimulate fundamental research in pure and applied analytical chemistry, and to illustrate the close relationship between academic and industrial analytical chemistry." In this second edition, the editors have revised and updated the material presented in the first edition, and have added sections on the many new methodologies now in use in analytical chemistry.

Volume 1 includes 14 chapters, grouped in four sections, A–D. Section A reviews the development of analytical chemistry as a discipline and discusses what should be included in an educational program preparing individuals to work in this field. Section B discusses the methods used in analytical chemistry and reviews sources of error, accuracy and precision, and sampling methodology. Section C examines chemical principles of importance for the analytical chemist including the chemistry of elements and compounds, atomic weight determination, chemical equilibria, reaction thermodynamics, the phase rule, and the use of kinetics and catalysis in analysis. Section D is concerned with solution equilibria, with transfer activity coefficients, electrode potentials, and pH determination. Volume 2 contains nine chapters which continue the presentation of Section D. In Volume 2, chapters include: graphic presentation of equilibrium data, evaluation of stability constants, acid and base concepts, acid-base equilibria in nonaqueous solutions, complexation reactions, masking and demasking procedures, oxidation-reduction reaction mechanism, and the use of induced reactions.

Each chapter includes an extensive reference list and a number of useful data tables. Each volume has an extended table of contents and a detailed subject index. A total of 28 authors contributed to the two-volume *Treatise*. The editors have done an excellent job of editing the *Treatise* and have presented a truly comprehensive account of this field.

DONALD F. LOGSDON, JR
Chapman College
Mather Air Force Base, CA

Food Safety. Edited by Howard R. Roberts. Published by Wiley—Interscience, John Wiley & Sons, Inc., New York, NY, 1981. 339 pp. Price $39.50. ISBN 0-471-06458-0.

Food Safety presents an overview of current concepts and concerns associated with today's food supply. The authors do a creditable job of providing a proper perspective on the potential hazards that are associated with the multitude of diverse food products offered to today's consumer. This reference book is directed at professionals involved with food production and regulation, at students in the field, and at the educated, concerned public. It is not intended for use by those with in-depth expertise in food safety assessment. The book is well written, readable, and understandable. It systematically discusses safety issues associated with foodborne hazards of microbial origin, nutritional hazards, environmental contaminants, food hazards of natural origin, food additives, and food safety and toxicology. An introductory chapter briefly covers historical aspects of food safety and food processing.

Particularly well written are the chapters on food additives, food safety and toxicology, and nutritional hazards. The complex scientific, legal, and consumer concerns regarding the risk/benefit of direct and indirect food additives are well presented, as is the role of toxicology in food safety. The question of the need for revision of food safety policy is discussed in some depth. Nutritional hazards—excesses as well as deficiencies—are covered; also presented in this chapter is a good overview of concerns with today's diet, including dietary trends and fad diets, food fortification, and dietary goals and guidelines. The incidence of foodborne illness, causative organisms and factors influencing microbial growth and control in foods is discussed in the chapter dealing with foodborne hazards of microbial origin. A particularly good section on mycotoxins is included in the chapter on food hazards of natural origin; this chapter also presents information on other groups of substances including intrinsic components of foods of plant origin, natural constituents of soil and water that accumulate in foods, and compounds of natural origin contaminating edible by-products. Industrial chemicals including PCBs and dioxins, and substances from natural sources including heavy metals, their safety and regulatory control, are treated in the chapter dealing with environmental contaminants.

Occasional weak points do occur; no significant mention is made of today's interest in health effects of dietary salt or the possible association between food additives and hyperkinesis. Because of the extent of public interest in potential hazards of pesticide chemicals, this area, in the reviewer's judgment, should have been treated in greater depth. The epidemiology of foodborne illnesses discussed in the chapter on foodborne hazards of microbial origin is not clearly presented and the style of data presentation in this chapter makes review tedious; however, these weaknesses detract little from the overall value of the book.

In summary, this book provides a good overview of current issues of food safety. It should be a valuable reference for food professionals in government and industry, laboratory personnel, students, and the educated consumer.

H. MICHAEL WEHR
Oregon Department of Agriculture
Laboratory Services Division
Salem, OR 97310

Flavor Microbiology. By Pinhas Z. Margalith. Published by Charles C Thomas, Springfield, IL, 1981. 309 pp. Price $31.50. ISBN 0-398-04083-4.

Effects on the flavor of foods and beverages caused by natural substances produced through the biochemical activity of microorganisms, especially fermentation products of yeasts and lactic acid bacteria, have long been recognized. However, many nuances of flavor which result from microbial activity are less well understood and continue to be the subject of intense research. The

author has produced a well organized text summarizing the scientific literature on the subject of flavor production by microorganisms.

The author's objective in preparing this text was to emphasize the role of microorganisms in flavor production, a subject generally given less emphasis in standard texts on food technology and microbiology. Background information is provided in the initial chapters for readers unfamiliar with general microbiology and flavor sensation. Subsequent chapters describe the effects of microbial action on flavor production in dairy products, meats, vegetables, bread, wine, and beer. The final chapter deals with the flavor components of mushrooms and the commercial production of flavor-enhancing substances such as monosodium glutamate. Extensive bibliographies follow each chapter, and author, subject, and organism indexes are provided for easy reference.

Overall, the book is well written and informative, and should serve as a useful reference for regulators and researchers concerned with flavor technology, microbiology, and chemistry.

JAMES H. MARYANSKI
Food and Drug Administration
Division of Food and Color Additives
Washington, DC 20204

Handbook of Anion Determination. By W. John Williams. Available through Butterworth Inc., 10 Tower Office Park, Woburn, MA 01801, 1979. 630 pp. Price $130.00. ISBN 0-408-71306-2.

This unique book contains over 110 practical procedures for the determination of 69 anions divided into 4 groups: general, sulfur, and halogen anions, and phosphorus oxyanions. Each section is similarily structured covering a range of analytical techniques for separation, gravimetry, titrimetry, spectroscopic methods, and electroanalytical methods followed by catalytic, kinetic, radiochemical, and thermal methods. This simplifies finding a particular method. Another important feature is the separation of procedures for determination according to particular anions.

Methods given are for samples in solution. If a sample to be analyzed is not in solution, the reader is referred to two monographs on decomposition techniques for inorganic and organic samples. It may be argued, however, that solubilization techniques may be important enough to require that the author provide specifics rather than refer the reader to other works.

Step-by-step procedures are given for each anion and method, required reagents are specified, and special apparatus is pictured or described when pertinent. The book is well written and is a fairly complete guide to important and useful methods including older but useful and reliable procedures of earlier years. Frequent references are made to procedures that are in recent editions of *Official Methods of Analysis of the AOAC.*

This book should be a part of the library of every analytical chemist dealing with assays that include most anions.

J. BENTON JONES, JR
University of Georgia
Department of Horticulture
Athens, GA 30602

NEW PUBLICATIONS

Beverages: Carbonated and Noncarbonated, Revised Edition. By Jasper G. Woodroof. Published by AVI Publishing Co. Inc., PO Box 831—250 Post Rd, East Westport, CT 06881, 1981. 524 pp. Price: U.S. & Can. $45.00, other countries $49.50. ISBN-0-87055-381-X.

A standard reference for the beverage industry, this book has been updated with new information on ingredients including acids, flavors, colors, and sweeteners. Recent developments in beverage technology cover quality control, equipment regulations, and waste disposal. Evaluations of opportunities in future markets are included as are discussions of the formulation and production of beverages, plant layout, production methods and carbonation and cooling, packaging technology, sanitation, product development, and marketing.

Commercial Winemaking, Processing and Controls. By Richard P. Vine. Published by AVI Publishing Co., Inc., PO Box 831—250 Post Rd, East Westport, CT 06881, 1981. 494 pp. Price: U.S. & Can. $24.50, other countries $29.50. ISBN-0-87055-376-3.

Commercial Winemaking is designed to assist new or existing small wineries in establishing, maintaining, and upgrading quality control methods, and to clarify technical information and government regulations. It can also be used as a textbook for introductory winemaking courses. Coverage of basic operations in the winery and laboratory include discussions of grapes, fermentation, aging, bottling, filling, corking, storing, microbiological controls, record keeping and analytical methods. Methods are presented for organizing samplings and analyses essential for quality control. U.S. Bureau of Alcohol, Tobacco and Firearms regulations are stressed and coverage of regulations includes approved laboratory equipment, procedures, wine identity, labeling requirements and certification, advertising and promotion of wine, standards of fill, and related provisions.

Food Law Handbook. By H. W. Schultz. Published by AVI Publishing Co., Inc., PO Box 831—250 Post Rd, East Westport, CT 06881, 1981. Approx. 675 pp. Price: U.S. & Can. $79.50, other countries $87.50. ISBN-0-87055-372-0.

Food Law Handbook describes the publication, documentation, and codification of federal food laws and regulations. Twenty food laws in the U.S. in addition to the Federal Food, Drug & Cosmetic Act are extensively discussed. Laws relating to the environment, trade practices, government operations, occupational safety and health, and public health are selectively included. Emphasis is placed on description of food law rather than interpretation. The book is designed to provide the knowledge and means needed to locate legal information on any subject relating to food. While specific regulations are included only as examples, references and step-by-step procedures are given for use of *Federal Register* and *Code of Federal Regulations* to provide current regulatory information and proposals relating to food. The book is aimed primarily at federal and state government employees and officials involved in food regulation, and secondarily at educators in food science and technology, agriculture, and packaging, and at those involved in food industry management, marketing, and product research and development.

GENERAL REFEREE REPORTS: COMMITTEE A

Report on Feeds

CLYDE E. JONES
State Department of Agriculture, 2331 W 31st Ave, Denver, CO 80211

One collaborative study on crude fiber was completed this year. No other collaborative work was reported, but some method development work has been done.

Crude Fat in Pet Foods.—(No Associate Referee). Although there is still a controversy concerning the combination of mixed pelleted and expanded products, no one contacted volunteered to become Associate Referee. A recommendation is made again this year that would allow regulatory laboratories the decision as to which fat method they would use in both pet foods and milk replacers.

Although it is questionable that methods should be modified to accommodate one or two manufacturers of pet foods, it seems reasonable that milk replacers should be analyzed by method **7.059**. The General Referee therefore suggests removal of the word "entirely" from instructions for crude fat or other extract, **7.055-7.060,** to read "Use Method **7.056** or **7.057** for mixed feeds other than (*1*) baked and/or expanded, (2) dried milk products, or (3) contg urea." For further discussion, see last year's General Referee report.

Crude Fiber.—A collaborative study on ceramic fiber used to replace asbestos as a filter aid in crude fiber determinations, by David O. Holst, showed that ceramic fiber was a suitable replacement for asbestos. Adoption as official first action is recommended. Associate Referee Holst has agreed to chair a working group for ISO to study filtration problems of crude fiber and cell wall constituents, with a report due at the 1982 meeting in Budapest on feeds.

Crude Fiber in Milk Replacers.—Jim Pierce continues to work with the Iowa Department of Agriculture on a revised method. Problems were again experienced with a new extraction system, but work will continue.

Minerals in Feeds.—Dianne Gehrke was appointed as a new Associate Referee this year; she is surveying the needs for method improvements in mineral analysis in feeds and seeking input and suggestions.

Non-Nutritive Residues in Feeds.—Peter J. Van Soest continues his work with ISO.

Protein.—Rodney Noel plans to conduct a collaborative study using copper catalyst for crude protein, but needs additional collaborators. There is some concern about how to treat the block digestion in the study.

Sampling.—V. C. Midkiff has been appointed the new Associate Referee. No collaborative study was conducted this year, but input was provided to ISO on their proposed feed sampling protocol.

Amino Acid Analysis in Mixed Feeds and Infrared Reflection Techniques in Mixed Feeds.—Interest has been expressed in both these fields, but as yet no one has volunteered to become an Associate Referee.

Several persons who are experts in iodine determinations in mixed feeds have expressed interest in improving iodine methods; work may be done in this field next year.

Recommendations

(1) Adopt as official first action the substitution of the ceramic fiber filter in the crude fiber method, **7.061-7.065**.

(2) Remove the word "entirely" from instructions for crude fat, **7.055-7.060**.

(3) Continue study on all topics.

This report of the General Referee was presented at the 95th Annual Meeting of the AOAC, Oct. 19-22, 1981, 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 for detailed recommendations, this issue.

Section numbers refer to *Official Methods of Analysis* (1980) 13th Ed.

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

Boron.—Associate Referee James R. Melton has reported on an extensive collaborative study comparing the AOAC titrimetric method for acid-soluble boron (**2.114-2.116**) with a colorimetric method using azomethine H as the developing agent. Blind duplicates of 4 matched pairs (16 samples) were used in the study by 11 collaborators. Evaluation of the results indicates promise of the azomethine H method as an alternative to the AOAC official titrimetric method. The Associate Referee recommends adoption of the colorimetric method as official first action and the General Referee concurs.

Iron.—Associate Referee James R. Silkey continues to evaluate procedures for the determination of chelated iron. Communication received from the Associate Referee indicates progress toward the development of a method for collaborative testing.

Sodium.—Associate Referee Luis F. Corominas has indicated that plans were underway for a collaborative comparison of the atomic absorption spectrophotometric method (*J. Assoc. Off. Anal. Chem.* **64,** 704–708 (1981) with the AOAC flame emission method (**2.147-2.150**). However, the report on this collaborative work has not been received.

No other reports have been received.

Recommendations

(1) Adopt as official first action the azomethine H colorimetric method as proposed by the Associate Referee.

(2) Appoint an Associate Referee for Molybdenum and continue study.

(3) Proceed with planned collaborative study on atomic absorption method for sodium.

(4) Continue official first action status of the following methods: water-insoluble nitrogen, Method II (**2.073-2.074**); modified comprehensive nitrogen (**2.061-2.062**); sulfur (**2.A01**).

(5) Continue study on all topics.

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Section numbers refer to *Official Methods of Analysis* (1980) 13th Ed.; "Changes in Methods," *J. Assoc. Off. Anal. Chem.* (1980) **63,** 374–423.

Report on Pesticide Formulations: Carbamate and Substituted Urea Insecticides

PAUL D. JUNG
Environmental Protection Agency, Office of Pesticide Programs, Beltsville, MD 20705

This past year has been the most productive in recent memory regarding work by pesticides Associate Referees. Our own laboratory has been involved with 8 individual collaborative studies relating to pesticide formulation analysis and we are currently committed to participating in 3 more studies. In most cases the technique of choice is HPLC with an internal standard.

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

Bendiocarb.—Peter L. Carter has now narrowed the internal standard HPLC method search and will probably initiate an AOAC-CIPAC method this year.

Carbofuran.—Edward J. Kikta, Jr, the new Associate Referee, has initiated an internal standard

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HPLC method for this chemical. He anticipated that samples would be sent in October 1981.

Carbosulfan.—Edward J. Kikta, Jr, the newly appointed Associate Referee for this new topic, is considering potential methods for collaboration.

Methiocarb.—Charles J. Cohen, the newly appointed Associate Referee, is considering all options for a collaborative method.

Methomyl.—James E. Conaway, Jr, has completed study of an internal standard HPLC method for formulations containing this active ingredient and is evaluating the data.

Oxamyl.—Glenn A. Sherwood, Jr, the newly appointed Associate Referee, has completed study of an HPLC method for formulations containing this commodity, and analytical data are being evaluated.

Pirimicarb.—Peter D. Bland completed an internal standard GLC collaborative study and the method was adopted interim official first action. The Associate Referee recommends and the General Referee concurs that the method be adopted official first action.

Propoxur.—Charles J. Cohen, the newly appointed Associate Referee, is considering a derivatization method for collaboration.

Other Associate Referees reported continued study on their topics.

Recommendations

(1) Adopt as official first action the interim first action GLC method for the determination of pirimicarb.

(2) Continue study on all topics.

Report on Pesticide Formulations: Fungicides and Disinfectants

THOMAS L. JENSEN
State Department of Agriculture, 3703 S 14th St, Lincoln, NE 68502

In this area, several topics have no Associate Referees. However, a lot of work has been done in the past and several methods are included in the current book of methods.

Brian Korsch of Diamond Shamrock has been appointed Associate Referee for Chlorothalonil, and is welcomed to the group. Also, it is hoped that through contacts made, new Associate Referees can be appointed.

The following is the status report of selected topics contained in this section:

Benomyl.—Associate Referee Lilia Rivera reports difficulties with the LC method being developed.

Chlorothalonil.—Associate Referee Brian H. Korsch reports that a method will be ready for collaborative study in the near future.

Pentachloronitrobenzene.—Associate Referee Alan R. Hanks has completed a collaborative study and has submitted the results to AOAC.

Recommendations

(1) Adopt as official first action the GLC method for pentachloronitrobenzene.

(2) Adopt as official final action the official first action GLC (**6.215-6.219**) and HPLC (**6.A09-6.A14**) methods for captan. Note that while both methods offer the same accuracy, the precision of the HPLC method is superior to that of the GLC method.

(3) Adopt as official final action the titrimetric and electrolytic methods for copper naphthenate, **6.065-6.066**.

(4) Adopt as official final action the potentiometric method for triphenyltin, **6.436-6.439**.

(5) Appoint Associate Referees in areas where official methods do not exist.

(6) Continue study in all areas, especially for compounds where a specific test may be available.

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The recommendations of the General Referee were approved by Committee A and were adopted by the Association. See the report of the committee for detailed recommendations, this issue.

Section numbers refer to *Official Methods of Analysis* (1980) 13th Ed.; "Changes in Methods," *J. Assoc. Off. Anal. Chem.* (1980) 63, 374-423.

Report on Pesticide Formulations: Halogenated and Other Insecticides, Synergists, and Insect Repellants

JAMES E. LAUNER
State Department of Agriculture, Laboratory Services, Salem, OR 97310

Considerable activity occurred under this assignment this year; however, very few collaborative reports were submitted.

At the 1981 Collaborative International Pesticides Analytical Council (CIPAC) meeting in Gembloux, Belgium, the AOAC-CIPAC provisional method for *n*-octyl bicycloheptenedicarboximide (MGK 264) (**6.A15-6.A17**) was adopted as a full AOAC-CIPAC method. CIPAC also agreed that, for method evaluation, AOAC statistical procedures should be followed; however, any statistically sound procedure which rejects aberrant results could be used.

Seven Associate Referees were appointed during the past year: Alan M. Rothman, Dicofol; Ron D. Collins, Fenvalerate; Lorraine Kroposki, Fumigants; George S. Walser, Methoxychlor; Spencer K. Carrigan, Nicotine; Anne Ochs, Perthane and Trichlorfon.

Since AG chlordane is no longer produced, the Associate Referee recommends declaring surplus the following final action methods: AG chlordane (**6.232-6.235**), AG chlordane in granulated formulations (**6.236-6.240**), and heptachlor in AG chlordane (**6.241-6.244**). Likewise, the final action colorimetric method for chlordane (**6.223-6.227**) is empirical and subject to analyst's expertise and familiarity with the method; therefore, the Associate Referee recommends it be declared a surplus method. The General Referee concurs.

Perthane, Ruelene, and Chlordecone no longer are produced in this country and little interest is indicated by regulatory agencies; therefore, the General Referee recommends discontinuing these topics.

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

Chlordane.—J. Forrette will study the combination of total chloride with infrared to determine chlordane and heptachlor in mixtures.

Chlordimeform.—A. Hofberg has initiated a collaborative study of a GLC method.

Dicofol (*1,1-Bis(chlorophenyl)-2,2,2-trichloroethanol*).—A. Rothman will initiate a collaborative study of an HPLC method.

Endosulfan.—R. Watson is investigating the GLC method accepted as a full CIPAC method.

Fenvalerate.—R. Collins is cooperating with CIPAC on a GLC study.

Fumigants.—L. Kroposki will study a GLC method for sulfuryl fluoride.

Methoxychlor.—G. Walser participated in a collaborative study with CIPAC.

N-Octyl Bicycloheptenedicarboximide (*MGK 264*).—V. Meinen recommends discontinuing this topic with the satisfactory official final action method (**6.A15-6.A17**).

Permethrin.—H. Morris is cooperating with CIPAC on a GLC study.

Piperonyl Butoxide and Pyrethrins.—D. Kassera has completed a collaborative study of a GLC method and recommends adoption as official first action. The General Referee concurs.

Rotenone.—R. Bushway plans a collaborative study of an HPLC method.

Resmethrin.—M. Law plans a collaborative study of a GLC method.

Tetradifon.—A. Martijn recommends adoption of the official first action gas-liquid chromatographic CIPAC-AOAC method (**6.B09-6.B14**) as official final action. The General Referee concurs.

Toxaphene.—W. Clark will initiate a study of an infrared method.

Trichlorfon (*Dylox*).—A. Ochs is investigating GLC and HPLC methods.

Recommendations

(1) Continue official first action status of the following items: (a) the GLC method for technical allethrin (**6.149-6.154**); (b) the radioactive tracer method for benzene hexachloride (**6.202**); (c) the hydrolyzable chloride method for dicofol (**6.283-6.288**); (d) the GLC method for fumigants (**6.143-6.148**); (e) the infrared method for rotenone (**6.163-6.164**); (f) the UV method for sulfoxide (**6.419**).

This report of the General Referee was presented at the 95th Annual Meeting of the AOAC, Oct. 19-22, 1981, 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 for detailed recommendations, this issue.

Section numbers refer to *Official Methods of Analysis* (1980) 13th Ed., and "Changes in Methods," *J. Assoc. Off. Anal. Chem.* (1980) **63**, 374-423; (1981) **64**, 501-540.

(2) Adopt as official first action the GLC method for piperonyl butoxide and pyrethrins described by the Associate Referee.

(3) Adopt as official final action the official first action CIPAC-AOAC GLC method for tetradifon (**6.B09-6.B14**).

(4) Declare as surplus the following final action methods: (a) AG chlordane (**6.232-6.235**); (b) AG chlordane in granulated formulations—infrared method (**6.236-6.240**); (c) heptachlor in AG chlordane—GLC method (**6.241-6.244**); and (d) colorimetric method for chlordane (**6.223-6.227**).

(5) Discontinue the topics: (a) Chlordecone; (b) *N*-Octyl Bicycloheptenedicarboximide (MGK Repellant 264); (c) Perthane; and (d) Ruelene.

(6) Transfer the topic Dinocap (2,4-Dinitro-6-octyl Phenyl Crotonate) to the General Referee for Pesticide Formulations: Fungicides and Disinfectants.

(7) Transfer the topic Pentachlorophenol to the General Referee for Herbicides I.

(8) Continue the movement towards the goal of deletion of total halide methods as rapidly as possible.

(9) Continue study on all other topics.

Report on Pesticide Formulations: Herbicides II

LASZLO TORMA

Montana Department of Agriculture, Laboratory Bureau, Montana State University, Bozeman, MT 59717

With the appointment of new General Referees for pesticide formulations the Association is hoping to increase the number of completed collaborative studies. AOAC proposes to do this by encouraging industry and regulatory chemists to initiate and participate in needed collaborative studies. The past year, the General Referee's primary objectives were to familiarize himself with the problems in the assigned area of AOAC work and obtain Associate Referees for the various topics. All existing Associate Referees were contacted and asked about their plans for future study. Responses from Associate Referees were slow and incomplete. New Associate Referees were obtained for some topics which were not previously assigned. Additional Referees will be needed to establish needed official methods.

Newly appointed Associate Referees and their topics are: G. Fuller, Alanap; B. H. Korsch, Dimethyl Tetrachloroterephthalate; G. A. Sherwood, Diuron, Linuron, and Siduron; L. R. Hageman, Paraquat.

The General Referee is still seeking Associate Referees for Barban, Benzoypropyl-ethyl, Dinoseb, Monuron, Oryzalin, and Penoxalin.

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

Alanap.—G. Fuller is investigating an HPLC method and plans a collaborative study.

Benefin, Fluchloralin, and Trifluralin.—G. S. Grimes is completing preliminary work on an HPLC method for these compounds. He is planning a collaborative study during the coming year. Indications are that the proposed method can also be used for the determination of profluralin.

Bromacil.—P. K. Tseng is ready to conduct a collaborative study of a proposed HPLC method.

Dimethyl Tetrachloroterephthalate.—B. H. Korsch is proposing a GLC collaborative study to resolve possible problems between existing official GLC and IR methods.

Paraquat.—L. R. Hageman is studying an HPLC method.

Recommendations

(1) Adopt as official first action the interim official IR method for methazole (*J. Assoc. Off. Anal. Chem.* **64**, 1185-1186 (1981)).

(2) Discontinue the topic Dinitramine. According to U.S. Borox Research Corp., this product is no longer marketed.

(3) Discontinue the topic Lenacil. This product is not marketed in the United States.

This report of the General Referee was presented at the 95th Annual Meeting of the AOAC, Oct. 19-22, 1981, 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 for detailed recommendations, this issue.

Report on Pesticide Formulations: Herbicides III

THOMAS L. JENSEN
State Department of Agriculture, 3703 S 14th St, Lincoln, NE 68502

This section has been an extremely active one during the past year, and the General Referee thanks the people involved in all areas. The Associate Referees have been most helpful in providing needed information on the different topics.

Several people have joined the ranks, including: L. A. Furer of Monsanto, for Alachlor and Propachlor; Tim Stevens of Dow, for Dalapon; William Betker of Mobay, for Metribuzin; Delmas Pennington of Rohm & Haas, for Propanil. Also, a possible referee for DMSA and MSMA has been contacted.

This leaves Bentazone, Cacodylic Acid, Metalochlor, and Terbuthylazine without methods or Associate Referees. The General Referee is optimistic of being able to find Associate Referees for the remaining topics, considering the industry support for this area.

Special thanks also go to the persons who, for various reasons, resigned as Associate Referees. In each and every case, a new Associate Referee was found directly from contacts made before resigning, and this was greatly appreciated.

The following is a status report of selected topics in this section:

Alachlor and Propachlor.—Associate Referee L. A. Furer is working on methods for both compounds, and intends to conduct a collaborative study soon.

Bromoxynil.—Associate Referee L. J. Helfant is looking at other formulations of the compound to determine if further work is necessary.

Dalapon.—Associate Referee T. Stevens is conducting a collaborative study on this compound.

Glyphosate.—Associate Referee A. J. Burns is conducting a collaborative study on this compound.

Metribuzin.—Associate Referee William Betker has a method that, with a few changes, will be submitted for collaborative study.

Propanil.—Associate Referee Delmas Pennington has proposed a method which will be collaboratively studied.

Recommendations

(1) Include Butachlor in the refereeship of Mr. Furer, now Associate Referee for Alachlor and Propachlor.

(2) Appoint an Associate Referee to investigate the need to update the method for amitrole.

(3) As a triazine, atrazine is included in another section under the refereeship of Mr. Hofberg. Therefore, Atrazine should be deleted as a separate topic.

(4) Appoint Associate Referees in areas where AOAC methods are lacking.

(5) Continue study in areas with emphasis in completing developmental work and collaborative studies now underway.

This report of the General Referee was presented at the 95th Annual Meeting of the AOAC, Oct. 19–22, 1981, 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 for detailed recommendations, this issue.

Report on Pesticide Formulations: Organothiophosphate Pesticides

EDWIN R. JACKSON
Mississippi State Chemical Laboratory, Box CR, Mississippi State, MS 39762

Progress in developing AOAC methods for organothiophosphate pesticide formulations has been slow during the past year. Many Associate Referees report that it is extremely difficult to find the necessary time to carry out their assignments. In addition, 3 Associate Referees found it necessary to resign their positions because they were no longer involved in work related to their assigned pesticide. Several additional topics have also been established and Associate Referees are needed to work in these areas.

James W. Miles, Centers for Disease Control, has completed a joint CIPAC-AOAC collaborative study on temephos (Abate). The study was successful, and Dr. Miles has submitted the method for adoption by AOAC.

The following is a summation of the activities and recommendations of the various Associate Referees:

Acephate.—Associate Referee T. C. Arnst resigned because of a change in job emphasis.

Chlorpyriphos.—An HPLC method was adopted official first action last year. Associate Referee N. E. Skelly recommends that this method be adopted official final action.

Coumaphos.—Mark McDonald has changed employers; it is anticipated that his replacement will be available to serve as Associate Referee for this pesticide.

Diazinon.—(A. H. Hofberg). The GLC method was adopted official first action but a modification of method is under consideration.

Dimethoate.—Associate Referee R. S. Wayne has developed an HPLC method and plans a joint AOAC-CIPAC study this winter.

Dioxathion.—An HPLC method is currently being used by Associate Referee W. H. Clark for quality control. He will evaluate the possibility of studying it collaboratively.

Encapsulated Organophosphorus Pesticides.—Associate Referee J. J. Karr conducted a collaborative study on encapsulated diazinon (Knox Out 2FM). He recommends that the method be adopted official first action.

EPN.—Associate Referee J. E. Forrette found it necessary to identify an interfering impurity in technical EPN. When this is done he will be ready to conduct a study on an HPLC method.

Ethoprop.—Associate Referee Chan Caldwell is no longer associated with crop chemicals; this product was sold to another company. A chemist employed by the purchaser has agreed to serve as Associate Referee if he is authorized to do so by his supervisors.

Fensulfothion.—An HPLC collaborative study is nearing completion and results look promising. Associate Referee Margie Owen may obtain interim first action approval during the coming year.

Fenthion.—Associate Referee W. G. Boyd plans to evaluate existing GLC methods for this compound.

Fonophos.—Associate Referee H. Moya has evaluated GLC methods and hopes to submit one to a collaborative study in 1982.

Parathion and Methyl Parathion.—Associate Referee E. R. Jackson plans a collaborative study to include parathion dusts and methyl parathion-parathion mixtures.

Phorate.—Associate Referee R. Grypa is studying a GLC method for possible collaborative study.

Recommendations

(1) Adopt as official final action the official first action method for chlorpyrifos (HPLC) (**6.B15-6.B19**) and encapsulated methyl parathion or parathion (GLC) (**6.409-6.414**).

(2) Adopt as official first action the GLC method for encapsulated diazinon (*J. Assoc. Off. Anal. Chem.* **65,** 115-118 (1982)).

(3) Adopt the proposed CIPAC-AOAC HPLC method for temephos as official first action.

(4) Continue study on all topics.

This report of the General Referee was presented at the 95th Annual Meeting of the AOAC, Oct. 19-22, 1981, 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 for detailed recommendations, this issue.

Section numbers refer to *Official Methods of Analysis* (1980) 13th Ed.; "Changes in Methods," *J. Assoc. Off. Anal. Chem.* (1981) **64,** 501-540.

Report on Pesticide Formulations: Other Organosphosphate Insecticides, Rodenticides, and Miscellaneous Pesticides

G. MARSHALL GENTRY
Florida Department of Agriculture and Consumer Services, Tallahassee, FL 32301

The Associate Referees have experienced heavy workloads in their area of responsibility and have had little time to devote to collaborative studies.

Associate Referee Elmer H. Hayes has requested to be relieved of the responsibility of *all* rodenticides and will be the Associate Referee on Warfarin. Associate Referees are needed for Chlorophacinone, α-Naphthylthiourea, Vacor, Strychnine, Crufomate, and Dicrotophos. Please contact the General Referee if you are interested in any of the above vacancies.

The following is the present status of selected topics under this General Refereeship:

Crotoxyphos.—Associate Referee W. King is investigating a GLC method.

Dichlorvos.—Associate Referee N. Epstein is investigating methods.

Mevinphos.—Associate Referee H. Holly has been investigating a GLC method and expects to conduct a collaborative study during the next year.

Monocrotophos.—Associate Referee G. Winstead is investigating GLC and HPLC methods.

Naled.—Associate Referee A. Carlstrom has investigated a GLC method with a capillary column and has experienced some difficulty. As soon as these problems have been overcome, a collaborative study will begin.

Tetrachlorvinphos.—Associate Referee N. Epstein is investigating methods.

Brodifacoum (Talon).—Associate Referee P. Bland is conducting a collaborative study of an HPLC method with a Zorbax ODS column.

Diphacinone.—Associate Referee V. Stevens is investigating an HPLC method.

Recommendations

(1) Appoint Associate Referee for Warfarin and delete the topic Rodenticides.

(2) Continue study on all topics.

This report of the General Referee was presented at the 95th Annual Meeting of the AOAC, Oct 19-22, 1981, 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 for detailed recommendations, this issue.

Report on Plants

J. BENTON JONES, JR
University of Georgia, Department of Horticulture, Athens, GA 30602

Fluorine.—A collaborative study, presented at the 1981 meeting, concluded that "although there has been considerable improvement in speed and simplicity of fluoride analyses during the last decade, there has been no improvement between laboratories. This is caused by the variety of methods and techniques in use and as indicated in this study by inherent differences between methods." Additional study is needed to identify the reasons for differences in results between methods and for the influence of different types of vegetation samples on the performance of analytical methods.

Chromium.—A collaborative study will begin in 1982 to evaluate a new method for determining chromium in a wide range of plant materials; the method has proven reliable in initial tests.

Boron.—It has been recommended that the azomethine-H procedure for boron in fertilizers be collaboratively studied for plants. This sim-

ple technique was first introduced for the determination of boron in waters and soil solution extracts.

Recommendations

(1) Change the title of Emission Spectroscopy (Plants) to Plasma and Spark Emission Spectroscopy (Plants) and appoint a new Associate Referee.

(2) Continue study on all other topics.

This report of the General Referee was presented at the 95th Annual Meeting of the AOAC, Oct. 19-22, 1981, 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 for detailed recommendations, this issue.

Report on Reference Materials and Standard Solutions

ROBERT ALVAREZ
National Bureau of Standards, Office of Standard Reference Materials, Washington, DC 20234

Certified Reference Materials (CRMs) are homogeneous materials used in determining the accuracy of methods, calibrating instruments, and validating and referring experimental data to a common base. CRMs are issued by organizations generally accepted as technically competent. The certificates for the CRMs list values of the certified concentration or properties.

The ISO Council Committee on Reference Materials has issued a *Directory of Certified Reference Materials* listing worldwide sources of supply and suggested uses. Categories of interest to AOAC include: environmental; biological, botanical, foods; biomedical, pharmaceuticals; clinical chemistry; and nuclear/radioactivity. The ISO publication is available from ISO Central Secretariat, Attention REMCO Directory, Case Postale 56, CH-1211, Geneve 20, Suisse (Switzerland).

AOAC-related, CRM activities of several organizations are noted below.

The National Physical Laboratory, Teddington, Middlesex, UK, TW11 OLW, issues CRMs which include 86 pesticides of certified purity, 10 substances with certified melting points, 6 substances for calibration of differential scanning calorimeters, and 8 powders certified for specific surface area.

The International Atomic Energy Agency, PO Box 590, A 1011 Vienna, Austria, issues both CRMs and Reference Materials (RMs). Materials are issued as RMs instead of 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) and SL-1 (lake sediment) are available as CRMs for trace element determinations; in preparation are V-9 (cotton cellulose), A-12 (animal bone), and A-13 (dried animal blood), also for the same use.

The Marine Analytical Chemistry Standards Program, National Research Council, Montreal Rd, Ottawa, Ontario, Canada K1A 0R9, has announced the availability of 2 marine sediment reference materials. Values for trace metals and minor and major constituents are provided on the certificates.

CRMs issued by the National Bureau of Standards (NBS) are known as Standard Reference Materials. A new edition of the SRM catalog will be available in 1982.

Recent developments in SRMs applicable to AOAC activities are listed below.

Biological, Botanical, Foods

(1) Citrus Leaves, SRM 1572, has been analyzed and data are being evaluated statistically. This SRM, which is certified for elements of nutritional and environmental significance, will be available during the first quarter of 1982.

(2) Wheat Flour, SRM 1567, and Rice Flour, SRM 1568, have been analyzed for Pb by isotopic dilution mass spectrometry and polarography. The revised certificates will show the Pb certified concentrations which are 0.02 and 0.04 $\mu g/g$, respectively. The values have an estimated uncertainty of $\pm$ 0.01 $\mu g/g$.

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The recommendations of the General Referee were approved by Committee A and were accepted by the Association. See the report of the committee for detailed recommendations, this issue.

Clinical

(1) Human Serum, SRM 909, was analyzed for cholesterol and magnesium by definitive methods—isotopic dilution mass spectrometry. An addendum to the certificate provides certified concentration values for these 2 analytes in addition to the 6 analytes certified previously. A collaborative study to determine the concentrations of 7 enzymes in this SRM by "best available" methodology has been completed. The enzymes are: acid phosphatase (residual), alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, creatine kinase, lactic dehydrogenase, and γ-glutamyltransferase. The results were evaluated statistically for inclusion in a revised certificate. The certificate will also include an uncertified concentration value for the protein content determined at NBS.

(2) Sodium Pyruvate, SRM 910, has been issued as a chemical of known purity. It is intended primarily for use in the calibration and standardization of procedures for pyruvate, lactic dehydrogenase, and glutamic–pyruvic transaminase determinations.

(3) 4-Nitrophenol, SRM 938, has been issued for use in calibrating spectrophotometers for clinical analysis in which 4-nitrophenol is the chromogenic compound formed.

(4) Anticonvulsant Drug Level Assay, SRM 1599, is being analyzed for carbamazepine and valproic acid by HPLC and GC methods. Low, medium, and high levels of these drugs are provided.

Environmental

(1) Priority Pollutant Polynuclear Aromatic Hydrocarbons, SRM 1647, has been prepared and analyzed. The SRM consists of ampuled solutions of 16 polynuclear aromatic hydrocarbons in acetonitrile. The certified concentrations of these EPA priority pollutants range from approximately 3 to 20 μg/mL. The principal intended use of this SRM is calibration of GC and HPLC instrumentation.

(2) Generator Columns for Polynuclear Aromatic Hydrocarbons, SRM 1644, was developed to generate known, accurate concentrations of anthracene, benzo(a)anthracene, and benzo(a)pyrene in water.

(3) Polychlorinated Biphenyls in Oil, SRM 1581, has been ampuled and is being analyzed for issue in 1982. The Certificate of Analysis will provide certified concentration values, at approximately 100 μg/g, of Aroclor 1242 and Aroclor 1260 in transformer oil and motor oil. A bottle of each matrix oil will be included as a diluent.

(4) Organics in Shale Oil, SRM 1580, has been analyzed for additional compounds of environmental significance. The revised certificate lists certified concentrations of fluoranthene, pyrene, benzo(a)pyrene, benzo(e)pyrene, perylene, phenol, *o*-cresol, 2,6-dimethylphenol, and benzo(f)quinoline (5,6-benzoquinoline) at μg/g levels.

(5) Estuarine Sediment, SRM 1646, has been analyzed and data are being evaluated statistically. The certificate for the SRM, available early in 1982, will provide certified concentration values of environmentally important elements, such as Cd, Pb, and elements of interest to marine biologists.

The Associate Referee on Stability of Organophosphorous Pesticide Standards, G. M. Doose, Food and Drug Administration, Los Angeles, CA, has completed one phase of his study on degradation of these pesticides under different experimental conditions. He has written a report, copies of which are available from him on request.

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 the General Referee of their plans to conduct collaborative studies. When analytes and matrix are sufficiently stable, the use of test samples representative of larger lots of materials in these studies will enable the materials to be used as RMs or possibly as CRMs.

(3) Encourage the Associate Referee to submit his report on the stability of organophosphorus pesticide standards for possible publication in the AOAC Journal.

GENERAL REFEREE REPORTS: COMMITTEE B

Report on Drugs, Acidic and Neutral Nitrogenous Organics

JAMES W. FITZGERALD
Food and Drug Administration, 109 Holton St, Winchester, MA 01890

Acetaminophen in Drug Mixtures.—David J. Krieger has developed an HPLC procedure for the identification and determination of acetaminophen in analgesic preparations and will present his work at the Annual Meeting. Using a C_{18} column with a methanol–0.75% acetic acid (1 + 3) mobile phase, he was able to separate acetaminophen from chlorpheniramine maleate, phenylephrine hydrochloride, caffeine, salicylamide, aspirin, salicylic acid, and phenacetin.

Benzothiazine Derivatives.—Stephen T. Hauser reported on his work on the separation of chlorothiazide, hydrochlorothiazide, benzthiazide, polythiazide, acetazolamide, and quinethazone by the HPLC method originally developed by Moskalyk et al. (*J. Pharm. Sci.* **64**, 1406–1408 (1975). Using a phenyl μBondapak column with a mobile phase of acetonitrile–water (35 + 65) and a flow rate of 2 mL/min, he obtained good separation of all these drugs except hydrochlorothiazide and quinethazone. The Associate Referee expects that with additional work the method will be suitable for separating all 6 drugs.

Methyldopa.—Susan Ting reported on the development of an HPLC procedure for separating methyldopa from its 3-O-methyldopa impurity and its combination drugs hydrochlorothiazide and chlorothiazide, as well as from 4-amino-1-chloro-1,3-benzenedisulfonamide, which is an impurity in hydrochlorothiazide and chlorothiazide. The method specifies use of a μBondapak C_{18} column with 4% methanol in 0.3% acetic acid in water mobile phase, a flow rate of 1.5 mL/min, and detection at 280 nm. Linearity and reproducibility studies have been conducted and results for the proposed procedure on 6 commercial products compare favorably with results obtained by other standard methods.

Primidone.—Stanley E. Roberts reported that he received results from 6 collaborators who performed the HPLC method. The results look very good, and he expects to submit a full report on the collaborative study.

Thyroid, and Thyroxine-Related Compounds.—A new Associate Referee, Donald J. Smith, was appointed to continue the work of Mae E. Biesemeyer.

Thyroid by DPP (Differential Pulse Polarography).—Walter Holak completed the collaborative study and will present his findings at the Annual Meeting.

Other Associate Referees report little or no progress because of other commitments.

Recommendations

(1) Adopt as official final action the official first action semiautomated method, **39.A01–39.A07,** for the determination of nitroglycerin in sublingual tablets.

(2) Adopt as official first action the differential pulse polarographic (DPP) method described by the Associate Referee for the determination of iodine in thyroid tablets.

(3) Combine the topics Barbiturates (General Methods) and Barbiturates (Liquid Chromatography) into a single topic Barbiturates, under the direction of the present Associate Referee for Barbiturates (Liquid Chromatography).

(4) Continue study on all other topics.

This report of the General Referee was presented at the 95th Annual Meeting of the AOAC, Oct. 19–22, 1981, 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 for detailed recommendations and "Changes in Methods," this issue.

Section numbers refer to "Changes in Methods," *J. Assoc. Off. Anal. Chem.* **63**, 374–423 (1980).

Report on Drugs, Alkaloids

EDWARD SMITH
Food and Drug Administration, Division of Drug Chemistry, Washington, DC 20204

Atropine in Morphine and Atropine Tablets and Injections.—I. J. Holcomb reported that progress has been made in his investigation of chromatographic procedures for the separation and determination of atropine and morphine in these pharmaceuticals. Initial results for a gas chromatographic procedure show good resolution and adequate sensitivity for the simultaneous determination of morphine and atropine.

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, for determining other belladonna alkaloids that might be present, and for ensuring the suitability of the procedure to detect decomposition products.

Colchicine in Tablets.—Richard D. Thompson reported that he has developed a suitable liquid chromatographic procedure for the determination of colchicine. The proposed HPLC system resolves colchicine from 13 associated compounds, including the related alkaloids and phototransformation products of colchicine. He has applied the procedure to samples of bulk drug to dosage forms that contain colchicine alone and in combination with probenecid. One decomposition product, colchiceine, does not chromatograph with the proposed HPLC procedure. Either a TLC limits test or an alternative chromatographic procedure will have to be developed for that determination.

Curare Alkaloids.—J. R. Hohmann has successfully applied the revised HPLC procedure to currently marketed samples of tubocurarine chloride injection; results agreed with the current USP bioassay. He continues to seek collaborators to study the proposed HPLC procedure.

Ephedrine.—Charles C. Clark reported no further work on this topic. No adverse comments have been received on the official first action on-column periodate reaction method for ephedrine sulfate in solid dosage forms, **38.A01-38.A05** (*J. Assoc. Off. Anal. Chem.* **63,** 692-695), and the Associate Referee recommends that the method be adopted official final action.

Ergot Alkaloids.—T. C. Knott reported the results of the collaborative study of his reverse phase HPLC procedure at this meeting (1). The method was applied to the determination of ergotamine tartrate in tablets and of methysergide maleate in tablets. The fact that the method is stability-indicating was illustrated by the results presented last year (2) for the analysis of ergotamine tartrate injection. The collaborative study of ergotamine tartrate injection with intact sealed samples (vs pooled reconstituted samples that were used in the previous study) was delayed because no samples were available.

Neostigmine.—R. E. Kling reported that no further work was done on this project.

Physostigmine and Its Salts.—N. W. Tymes reported at least year's meeting (3) on the collaborative study of his reverse phase HPLC procedure for analyzing physostigmine salicylate injection and solution, and physostigmine sulfate ointment. The proposed HPLC method (*J. Assoc. Off. Anal. Chem.* **65,** 132-137 (1982)) gives good resolution between physostigmine and any breakdown products, preservatives, and the internal standard flurazepam hydrochloride. This year's written report includes the results of an additional collaborator. The Associate Referee recommends that the method be adopted official first action for determining physostigmine salicylate and physostigmine sulfate in pharmaceuticals.

Pilocarpine.—I. W. Wainer is continuing his investigation of a proposed reverse phase HPLC method for the simultaneous determination of pilocarpine, isopilocarpine, pilocarpic acid, and isopilocarpic acid, using a UV detector instead of the refractive index detector proposed in the original method (4). He has completed validation of the method with commercial samples that contain a variety of adjuvants and preservatives. Preliminary work indicates that adequate reso-

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This report of the General Referee was presented at the 95th Annual Meeting of the AOAC, Oct. 19-22, 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 for detailed recommendations and "Changes in Methods," this issue.

Section numbers refer to "Changes in Methods," *J. Assoc. Off. Anal. Chem.* **63,** 374-423 (1980).

lution is obtained with one brand of ODS microparticulate support but not with others. Application of the procedure with commercial columns of the current vintage of that brand of support failed to give the desired resolution. The manufacturer of the support acknowledged that the method of preparing the reverse phase had been changed. The original investigator subsequently reported (5) on the use of a different support. Application of that brand also failed to give the desired resolution. Modifying the mobile phase with acetonitrile instead of methanol together with an alkylphenyl microparticulate column gave the desired resolution together with a change in order of elution. A collaborative study is recommended following the completion of an intralaboratory validation.

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

Rauwolfia serpentina.—U. Cieri continues to investigate the development of a suitable single tablet assay for determining reserpine-rescinnamine in *Rauwolfia serpentina* tablets. He has initially applied normal phase HPLC with a fluorometric detector.

Recommendations

(1) Adopt as official final action the official first action method, **38.A01-38.A05,** for determination of ephedrine sulfate in solid dosage forms.

(2) Adopt as official first action the proposed HPLC method for physostigmine salicylate and physostigmine sulfate for solution dosage forms only.

(3) Continue study on all topics as indicated.

REFERENCES

(1) Knott, T. C. (1981) 95th Annual Meeting of the AOAC, Washington, DC, Abstract 44
(2) Knott, T. C. (1980) 94th Annual Meeting of the AOAC, Washington, DC, Abstract 91
(3) Tymes, N. W. (1980) 94th Annual Meeting of the AOAC, Washington, DC Abstract 92
(4) Noordam, A., Waliszewski, K., Olieman, C., Maat, L., & Beyerman, H. C. (1978) *J. Chromatogr.* **153,** 271-273
(5) Noordam, A., Maat, L., & Beyerman, H. C. (1981) *J. Pharm. Sci.* **70,** 96-97

Report on Drugs, Miscellaneous

TED M. HOPES
Food and Drug Administration, 850 Third Ave, Brooklyn, NY 11232

Benzoyl Peroxide.—The Associate Referee has written an HPLC method as modification of a method that appeared in the USP publication *Pharmacopoeial Forum,* and has submitted it to his FDA headquarters for forwarding to the USP. The Associate Referee feels that no further work is necessary unless the USP requests a collaborative study.

Chlorinated Hydrocarbons in Drugs.—This project was originally developed when chloroform and other chlorinated hydrocarbons were permitted ingredients in drugs. This is no longer the case in this country, and instances of the occurrence of those solvents in drugs are reduced to rare formulations or contaminations which are not subject to the standard procedure that was contemplated. A survey of a number of people involved in regulatory drug analysis uncovered no known problems of this type and no need for methods to determine chlorinated hydrocarbons in drugs. The General Referee solicits comments on the proposal to drop this project and surplus the current official first action AOAC method **36.013-36.017.**

Disulfiram.—A method was adopted as official first action (**36.B01-36.B04**) at the last meeting. It is recommended that the method remain in first action status for at least one more year. Any comments on this method are solicited.

Ethylene Oxide.—The Associate Referee encountered difficulties in preparing reproducible samples for collaborative study, and in obtaining

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commercially prepared columns that can be used at the high sensitivity levels required by the method. He will deal with these problems in the coming year.

Fluoride.—The planned collaborative study was not carried out because of a lack of time. Time has been planned for later this year.

Identification of Drugs (Mass Spectroscopy).—No report was received.

Medicinal Gases.—The planned collaborative study was not carried out because of various problems with equipment and a problem encountered with linearity of responses from commercially pure reference gas. These problems will be investigated further in the coming year.

Menadiol Sodium Diphosphate Injection.—Time for the planned collaborative study was not available in 1981. Sufficient time has been set aside in 1982 and completion of the study is expected.

Mercurial Diuretics.—The planned collaborative study was not conducted because of a lack of time. Time has been planned for later this year.

Mercury-Containing Drugs.—The Associate Referee has completed a collaborative study of his atomic absorption method for total mercury in mercury-containing drugs which was reported at the 1979 meeting of the AOAC. The study appears to have been successful and the results will be forwarded to the statistician for his review and evaluation. This method for the analysis of dosage forms containing thimerosal, phenylmercuric acetate, ammoniated mercury, or mersalyl will be submitted for interim adoption if the evaluation is favorable.

Metals in Bulk Drug Powders.—The Associate Referee, who presented a paper last year on preliminary work on this project, has resigned. Walter Holak, FDA New York Regional Laboratory, will be recommended for appointment.

Microcrystalline Tests.—This topic has been vacant for several years. An Associate Referee is needed to review and improve these methods.

Protein Nitrogen Units in Allergenic Extracts.—Associate Referee Joan May, Bureau of Biologics, FDA, has conducted a collaborative study on a procedure involving precipitation of allergenic extracts by phosphotungstic acid followed by a Kjeldahl nitrogen determination (*J. Assoc. Off. Anal. Chem.* **64**, 1435-1438 (1981)). The method was satisfactory for the type of sample being analyzed, and was granted interim first action status (*J. Assoc. Off. Anal. Chem.* **64**, 1031 (1981)). I concur with the Associate Referee's recommendation for adoption of the method official first action.

Recommendations

(1) Adopt as official first action the interim first action protein nitrogen unit precipitation method for allergenic extracts.

(2) Continue study on all other topics.

Report on Drugs, Narcotic and Dangerous

CHARLES C. CLARK
Drug Enforcement Administration, Southeast Regional Laboratory, Miami, FL 33166

The Associate Referee for benzodiazepines has developed an HPLC procedure for the determination of certain benzodiazepines. A collaborative study will be initiated when a suitable internal standard is found.

The Associate Referee for heroin has completed the method development portion of the project, and is presently preparing samples suitable for a collaborative study. A request for collaborators will be made in the near future.

No progress reports were received on the following topics: chemical microscopy; cocaine, dimethyltryptamine, diethyltryptamine, and dipropyltryptamine; marihuana and synthetic tetrahydrocannabinol; methadone; methamphetamine; methaqualone; methylphenidate; optical crystallographic properties of drugs; and phencyclidine.

Recommendation

It is recommended that study be continued on all topics.

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Report on Drugs, Nonalkaloid Organic Nitrogenous Bases

THOMAS G. ALEXANDER
Food and Drug Administration, National Center for Antibiotic Analysis, Washington, DC 20204

Aminacrine.—The Associate Referee has developed a TLC identity test and is now investigating means of isolating aminacrine from common matrices before assay. The presence of trace amounts of a contaminant in the aminacrine samples has made the study more complex. However, it is anticipated that in the near future, the assay method will be developed and work will proceed on a collaborative study.

Chlorpheniramine Maleate Tablets (Semiautomated Analysis).—This method was adopted official first action 2 years ago (**38.A06-38.A13**) and no adverse comments have been received. Adoption as official final action and discontinuation of the topic are recommended.

Epinephrine and Related Compounds by HPLC-Electrochemical Detectors.—The Associate Referee has developed a method using a polarographic detector and has been studying the determination of epinephrine and its breakdown products in various pharmaceutical products. He expects to conduct a collaborative study soon.

Homatropine Methyl Bromide in Tablets.—The new Associate Referee is also working with picrate derivatives, but is now developing an HPLC analysis to be applied after derivatization.

Phenethylamine Drugs—Semiautomated Individual Unit Analysis.—The Associate Referee is developing a colorimetric method for use with this class of compounds on a high sample-volume basis.

Pheniramine with Pyrilamine Phenyl, Propanolamine, and Phenylephrine.—The Associate Referee has experienced difficulties with syrup preparations containing parabens; tablet preparations assayed satisfactorily. He expects to resolve the problem and proceed with a collaborative study soon.

Other Topics.—(*1*) *Quinacrine Hydrochloride.*—In a recent interlaboratory study, the former Associate Referee found that some analysts were experiencing high background interference and were not compensating properly in carrying out the determination of quinacrine hydrochloride in pharmaceuticals, **38.231-38.235**. This resulted in erroneous, high emission values. The prescribed running of the complete emission scans should be followed to properly compensate for high backgrounds. It is recommended that **38.234** be editorially revised to ensure compensation for high background interference.

(*2*) *Ion Exchange Chromatography for Selected Drug Combinations.*—Method **38.111-38.117** for the analysis of selected drug combinations by ion exchange chromatography was adopted official final action (*J. Assoc. Off. Anal. Chem.* **61**, 472 (1978)) and is so designated in *Official Methods of Analysis* (1980) 13th Edition. Recent reports of Committee B refer to this method as being official first action. The Referee recommends that the Committee declare this method to indeed be official final action.

(*3*) *Amphetamine (Spectrophotometric Method).*—Method **38.122-38.126** is not specific for amphetamine and there are valid official AOAC methods for its assay. The Referee recommends deletion.

(*4*) *Phenothiazine.*—Method **38.185-38.186** for assaying phenothiazine has been in official first action status for some years; it appears to be a sound method. It is recommended that the method be adopted official final action. This is a veterinary product and the method rightfully belongs in Chapter **42**.

(*5*) *Procainamide Hydrochloride.*—This method was successfully collaborated several years ago and was adopted official first action (**38.224-38.227**). No adverse comments have been received. It is recommended that the method be adopted official final action.

Recommendations

(1) Adopt the official first action semiautomated method for chlorpheniramine maleate tablets, **38.A06-38.A13**, as official final action; discontinue topic.

(2) Editorially revise method **38.231-38.235**, fluorometric determination of quinacrine hy-

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drochloride in pharmaceuticals, so that full baseline scans are obtained. Change **38.234** to read:

38.234 ***Determination***

Adjust spectrophotofluorometer to ca 80% fluorescence intensity (*F*) at 500 nm with std soln. Transfer ca 3 mL 0.1N HCl to clean 10 × 10 mm cell and record the blank scan between 350 and 650 nm. Repeat with std and sample solns. In each case, draw baseline from 350 to 650 nm. Det. %*F* at peak max. (ca 500 nm) of sample and std solns relative to 0.1N HCl blank.

(3) Reiterate official final action status of method **38.111-38.117,** ion exchange chromatography for selected drug combinations.

(4) Delete the official first action spectrophotometric method for amphetamine (**38.122-38.126**).

(5) Adopt as official final action the official first action method for phenothiazine (**38.185-38.186**) and transfer the method to Chapter 42.

(6) Adopt as official final action the official first action method for procainamide hydrochloride (**38.224-38.227**).

(7) Continue study on all other topics.

AOAC Regional Section Meetings

AOAC Midwest Regional Section Meeting, June 2–3, 1982, Ames, IA. For more information, contact H. Michael Stahr, Iowa State University, 515/294-1950

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AOAC Pacific Northwest Regional Section Meeting, June 16–17, 1982, Olympia, WA. For more information, contact H. Michael Wehr, Oregon Department of Agriculture, 503/378-3793

■

AOAC Northeast Regional Section Meeting, June 22–23, 1982, Syracuse, NY. For more information, contact Audrey Gardner, NY State Agriculture Experiment Station, 315/787-2281

GENERAL REFEREE REPORTS: COMMITTEE C

Report on Coffee and Tea

ROBERT H. DICK
Food and Drug Administration, 850 Third Ave, Brooklyn, NY 11232

The collaborative study on ash and moisture in instant tea, carried out by the ISO Indian group, was unsatisfactory because of a lack of reproducibility of results between laboratories. The group suggested that nations most interested in these analyses be responsible for a new study. F. Farrell, Associate Referee on Ash in Instant Tea, has started a new study, combining a study of ash and moisture. W. P. Clinton, Associate Referee on Moisture in Coffee and Tea, concurs in making it a double study.

J. A. Yeransian, Associate Referee on Chlorogenic Acid in Coffee, has not been able to find a sufficient number of collaborators for his automated method except in his own company. He hesitates to start a study without a more widely based group of collaborators.

D. Page, Associate Referee on Solvent Residues in Decaffeinated Coffee, did not submit a report.

The Associate Referee on Crude Fiber in Tea was forced to resign because of other work. The Associate Referee on Water Extract in Tea, E. DeLaTeja carried out a collaborative study on his method, but did not receive reports from all his collaborators in time for the results to be presented at the 1981 AOAC meeting. He states that the method appears to be satisfactory, but is waiting for a statistical analysis.

The Associate Referee on Caffeine in Coffee and Tea was busy on a method for caffeine in cola beverages and did no work on tea as planned.

The lack of a method for solvent residues in decaffeinated tea continues to be a problem.

A study of methyl xanthines in food containing these compounds is planned. If so, a method for the determination of theophyllin in tea should be developed.

Recommendations

It is recommended that work on all topics be continued, and Associate Referees be appointed for Crude Fiber in Tea, Solvents Residues in Decaffeinated Tea, and Theophyllin in Tea.

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Report on Dairy Products

ROBERT W. WEIK
Food and Drug Administration, Bureau of Foods, Washington, DC 20204

The Referee received no reports from Associate Referees recommending official action for the 1981 AOAC meeting. Some collaborative studies are in progress and it is possible there will be some recommendations for interim approval. It is hoped that several recommendations for official action will be made at the 1982 annual meeting of the AOAC.

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 the determination of total phosphorus content of cheese and processed cheese. Results of a collaborative study are being evaluated by the

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statisticians and we anticipate making a recommendation for interim action in the near future.

Last year, the Referee referred to a possible error in **16.059,** Roese-Gottlieb method for the determination of fat in milk. The present method required only one addition of ethanol to the sample, while the original Mojonnier method called for a second addition of ethanol before the second ether extraction. Some laboratories have reported problems of an emulsion forming during the second extraction without the additional ethanol. The method is being revised by the Joint IDF/ISO/AOAC Group E-31 and the current draft of the revision does specify a second addition of alcohol before the second ether extraction. The Referee anticipates completion of a collaborative study on the revised method possibly in time to recommend interim action before the 1982 meeting.

The Referee has noticed an error in **16.085** in the 13th edition of *Official Methods of Analysis.* The last sentence of the first paragraph has 2 reference numbers in parenthesis which are incorrect. **16.052** should read **16.056,** and **16.053** should read **16.057.**

For the past several years there have been 12 topics under "Dairy Products" that have not had Associate Referees. The recommendation of several annual meetings has been "appoint associate referee; continue study." However, the Referee has not been successful in appointing Associate Referees and recommends that the topics be discontinued. If an analyst is actively working in one of those areas, the topic can be reactivated.

The Referee has received a number of inquiries about the Cyroscopic Methods which appear on pages 249–251 of the 13th edition of *Official Methods of Analysis* (1980). The questions mainly pertain to the conversion of "Hortvet" to celsius degrees. There appears to be an error when freezing point in °H is converted to °C by the formula. The calculated results are different from the stated results. Also, there are reported differences in values for the standard solutions. We have also observed differences in the corresponding values in ISO Draft Proposal 5764.2.

The questions have been referred to the joint IDF/ISO/AOAC Group E-37 "Freezing Point of Milk." It is important that all 3 organizations agree on the values for the reference solutions and also agree on the formula. The Referee may be able to report a final solution to this problem at the next annual meeting.

Recommendations

(1) Discontinue 12 topics that do not have Associate Referees:. Carrageenan in Milk and Milk Products; Cheese Spreads, Gums In; Desserts, Frozen; Desserts, Frozen, Gums In; FAO and AOAC Methods, Comparison; Fat Content; Fats, Foreign (Sterol Acetate); Milk, Reconstituted; Phosphatase Test; Salt; Sampling, Fat and Moisture in Cheese; Titanium Dioxide in Cheese.

(2) Continue study on all other topics.

Report on Decomposition and Filth in Foods (Chemical Methods)

WALTER F. STARUSZKIEWICZ, JR
Food and Drug Administration, Division of Food Technology, Washington, DC 20204

Amines in Seafoods.—Associate Referee C. R. Rigby has resigned. The Referee recommends that the topic be discontinued.

Ammonia in Dogfish.—Associate Referee Beverly Smith reported on the application of an enzymatic procedure for the determination of ammonia in dogfish. Ammonia is used as a quality indicator in dogfish and limits have been established by Belgium and France in the range 50–100 mg/100 g fish. Reagents for the enzymatic test are available commercially in kit form. The procedure is based on the reductive amination of α-ketoglutarate by glutamate dehydrogenase and reduced nicotinamide adenine dinucleotide. The reaction is dependent on the ammonium ion. In the procedure, fish are homogenized with water and the mixture is filtered. An aliquot of the filtrate is added to a solution containing α-ketoglutarate and NADH followed by addition of L-glutamic dehydrogenase. Changes in ultraviolet absorption at 340 nm are used to quantitate ammonia, present in solution as the am-

monium ion, which is the limiting reactant in the procedure. The enzymatic procedure compared favorably with the AOAC colorimetric method, **18.027-18.030.** The new procedure requires fewer analytical steps and as a result is much faster. The Referee concurs with the recommendation of the Associate Referee that the enzymatic procedure be submitted to collaborative study.

Coprostanol.—Associate Referee James Stewart has reported on improvements to a gas chromatographic procedure for coprostanol in sea water. Coprostanol has been suggested as an indicator of fecal coliform contamination in growing areas used for producing oysters. The improved procedure eliminates the need for a thin layer chromatographic cleanup step previously used. The Referee concurs in the recommendation of the Associate Referee that the procedure be submitted to collaborative study.

Gas and Liquid Chromatography.—The gas chromatographic procedure (*J. Assoc. Off. Anal. Chem.* **64,** 584-591 (1981)) for the determination of cadaverine and putrescine has been submitted to collaborative study. The samples under analysis include yellowfin, skipjack and albacore tuna, shrimp, and dolphin (mahimahi). The first data received show good precision and accuracy. The GLC procedure is being applied in a separate study to develop a data base for the diamines in acceptable and decomposed quality canned tuna. Initial data from the study show that 93% of the cans of acceptable quality tuna contained less than 2 μg cadaverine/g sample, and 100% contained less than 2 μg putrescine/g sample. All cans of decomposed tuna contained >2 μg cadaverine/g; 67% contained the amine in the range 50–300 μg/g. Eighty-nine percent of the same cans contained in excess of 2 μg putrescine/g. The diamines are also produced during decomposition in shrimp, dolphin, mackerel, halibut, salmon, and bluefish. The Referee recommends that study be continued.

Shellfish.—Associate Referee T. L. Chambers reported on a modification to the official GLC method for the determination of indole in shrimp, **18.075-18.078,** and on the status of experiments testing the effects of cooking and chlorination on chemical indicators of decomposition in shrimp. When the current AOAC GLC method for indole was adopted in 1973, the primary indole level of interest was 50 μg/100 g sample for regulatory applications. During the past 2 years, applications at a level of 25 μg/100 g have increased and earlier proposals (*J. Assoc. Off. Anal. Chem.* **61,** 136-138 (1978)) to further strengthen the method took on added significance. The modifications included standardizing the activity of the silica gel cleanup column by drying 2 h at 125°C followed by equilibration with 3 g water/25 g silica gel. Also, concentrated ethyl acetate extracts were treated with anhydrous Na_2SO_4 before column cleanup, and the strength of the eluting solvent was increased to 15% diethyl ether in hexane. A reduced amount of internal standard, 2-methylindole, was used to improve peak measurements at the 25 μg/100 g level. Comparisons of the modified method with the AOAC method showed improved precision at all levels of indole with a coefficient of variation at 25 μg/100 g of 3.9. In terms of practical applications, the sample results (at a 95% confidence level) were the same for each sample pair by either the current AOAC method or the modified method. The modifications, however, add an increased measure of reliability and will make the AOAC method more rugged.

In the study on cooking and chlorination effects, the Associate Referee reported on changes which were monitored in samples of decomposed shrimp ranging in size from 40 count to 200 count. Although cooking did not influence indole levels, chlorination significantly reduced the amount of indole which had been present due to decomposition. The largest change resulted in a decrease from a level of 184 to 26 μg/100 g. Putrescine levels were not lowered by either cooking or chlorination.

The Referee concurs in the recommendations of the Associate Referee that the modifications listed above be incorporated into the AOAC GLC method for indole, **18.075-18.078,** and that the chlorination study be continued.

TLC Identification of Indole in Shrimp.—The status of this topic was affected significantly by the adoption at the 1980 annual meeting of the HPLC method for indole, **18.B01-18.B05.** In the HPLC method, no sample extract concentrations or cleanup steps are required and indole is quantitated from the filtered homogenate of shrimp. Any TLC procedure would require more extensive sample workup to permit quantitation of the required level of 25 μg/100 g. In discussions with Associate Referee T. R. Weber, it was determined the need is greater for a rapid screening test for a group of amines currently determined by several procedures, each requiring extract concentrations and cleanup steps before quantitation. The Referee therefore recommends that the topic be retitled TLC Identification of Amines in Fishery Products.

Although there were no progress reports on the topics Crabmeat, Diacetyl in Citrus Products, Ethanol in Seafoods, and Tomatoes, the Referee recommends that they be continued.

Recommendations

(1) Incorporate the modifications to the GLC method for the determination of indole, **18.075-18.078**.

(2) Adopt as official final action the HPLC method for the determination of indole, **18.B01-18.B05**.

(3) Change the topic TLC Identification of Indole in Shrimp to TLC Identification of Amines in Fishery Products.

(4) Discontinue the topics Amines in Seafoods; Apple Products; Eggs, Dried and Frozen; Fish Products; and Nuts, Rancidity.

(5) Continue study on all other topics.

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Report on Eggs and Egg Products

WALLACE S. BRAMMELL
Food and Drug Administration, Division of Color Technology, Washington, DC 20204

Color.—Marvin E. Winston reports that he is making a literature search for methods of analysis to detect the addition of artificial and other new types of colored compounds which might be added to eggs illegally to enhance a bright yellow appearance. He plans to obtain samples of various coloring compounds and then investigate the adequacy of methods for their detection in eggs.

Phosphorus.—Associate Referee Wallace S. Brammell has developed a spectrometric molybdovanadophosphate method for determining phosphorus content of eggs and egg products. It will be submitted soon for publication.

Sterols (Gas Chromatography).—Associate Referee Alan J. Sheppard reports that he has completed a comparison study of the gas chromatographic method for measuring egg cholesterol and an enzymatic cholesterol method. The enzymatic method gave 97.4 ± 1.6% recovery of cholesterol added to egg yolks. Determinations by the enzymatic method were in excellent agreement with those obtained by the gas chromatographic method.

No other reports were received.

Recommendation

Continue study on all topics.

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Report on Fish and Other Marine Products

LOUIS L. GERSHMAN
Food and Drug Administration, 585 Commercial St, Boston, MA 02109

Crabmeat Identification.—Associate Referee Judith Krzynowek continued her studies on this topic this year. The method she developed to determine the genus of cooked crabmeat was successfully collaborated last year and adopted official first action, **18.B06-18.B11.** She is now investigating the suitability of replacing the polyacrylamide gel with agarose gel as the medium for isoelectric focusing because of the known toxicity of acrylamide. Although the agarose medium shows promise, some difficulties were encountered; modifications in the extraction technique, pH gradients, stains, and focusing parameters have been made in the first action method to increase the reproducibility of the agarose gel patterns for cooked crabmeat obtained from different genera and species.

Drained Weight of Block Frozen, Raw, Peeled Shrimp.—Associate Referee Frederick King could not conduct any drained weight measurements because the needed samples were not received from the American Shrimp Canners and Producers Association. The Association was unable to obtain funds to purchase and transport the samples needed for the study.

Drip Fluid in Fish Fillets and Fish Fillet Blocks, Quantitation.—Associate Referee Frederick King is continuing to develop a procedure to measure the "cook drip" (fluid obtained from cooked samples) by using a modified tuna press and by using moisture content (AOAC **24.003(a)**). However, his progress has been slow because of personnel and budget limitations.

Fish Species Identification (Thin Layer Isoelectric Focusing).—Associate Referee Ronald Lundstrom conducted a collaborative study of agarose gel isoelectric focusing to determine whether unknown fish species can be identified by comparing the protein patterns with a photograph of protein patterns obtained from authentic species. Fourteen collaborators identified a total of 276 unknown samples with 84% accuracy. The results of this study showed more variability than those for the present official final action method, **18.A01-18.A04,** using polyacrylamide gel isoelectric focusing (*J. Assoc. Off. Anal. Chem.* **62,** 624-629 (1979)). In the latter method, 93% of the unknown species were correctly identified. Agarose gel isoelectric focusing has several distinct advantages over the polyacrylamide method, such as elimination of the toxicity hazard associated with the use of polyacrylamide, its speed, and its relatively low costs. The Associate Referee intends to evaluate different commercially available agarose-carrier ampholyte systems to determine which are most reproducible. He also intends to identify a suitable replacement for the discontinued Coomassie Blue R-250 stain to ensure reproducible staining results. Another collaborative study will be initiated when a method is devised, based on this additional work.

Net Weight of Glazed Shrimp.—Associate Referee Raymond Bradshaw was relocated in 1980 and has been unable to conduct any further work in this area. He has suggested that this topic be discontinued until work can be resumed again.

Nitrites in Smoked Fish.—Associate Referee Charles Cardile obtained results with a specific ion electrode method which compared favorably with results for the colorimetric method, **24.041-24.042.** The proposed method was tested on lyophilized samples of smoked fish with known amounts of added nitrite. Recoveries were in close agreement with those obtained using the official method. A collaborative study is planned for next year.

Recommendations

(1) Collaboratively study the agarose gel isoelectric focusing method investigated by the Associate Referee as an alternative to the official polyacrylamide gel isoelectric focusing method.

(2) Subject the proposed specific ion electrode method for nitrites in smoked fish to collaborative study.

(3) Discontinue the topic Net Weight of Glazed Shrimp.

(4) Continue study on all other topics.

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Report on Food Additives

THOMAS FAZIO
Food and Drug Administration, Division of Chemistry and Physics, Washington, DC 20204

Anticaking Agents.—The General Referee was unable to obtain an Associate Referee for this topic during the past year.

Antioxidants.—The General Referee is currently negotiating filling this position. A collaborative study has been conducted of a liquid chromatographic method developed by Dr. D. Page of Canada for determining 7 antioxidants in fats and oils. The results of his study were presented by Dr. Page at the AOAC meeting. Briefly, the data show that the method is capable of determining 7 antioxidants (BHA, BHT, PG, NDGA, THBP, TBHQ, and Ionox-100). Recoveries of these antioxidants added at 20–200 ppm to oil and lard ranged from 85 to 98%, and the coefficients of variation (CV) ranged from 4 to 9%. TBHQ was the only antioxidant with a CV as high as 23%. This was apparently due to problems with evaporation that some laboratories encountered, and has since been rectified. The study was successful and the method is recommended for official first action adoption.

Brominated Oils.—The General Referee was unable to obtain an Associate Referee during the past year to continue work on this topic.

Chloride Titrator.—A report was not received from the Associate Referee, Alfred H. Free.

Chlorobutanol in Milk.—A status report was not received from the Associate Referee.

Dichlorodifluoromethane in Frozen Foods.—Little progress has been made by the Associate Referee during the past year due to higher priority commitments.

Dilaurylthiodipropionate.—The General Referee was unable to obtain an Associate Referee during the past year.

Dimethylpolysiloxane.—A report was not received from the Associate Referee, R. Jameison.

Dressings.—Associate Referee Warner reports the development of a colorimetric procedure for determining emulsifiers in salad dressings based upon the complexation ability of the polyoxyethylene chains in ethoxylates. A report describing the method and findings, which has been accepted by the AOAC, includes a TLC system for semiquantitative confirmation of the colorimetric results. Recoveries were about 85% at the permitted level of 0.2%.

EDTA in Food Products.—The Associate Referee 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 has been initiated.

A limited collaborative study involving 4 laboratories has just been completed. Unfortunately, because of spoilage of the food samples, the recoveries were low. For crabmeat the recoveries at all 3 spiking levels tested ranged from 62 to 68%. The within-laboratory coefficient of variation (repeatability) was 7–10%. Among laboratories the coefficient of variation (reproducibility) was 9–13%. For mayonnaise the within-laboratory coefficient of variation was 3–5.5%. At the one-half tolerance and the tolerance levels the recoveries were 75% and 69%, respectively. Among laboratories the coefficient of variation was about 28%. At the twice tolerance level, recovery averaged 88% and the coefficient of variation among laboratories was 4%. There were no outlier laboratories according to the Youden Rank Sum test. In the more extensive collaborative study to be initiated, more care will be taken to prevent spoilage of the samples. Also, calibration will be done by the method of standard addition to negate the effects of any spoilage that does occur.

Ethoxyquin in Foods.—The Associate Referee, Gracia Perfetti, reported that a manuscript describing the determination of ethoxyquin in spices by HPLC with ultraviolet detection has been published (*J. Assoc. Off. Anal. Chem.* (1981) **64**, 1453–1456).

Work has continued to adapt the basic method (with a change to fluorescence detection) for the determination of ethoxyquin in milk and eggs at levels below 1 ppm.

To date a sample cleanup procedure has been developed for ethoxyquin in milk at the 10 ppb level. Recovery studies from fortified samples

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This report of the General Referee was presented at the 95th Annual Meeting of the AOAC, Oct. 19–22, 1981, at Washington, DC.

The recommendations of the General Referee were approved by Committee C with the exception of Recommendation 1; the Committee recommended continued study with a view to interim first action adoption. The recommendations, with this exception, were adopted by the Association. See the report of the committee for detailed recommendations, this issue.

averaged 90%. The chromatograms are free of interferences. Some problems were encountered with changing peak heights, but have been resolved by sparging the mobile phase with helium and by pumping large volumes of mobile phase through the column before injecting samples.

Gums.—The General Referee has been unsuccessful in finding an Associate Referee for this topic during the past year.

Indirect Additives from Food Packaging.—The Associate Referee reports the following progress:

Vinyl chloride: Work has continued in ASTM Committee D-20 on Plastics on the development of a rapid, quality control (QC) method to determine residual vinyl chloride in PVC at the ~5 ppb level. A round-robin study was carried out using several new, industry-developed methods. Three of these methods (Dow, Union Carbide, and Ethyl) showed potential as QC methods. The major difference between them was the GC detector used: FID, Hall electro-conductivity, and PID, respectively. As a result, a draft of a new ASTM method has been written in which the analyst is given a choice of detectors depending upon his particular sample matrix and equipment. The draft of the method is currently undergoing review by the method development task group. After review, a second draft will be prepared and a round-robin evaluation will be made of the method.

Plasticizers in food grade PVC: A procedure was developed to isolate and identify various plasticizers in PVC meat wrap and produce wrap. The procedure involves extraction of the wraps with toluene, isolation of the plasticizer fraction of the extract with size exclusion chromatography, and determination of the plasticizers in the plasticizer fraction by capillary column gas chromatography. GC/MS and NMR were used to confirm the identity of the plasticizers found. Two surveys were conducted of locally purchased hamburger and produce wraps. Plasticizers found include di-2-ethyl hexyl adipate, di-2-ethyl hexyl phthalate, linear di-C_7-C_9 mixed adipate, and some 2-methyl alkyl adipates.

Acrylonitrile: A new method has been developed to determine acrylonitrile in 3% acetic acid extracts down to a level of ~50 parts per trillion. The method utilizes headspace analysis of an enclosed sample of acetic acid extract and Na_2SO_4. A manuscript describing the method has been accepted for publication in *J. Assoc. Off. Anal. Chem.* and was presented at the 95th Annual Meeting.

Terephthalic acid: Method development is underway for the determination of residual terephthalic acid in PET beverage bottles. The approach utilizes derivatization of the terephthalic acid followed by GC analysis. More details will be made known as the method is developed.

Styrene: A method has been developed to determine styrene in margarine. The styrene is azeotropically distilled out of the margarine and the distillate is analyzed by GC. A survey of locally purchased margarines is planned, as well as publication of the method and results.

Toluene diamines: An HPLC method has been developed to determine 2,4- and 2,6-toluene diamines in retort pouch and boil-in-bag extracts at the 0.1 ppb level. These amines are confirmed by either (a) GC analysis of the isolated HPLC peaks using a nitrogen selective detector, (b) treatment of the isolated HPLC peaks with trifluoroacetic anhydride and determination of the trifluoro acetyl derivatives with GC and a nitrogen selective detector, or (c) GC/MS analysis of the trifluoro acetyl derivative. Several retort pouches and boil-in-bag containers have been analyzed. A manuscript describing the HPLC determinative method has been submitted for publication and a manuscript is in progress describing the confirmation procedures. An ASTM-sponsored round-robin evaluation of the HPLC method is in progress.

Volatiles: Work continues on a dual capillary column GC method to determine volatiles in foods and food packaging. Retention indexes are being determined for a large number of compounds. A tabulation of these indexes will be made available when the work is completed.

ASTM is planning a round-robin study to evaluate a headspace technique to determine residual solvents in food packaging films.

Hydrogen peroxide (H_2O_2): Several methods to determine H_2O_2 in distilled water extracts were evaluated for their usefulness in enforcing a 0.1 ppm limit for residual H_2O_2 in aseptic packaging. Two colorimetric methods, several polarographic methods, and a potentiometric titration method were studied. The titration method and a polarographic method, both by Boto and Williams (*Anal. Chim. Acta* (1976) 85, 179–183), were found suitable.

Total nonvolatile residues: An ASTM subcommittee studying retort pouches has been asked to consider a round-robin study of FDA's total nonvolatile residue method. It is hoped that this activity can be initiated in the near future.

Mineral Oil in Raisins.—A report was not received from the Associate Referee.

Nitrates (Selective Ion Electrodes).—A collaborative study has been completed for nitrate determination in food using a nitrate ion selective electrode. The data were statistically evaluated by the Associate Referee and did not warrant adoption of the method; in fact, the Associate Referee considers it not satisfactory for general use.

The General Referee also had the data statistically evaluated (as shown in the table below) and concluded the following:

1. Laboratories No. 2 and 7 throughout seem to be distorting the results, one being consistently low, the other consistently high. Unfortunately, rejection of their results means rejection of 20% of the data points. No matter how good the new CVs become, any method that generates such a high rejection rate needs to be re-examined. A simple ruggedness test may serve to identify the critical factors that need to be monitored and controlled more closely.

2. Huge biases exist between the laboratories, especially in measuring nitrates in carrots. The differences between laboratory average (with perhaps one exception) are much greater than can be accounted for by random fluctuations. It is almost as if the laboratories calibrated differently. Accuracy and precision are both poor, and revision is needed.

Results[a] of Collaborative Tests

	Carrots		*Ham*	
	Sample 1		Sample 4	
Av. Concn	122.70	118.06	120.22	104.29
CV_o, %	9.3	3.7	48.4	40.6
CV_x, %	24.4	9.5	70.6	53.6
	Sample 2		Sample 5	
Av. Concn	221.65	216.81	169.39	146.07
CV_o, %	9.8	2.7	47.4	13.1
CV_x, %	23.0	14.9	69.5	43.0
	Sample 3		Sample 6	
Av. Concn	311.00	293.31	220.06	204.43
CV_o, %	7.9	6.7	21.7	19.2
CV_x, %	30.7	17.6	42.3	27.0

[a] All readings retained except for Laboratory 5, Ham.

Nitrates and Nitrites.—The General Referee is currently negotiating with USDA to appoint Dr. Jay Fox as the Associate Referee for this topic.

Nitrosamines.—The General Referee reports excellent progress in this research area. A collaborative study has been conducted and several new methods have been developed during the past year. The results of these efforts are summarized below.

1. "Determination of *N*-Nitrosodimethylamine in Beer: An International Collaborative Study" by Sen and Seaman: The method for determining *N*-nitrosodimethylamine (NDMA) in beer reported by the Associate Referee at the 1980 Annual Meeting of the AOAC was studied collaboratively. Thirteen laboratories from 7 countries participated. The collaborators were asked to analyze a total of 10 randomly labeled samples of beer which consisted of the following duplicates: a naturally contaminated commercial beer; a beer extremely low (about 0.1 ppb) in NDMA; and the above low NDMA beer spiked with 0.5 ppb, 1.9 ppb, and 5.0 ppb NDMA. The pooled repeatability and reproducibility coefficients of variation (CV) for all samples were 17 and 27%, respectively. However, when the data from 2 laboratories (outliers) were omitted, the corresponding CV improved considerably (11 and 15%, respectively). Variance analysis showed the presence of a significant laboratory-sample interaction when all the data from the 2 outlying laboratories were excluded. The pooled percent recovery of the overall method (omitting the outliers) was found to be 101.4 ± 3.5. All the laboratories detected NDMA in the low DMA beer. It is recommended that the method be adopted official first action.

2. "An Investigation into the Possible Presence of Volatile *N*-Nitrosamines in Cooking Oils, Margarine, and Butter" by Sen and Seaman: Following a report of the occurrence of volatile nitrosamines in various vegetable oils and margarines in Germany, a study was carried out to determine the nitrosamine levels in such products sold in retail outlets in Canada. Thirty-eight samples of various vegetable oils, 10 of butter, 14 of margarine, and 6 of lard were analyzed. All the oils and lard were negative. Only 1 butter and 5 margarine samples contained trace levels (0.2–3.8 ppb) of *N*-nitrosodimethylamine and/or *N*-nitrosomorpholine. Further investigations at the plant level failed to uncover any definite source of the nitrosamine contamination detected in some of the margarine samples. Recent samples of margarine from these plants, however, either were negative or contained insignificant levels of volatile nitrosamines. It was concluded that nitrosamine levels in these products are either negative or negligible, and therefore should not be a matter of concern.

3. "A Rapid Liquid-Liquid Extraction Cleanup Method for the Determination of Volatile *N*-Nitrosamines in Cooked-out Bacon Fat" by Sen

and Seaman: A rapid liquid-liquid extraction method is described for the determination of volatile nitrosamines in cooked-out bacon fat. The method consists of partitioning of the nitrosamines between *n*-hexane and an acidic aqueous-methanol mixture containing small amounts of sulfamic acid. An aliquot of the aqueous phase is then extracted with dichloromethane; the dichloromethane extract is concentrated, and an aliquot of the concentrated extract is analyzed by GLC-thermal energy analyzer. The technique is very simple and rapid, and does not involve any distillation. The method gives results comparable to those obtained by an alkaline vacuum distillation method. The average percentage recoveries of *N*-nitrosodimethylamine, *N*-nitrosodiethylamine, *N*-nitrosopiperidine, *N*-nitrosopyrrolidine, and *N*-nitrosomorpholine when added to cooked-out bacon fat or lard at levels ranging between 5 and 20 ppb were 78.8, 77.8, 89.4, 100.3, and 97.4, respectively. The method uses *N*-nitrosazetidine as an internal standard and has an overall detection limit of 1 ppb for each of the above 5 nitrosamines. The average levels (uncorrected) of NDMA and NPYR detected in the 11 samples of cooked-out bacon fat were found to be 4.8 ppb and 21.1 ppb, respectively. These values are comparable to those observed in an earlier survey carried out during 1978 which indicated no change in nitrosamine levels.

4. "A Rapid Method for the Determination of Volatile Nitrosamines in Nonfat Dry Milk" by Havery, Hotchkiss, and Fazio: A rapid method has been developed for the analysis of volatile nitrosamines in nonfat dry milk. Nitrosodimethylamine added to nonfat dry milk at the 3 ppb level was recovered at an average of 96%. In a nationwide survey of nonfat dry milk, nitrosodimethylamine was found in 48 of 57 samples at an average level of 0.6 ppb. The presence of nitrosopyrrolidine and nitrosopiperidine was also indicated by gas chromatography-thermal energy analysis at average levels of approximately 0.1 ppb.

5. "The Determination and Conformation of *N*-Nitrosodimethylamine in Beer" by Andrzejewski, Havery, and Fazio: A method was developed for the confirmation of *N*-nitrosodimethylamine (NDMA) in beer by capillary gas chromatography-mass spectrometry (GC/MS) from full mass scans after quantitation by a gas chromatograph interfaced to a thermal energy analyzer (GC/TEA). The GC/MS method has a lower limit of confirmation of 5 ppb. In a survey of 22 imported and 42 domestic (U.S.) beers, NDMA was found by GC/TEA at levels ranging from below the sensitivity of the method to 7.7 ppb, and was confirmed by GC/MS in 5 samples.

Polycyclic Aromatic Hydrocarbons in Foods.—The Associate Referee reports that 2 methods have been developed for the analysis of food matrices for PAH content. The first involves the use of ultrasonics to facilitate extraction of PAH from ground-up barley samples. A time evaluation study of the procedure indicates that 4 samples can be completed in 2 days. Average recoveries of 11 PAH, including benzo(a)pyrene, benzo(b)-fluoranthene, indeno(1,2,3-c,d)pyrene, and benz(a) anthracene, added to 25 g samples at 2.5 and 5 ppb, ranged from 78 to 97% with a mean relative standard deviation of 6.6%. These studies indicated that the lower limit of reliable measurement was 2.5 ppb.

Thirty food samples were analyzed by this procedure. Apparent trace levels of benzo(a)-pyrene ($\leq$1 ppb) were found in 18 samples.

The second method is for the analysis of smoked foods. The procedure involves alcoholic-KOH saponification of the food product, dilutions of the resulting solution with water, extraction of the PAH into trichlorotrifluoroethane (TCTFE), column chromatography, liquid-liquid partitioning, concentration, and HPLC and/or GC analysis. A time study of this procedure indicates that 4 samples can be completely analyzed in 2 days.

Reproducibility studies of recovery data were conducted on blank solutions spiked in triplicate at 5 ppb with 11 PAH. Based on fluorescence data obtained at an excitation wavelength of 333 nm with a 370 nm emission cutoff filter, the recovery values ranged from 76 to 96% with a mean value of 90% and a CV of 6%.

Nineteen smoked food samples have been analyzed to date. The noncarcinogenic PAH, fluoranthene and pyrene, were found in all the samples analyzed at levels ranging from 0.2 to 4 ppb. Apparent benzo(a)pyrene (carcinogenic) was found in 7 samples at levels ranging from 0.1 to 2.3 ppb. Trace levels of other carcinogenic PAH, including benzo(k)fluoranthene and indeno(1,2,3-c,d)pyrene, were also apparent in several of the samples analyzed. This study is continuing.

Polysorbates.—See Dressings.

Propylene Chlorohydrin.—A report was not received from the Associate Referee.

Sodium Lauryl Sulfate.—The Associate Referee reports no progress due to other higher priority commitments.

General Referee Statement

Research Efforts

The General Referee has continually stressed to the Associate Referees the necessity and importance of active participation on their respective program areas. During the past year the General Referee has attempted to stimulate research efforts with considerable success as evidenced by the numerous papers submitted and the collaborative studies scheduled and completed.

Food Additive Analytical Manual (FAAM)

The revision of the FDA's *Food Additive Analytical Manual* (FAAM) is nearing completion of a significant milestone. Soon the first volume will be available (it is hoped by the end of the calendar year). This volume will consist of a series of monographs, each of which summarizes the regulatory status of the additive of interest and provides a step-by-step description of a method for the determination of this substance in at least 1 food matrix. As presently envisioned, the first volume will include 9 antioxidants, 6 preservatives, 7 indirect food additives, 2 emulsifiers, 8 miscellaneous additives, and approximately 15 substances which are found as contaminants in direct food additives or which could be found in food because of chemical processing or modification of food substances.

Recommendations

(1) Adopt the method for antioxidants in foods reported by D. Page as official first action; appoint an Associate Referee; continue study.

(2) Adopt the method for *N*-nitrosodimethylamine in beer, collaboratively studied by the Associate Referee as Method 1, official first action; continue study.

(3) Appoint Associate Referees on Anticaking Agents in Foods, Brominated Oils, Dilaurylthiodipropionate, Gums in Foods, and Nitrates and Nitrites.

(4) Continue study of all other topics.

Report on Meat and Meat Products

RICHARD L. ELLIS
U.S. Department of Agriculture, Food Safety and Inspection Service, Chemistry Division, Washington, DC 20250

Activity and progress on topics has been variable. The topics Automated Methods; Nitrates and Nitrites; Nitrosamines in Bacon and Fat; and Moisture Analysis, Rapid Methods have been quite active in method development activities and collaborative studies. Other topics have been relatively inactive.

Interest in nutritional analysis and food safety suggests additional topics for study. The U.S. Department of Agriculture (USDA), Food Safety and Inspection Service (FSIS), has taken new and renewed action on sodium and mechanically processed products, resulting in new programs in the Agency. Activities in several new topics relating to these agency needs should be established under the General Referee for Meat and Meat Products.

Presently, 4 topics are associated with nonmeat protein analysis: Nonmeat Protein in Meat, Peanut and Cottonseed Proteins in Meat Products, Sodium Caseinate in Meat Products, and Soy Products in Meat Products. These should be combined and an Associate Referee Committee should be established on nonmeat protein analysis and determination to coordinate activities in this area. This could improve effectiveness and avoid duplication of effort. Current Associate Referees should be members of this committee. Associate Referee J. D. Pettinati has tentatively offered to chair the committee, if the recommendation is adopted.

The second topic recommended for this refereeship is Total Fat in Meat Products, to address 2 areas: the pending adoption of the protein fat-free approach as a regulatory device for controlling product quality, and concerns with the final drying time and temperature in AOAC method **24.005**.

The increased emphasis on sodium consumption and the need for monitoring meat products

for sodium content has created a need for a collaboratively studied method. The new topic Sodium and Potassium in Meat Food Products should be initiated.

The rapid advancement in developing new analytical instrumentation for cations and anions by specific ion electrodes, and their application for determining sodium, potassium, nitrite, fluoride, and others should be evaluated. Therefore, I am recommending initiation of the topic Specific Ion Application to Meat Product Analysis.

On methods requiring AOAC action, 2 recommendations are made. On proteins in meat, **24.027-24.037**, no adverse reports have been received; however, the Associate Referee has recommended that the method be continued as official first action for an additional year and I concur. Second, I recommend that the interim mineral oil-vacuum distillation thermal energy analyzer (TEA) method to determine volatile nitrosamines in cooked bacon be adopted official first action.

Ashing Methods.—A vacancy still exists for an Associate Referee. The topic should be continued and more efforts should be made to fill that vacancy.

Automated Methods.—Associate Referee Jon L. Schermerhorn has completed mini-studies evaluating the block digestor for use in automated methods. He recommends a collaborative study and the Referee concurs.

Bone Content.—A new Associate Referee is needed to fill the vacancy created by the reassignment of Larry Menke. The Referee recommends Paul Corrao, USDA, FSIS, Science Program, Chemistry Division, who is planning a collaborative study on calcium analysis.

Chlorinated Hydrocarbons in Poultry.—High interest for rapid chlorinated hydrocarbon analyses continues. Although no report has been received on this topic, the Referee recommends this topic be continued for at least one more year.

Fat and Moisture Analysis, Rapid Methods.—Associate Referee J. D. Pettinati reports that a comparative study of meat samples prepared for analysis in a food cutter and a chopper was completed (results have been presented at the annual meeting by S. A. Akerman). Further, a collaborative study to determine moisture in meat and meat products with a microwave oven is in progress.

Fluoride in Deboned Meat and Poultry.—Associate Referee Thomas Dolan was unable to complete any additional work in this topic. However, R. W. Dabeka and A. D. McKenzie reported at the 1981 meeting on a collaborative study of fluoride in infant foods by a combination micro diffusion-fluoride specific ion electrode method (*J. Assoc. Off. Anal. Chem.* **64**, 1021-1026 (1981)). It is recommended that extension of this method to deboned meat and poultry be studied.

Identification of Meats, Serological Tests.—No report has been received this year. In light of the recent incident on imported meat, this topic should be continued. It is reasonable to assume that USDA, FSIS will increase emphasis on this topic in 1982.

Nitrates and Nitrites.—No report has been received. Emphasis on this topic continues, however, and study is included in USDA, FSIS program plans. The Referee recommends that F. B. Suhre be appointed Associate Referee on this topic. He currently is investigating ion chromatographic techniques for quantitating these anions.

Nitrosamines in Bacon.—The Referee recommends this topic be continued under Mr. Greenfield. Further, the collaborative study presented by Greenfield, A. J. Malanoski, and W. L. Smith on the mineral oil distillation TEA procedure should be adopted official first action.

Nonmeat Proteins in Meat.—J. D. Pettinati should be appointed Associate Referee on this topic and act as chairperson for topics associated with nonmeat protein analysis.

Peanut and Cottonseed Proteins in Meat Products.—No report has been received. This topic should be continued, however, because of the interest in nonmeat protein analysis. A review of the present Associate Referee's assignment is recommended.

Protein in Meat.-Associate Referee F. B. Suhre recommends that the block digestion-steam distillation method for protein analysis, **24.B01-24.B03**, continue as official first action for an additional year, although no adverse reports have been received; the Referee concurs.

Proximate Composition Relationships.—This topic should be continued with A. J. Malanoski appointed Associate Referee.

Sodium Caseinate in Meat Products.—No report has been received. This topic should be discontinued as a separate topic and incorporated under nonmeat proteins in meat.

Soy Proteins in Meat Products.—No report has been received. This topic should be continued because of interest in nonmeat protein analysis. The current Associate Referee's assignment should be reviewed.

Sugars and Sugar Alcohol.—This topic should be continued because of high interest in the nutrition and food safety area. A new Associate Referee engaged in this area of research and method development should be appointed.

Temperature, Minimum Processing.—Associate Referee J. D. Pettinati has been reassigned and the topic has been inactive. This topic should be reviewed, and if no interest is expressed, it should be deleted.

Recommendations

(1) Maintain official first action status for crude protein methods, **24.027-24.037**.

(2) Adopt as official first action the mineral oil distillation-TEA method for the determination of nitrosamines in bacon.

(3) Conduct a collaborative study on bone content.

(4) Initiate the following topics: Sodium and Potassium in Meat Products; Fat in Meat Products; Specific Ion Electrode Applications to Meat Products.

(5) Discontinue the topic Sodium Caseinate in Meat Products.

(6) Continue study on all other topics.

This report of the General Referee was presented at the 95th Annual Meeting of the AOAC, Oct. 19-22, 1981, 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 for detailed recommendations, this issue.

Section numbers refer to *Official Methods of Analysis* (1980) 13th Ed., and "Changes in Methods," *J. Assoc. Off. Anal. Chem.* (1981) **64**, 501-540.

Report on Mycotoxins

LEONARD STOLOFF
Food and Drug Administration, Division of Chemistry and Physics, Washington, DC 20204

The papers on mycotoxins offered for presentation at the 1981 annual session of the AOAC continue the trend observed over the last several years toward increased use of high performance liquid chromatography (HPLC) for the ultimate separation and quantitation of the analyte being sought. There is no doubt that HPLC is a powerful separation and measurement tool, the use of which is worth exploring, but at the current state of instrumentation, it is also an expensive tool. What is the implication of this situation for the future development and application of analytical methods for mycotoxins?

When the development of methods for aflatoxins started about 1963, the upcoming separation tool was thin layer chromatography (TLC). TLC was the natural successor to paper chromatography, and because of marked advantages in speed, equipment costs, space requirements, and resolution, it rapidly made paper chromatography obsolete. (It is difficult to conceive at this time that the first separation of aflatoxin from toxic peanut meal was made by paper chromatography (1).) The developers of methods for aflatoxins, and subsequently other mycotoxins such as sterigmatocystin, ochratoxin, patulin, and zearalenone, naturally utilized TLC as the preferred separation tool. The simplicity of the technique produced a side effect that could have been anticipated. There seemed to be no upper limit to the number of laboratories that set up to perform aflatoxin analyses, and no lower limit to their sophistication. One result was an explosion in production of information about aflatoxins, fired, obviously, by public health interests and ready availability of reference standards.

Returning to the original question, will the cost of equipment limit the application of the methods now being developed with an HPLC step? I would predict that it will. I expect that fewer laboratories that perform aflatoxin assays will be equipped to use the methods, and there will be fewer possible analyses within any one laboratory because of competition for time on a popular, effective separation tool. The remedy is in the hands of the equipment manufacturers. Now is the time for the stripped-down models that any laboratory can afford.

This report of the General Referee was presented at the 95th Annual Meeting of the AOAC, Oct. 19-22, 1981, at Washington, DC.

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

Section numbers refer to *Official Methods of Analysis* (1980) 13th Ed. and "Changes in Methods," *J. Assoc. Off. Anal. Chem.* (1980) 63, 374-423.

Let us examine to what extent the Associate Referees are following the trend.

Aflatoxin M.—Robert D. Stubblefield (U.S. Department of Agriculture (USDA), NRRC, Peoria, IL) reported on a repeat of the international collaborative study of a method for aflatoxins B_1 and M_1 in liver. This time the method was revised (2) to eliminate early elution problems from the silica gel cleanup column, and to include the use of coded spiking solutions to be added to fresh liver to avoid the development of interferences encountered in lyophilized liver. The study included 2 methods for confirmation of aflatoxin identity (**26.A15;** 3). Almost all the collaborators in the original study participated in the second study; analytical results were received from 16 laboratories. The recovery and precision values calculated from these results were an improvement over those from the initial study, and fell in a range that warranted a recommendation that the method be adopted official first action. The overspot and spray techniques for forming the acid-catalyzed identifying derivatives of aflatoxins B_1 and M_1 were equally effective. By either technique the few false negative observations were at the lowest aflatoxin levels, which approach the limit of determination, and the low incidence of false positives occurred most frequently with aflatoxin M_1, which was the more difficult of the 2 aflatoxins to separate from interferences. Both procedures for confirmation of identity were therefore included in the recommendation for adoption.

The method proposed for adoption has been used for a study (4) of the distribution of aflatoxins B_1, B_2, G_1, G_2, and M_1 in the tissues of a 300-pound steer given aflatoxins added to its normal ration. All aflatoxins detectable by the method were found in liver, kidney, spleen, heart, and loin muscle. Except for the kidney, B_1 levels exceeded M_1 levels; the M_1 level in the kidneys exceeded the B_1 level and the level of M_1 in any other tissue including the liver. Analyses of 14 separate liver sections showed generally uniform distribution except for the edge samples, which averaged 44% lower than the remainder of the lobe samples, supporting a prior report (5) that wedge cuts from a liver were representative of the total liver for aflatoxin levels.

The Associate Referee has also been working on modifications of the method for determination of aflatoxin M in fluid milk. One apparent improvement is in the extraction step. The modification, which is intended to eliminate emulsion problems, involves the use of a solid matrix, as described by Fukayama et al. (6), for liquid/liquid partition of the aflatoxins between extracting solvent and milk. The Associate Referee has converted completely to this procedure in his own laboratory and will recommend that it be made part of the official method if his observations are confirmed by other laboratories. He has also been comparing various proposed short methods using HPLC (7, 8; H. Chang, personal communication), and two screening methods using minicolumns (9, 10). Results with the HPLC methods are encouraging and warrant further testing toward eventual collaborative study of a selected HPLC method. The minicolumn screening methods require further modification, and work to this end is continuing with their originators.

Work has also continued on anomalies observed in the quantitation of alfatoxin B_1 and M_1 spots on 2-dimensional TLC plates. Because the reference standard on a 2-dimensional plate is developed in only one direction with only one of the solvents used for the 2 developments of the extract spot, the compared spots are theoretically subjected to different influences on their fluorescence intensity, and therefore are not strictly comparable. Preliminary experiments confirmed the theory. By selection of the order of developing solvents used and the dimension for the reference standard, the difference can be reduced to within the error of measurement, as was done with the method for aflatoxins B_1 and M_1 in liver. Because the effect is probably related more to the developing solvents than to the extracts involved, the observations are probably pertinent to other 2-dimensional TLC methods, but should be confirmed in each situation.

The Associate Referee calls attention to a report (11) of an aflatoxin M_1 artifact appearing in milk of cows on feed containing citrus pulp. Although separable from aflatoxin M_1 by TLC, the artifact migrates with M_1 on HPLC; nor does the confirming derivative separate from the modified artifact on HPLC, although the compounds do separate on TLC. In this situation, one separation technique should be used to support the other.

The application of all this effort to develop a reliable method for aflatoxin M was tested in a check sample study conducted by the International Agency for Research on Cancer (IARC). The material distributed for test was naturally contaminated lyophilized cow's milk containing aflatoxin M_1 at a mean determined level in the milk solids of 7.4 ng/g. The best reproducibility (19%) was attained by laboratories using HPLC detection, followed by those using AOAC

Method I (**26.A10-26.A14**) (35%); the overall reproducibility of the 80 laboratories that reported their analyses was 48%. Those laboratories that used reference standards supplied by IARC showed better between-laboratory precision than did those laboratories that used their own standards, a sad commentary on the quality of reference standard maintenance that has been repeated in other check sample studies.

The Associate Referee recommends that the Stubblefield and Shotwell method (2) for the determination of aflatoxins B_1 and M_1 in liver and the methods of the AOAC (**26.A15**) and of van Egmond and Stubblefield (3) for confirmation of identity be adopted as official first action; that all first action methods for aflatoxin M analysis and confirmation of identity (**26.A10-26.A14, 26.090-26.094**) be retained in first action status; that the partition column technique of Fukayama et al. (6) be tested in a number of other laboratories before submission for incorporation in current methods, and that work continue on the evaluation of HPLC and screening methods. The Referee concurs.

Aflatoxin Methods.—Alfred D. Campbell (FDA, Washington, DC) reports that all results have been received from a collaborative study of 2 methods for aflatoxin in peanut butter in which HPLC is used for the determinative step, but that compilation and statistical analysis of the data could not be completed in time for a report at this meeting. He recommends that the statistical part of the study be completed in time for an interim report and that all current methods for aflatoxins in peanut products (**26.026-26.031, 26.032-26.036**) be retained in current status. The Referee concurs.

Alternaria Toxins.—A. Douglas King, Jr. (USDA, Western Regional Research Center, Albany, CA) reports that his comprehensive review of *Alternaria* toxins and their relation to foods, presented in part in the 1980 report, is almost completed. In addition he has accumulated a good supply of copper tenuazonate in preparation for further work on a method for tenuazonic acid in which HPLC is used for the determination step.

The Associate Referee also calls attention to a number of reports that have appeared in the past year. One (12) is publication of the paper noted last year as an American Chemical Society meeting abstract reporting the detection of alternariol, alternariol methyl ether, altenuene, tenuazonic acid, and altertoxin I in naturally infected apples and, with the exception of altertoxin I, in tomatoes. All the preceding toxins were detected in oranges and lemons inoculated with *Alternaria citrii*. The same authors have followed up with a study (13) of the stability of these toxins in various fruit juices and whole wheat flour during storage, following pasteurization or sulfur dioxide treatment. Another paper (14) describes a procedure for isolation of large quantities of alternariol, alternariol methyl ether, and altenuene in which preparatory HPLC is used. A third report (15) provides information on the ability of *Alternaria alternata* culture extracts to elicit mutations in the Ames *Salmonella* system. Of the pure compounds tested, only alternariol methyl ether showed any mutagenic activity. This observation is supported by an independent unpublished report (R. Heimsch, University of Idaho, personal communication).

The Associate Referee recommends continued work toward a determination of the extent of the natural presence of toxins in fruits and vegetables subject to invasion by *Alternaria spp.* and as an approach toward a better understanding of that determination, further work on the stability of the known toxins both in the foods and as isolated entities. The Referee concurs.

Citrinin.—David M. Wilson (University of Georgia, Coastal Plains Station, Tifton, GA) reports that attempts to improve citrinin extraction by addition of chelating agents to the solvent systems, including salts of ethylenediamine tetraacetic acid (EDTA), have been ineffective. He is watching citrinin extraction studies at the FDA New Orleans Research Center, using ring-labelled citrinin, that are aimed at locating the unextractable portion. The Associate Referee recommends continuation of the extraction studies, and the Referee concurs.

Ergot Alkaloids.—Colette P. Levi (General Foods Corp., White Plains, NY) calls attention to 3 important papers published within the past year by Canadian laboratories. One is the first published analysis for ergot alkaloids in milled grain (16); HPLC with a fluorescence detector is used for the determinative step. Analyses for ergometrine, ergotamine, α-ergokryptine, ergocristine, ergosine, and ergocornine could be performed with no difficulty in the ng/g range. All these alkaloids could be detected in one or more of 2 samples of rye flour and 12 samples of wheat flour from commercial markets. The highest alkaloid level determined was in a rye flour which contained ergocristine at 62 ng/g and ergotamine at 37 ng/g, plus lesser quantities of each of the other alkaloids. Another laboratory, using the same basic detection technique, has explored the variability in alkaloid content and composition of ergots from rye (17), and from

wheat (18). These reports provide the Associate Referee with an excellent base from which to start her own work toward a method suitable for collaborative study. She recommends continued effort toward this end, and the Referee concurs.

Grains.—Odette L. Shotwell (USDA, NRRC, Peoria, IL) reports that she has continued the investigations of farm worker exposure to dust-borne aflatoxin in cooperation with William R. Burg, University of Cincinnati (19, 20). Samples from 3 farms of airborne dust generated by combines during harvest contained aflatoxin at levels generally related to the levels in the bulk corn samples taken from those farms. The highest level of total aflatoxins in the dust was 113 ng/g, related to 406 ng/g in the bulk corn. Aflatoxin was also found in settled dust (222 ng/g) collected at an elevator, and in dust generated during corn delivery (80 ng/g) and corn loading (340 ng/g). Significant aflatoxin levels were found in all the associated bulk corn.

Because a question had been raised as to whether aflatoxin levels in some corn shipped to the laboratory as undried ears were representative of field contamination, comparisons were made of dried and undried ears shipped from Georgia to Peoria (21). Additional observations were made of ears held for up to 6 weeks after arrival in Peoria, and of ears shipped and stored in a container with added "Monoprop" (2.5% propionic acid on vermiculite). There was no difference in incidence or level of aflatoxin contamination between dried and undried ears on arrival at Peoria; the average level had approximately doubled, but the incidence of contaminated ears was essentially unchanged. In the presence of "Monoprop" the incidence was also unchanged, but the average level had dropped significantly; the acidity was apparently sufficient to result in some aflatoxin alteration (22).

A 5-year study has been completed (23) of testing for aflatoxin, zearalenone, and ochratoxin A in samples of corn and wheat collected by the Federal Grain Inspection Service from trucks moving to elevators in the state of Virginia in the 5-year period 1976–1980. This was a followup to finding zearalenone at relatively high levels in 19 of 42 samples of Virginia wheat in 1975 (24). None of the mycotoxins was found in any sample of wheat taken during the subsequent 5 years, nor was zearalenone or ochratoxin A found in any of the corn samples, but aflatoxin was found in some corn every year; the yearly incidence of samples with levels exceeding 20 ng/g ranged from 18 to 62%.

Two variants of currently proposed analytical methodology for aflatoxins have been successfully applied to corn: in one (25) a small column of microparticulate silica gel (Sep-Pak®) is used for cleanup followed by either TLC or HPLC for determinative separation; in the other (26) methylene chloride is substituted for chloroform in the CB method and the 2 g silica gel column (**26.A05**) of the cottonseed method for the 10 g CB column. Both methods appear worth evaluating.

The Associate Referee recommends further study of method modifications and of procedures for prevention of mycotoxin formation in grain samples after collection. The Referee concurs.

Mixed Feeds.—Thomas R. Romer (Ralston Purina Co., St. Louis, MO) reports that he has continued the successful application to the determination of aflatoxin in mixed feeds of the system in which small reverse phase columns of silanized silica gel are used for cleanup of extracts prepared by the official minicolumn method (**26.014–26.019**). Extracts so prepared have been suitable for determinative separation by either TLC or HPLC. He has also been active in improving the method for deoxynivalenol in grains, first presented at the 1979 annual meeting of the AOAC (Paper No. 179), and further modified by Scott et al. (27). The Associate Referee recommends collaborative study of a selected method for aflatoxin in mixed feeds and continued study of the methods for trichothecenes in feeds. The Referee concurs.

Ochratoxin.—Stanley Nesheim (FDA, Washington, DC) reports that he has been unable to carry out sufficient work himself to make a choice among the various methods so far proposed for the determination of ochratoxin A in grains. From conversations with analysts in other laboratories, the preference appears to be for the method described by Thorpe et al. (28). Application of this method to grain and feed samples by the FDA Mycotoxins Analytical Laboratory in New Orleans has produced minor problems with recovery, emulsions, and filtration rate that have been overcome by using a Polytron instead of a Waring Blendor for mixing the sample and extracting solvent and by centrifuging this mixture before filtration. The Associate Referee now recommends that the modified Thorpe method be collaboratively studied. The Referee concurs.

Patulin.—Peter M. Scott (Canadian Health Protection Branch, Ottawa, Ontario, Canada) reports that with the decline in concern over patulin as a carcinogen, method development

has slackened. However, patulin continues to be included as one of the classical mycotoxins in multimycotoxin methods, one using high performance TLC with densitometric measurement of UV absorption (29) and another using fluorescent TLC plates with measurement of the fluorescence quenching (30). For routine control of patulin in apple juice in Sweden, where a 50 ng/mL action level is in effect, a rapid method has been developed, using cleanup on a micro scale and LC for determinative separation (31). The alkaline cleanup in this method, adapted from that of Tanner and Zanier (32), should be evaluated for possible patulin losses. The AOAC method for patulin in apple juice, **26.111-26.116,** has been further successfully tested in Poland (33). As a result of this experience and the decline in activity, the Associate Referee now recommends that the method be made official final action. The Referee concurs.

Penicillic Acid.—Charles W. Thorpe (FDA, Washington, DC) reports inclusion of penicillic acid as an analyte in the same multimycotoxin methods (29, 30) referred to by the Associate Referee on Patulin. The following interesting observations are pertinent to the analysis of penicillic acid: The TLC developing solvent for high performance silica gel plates was toluene-ethyl acetate-formic acid (30 + 6 + 0.5) with quantitation by UV reflectance scanning at 240 nm, detection on silica gel thin layer plates by fluorescence after exposure to ammonia fumes, and enhancement of the penicillic acid fluorescence by spraying the plate with paraffin-hexane (2 + 1). Two more methods, specifically for penicillic acid, have been published, one for the determination of penicillic acid in body fluids, in which HPLC is used for the determinative step (34); in the other (35), either HPLC or GLC is used for the determinative step after conversion of the penicillic acid to a pyrazoline derivative with diazomethane. The derivative produced a 2.5-fold increase in UV absorptivity.

The Associate Referee also reports completion of a preliminary collaborative study of a method first described at the 1978 AOAC meeting (36). The results have encouraged him to conduct a full collaborative study of the method in the coming year, and he so recommends. The Referee concurs.

Sterigmatocystin.—Octave J. Francis, Jr. (FDA, New Orleans, LA) reports that sterigmatocystin is included as an analyte in the multimycotoxin methods (29, 30) referred to by the Associate Referees on Patulin and on Penicillic Acid. He also calls attention to a method (37), in which HPLC is used for the determinative step, that has been applied to the analysis of moldy rice. None of these methods significantly lowers the limit of determination.

More important is the finding of sterigmatocystin in 9 of 39 samples of rind from hard cheeses ripening in Dutch warehouses (38). These findings were associated with surface outgrowths of *Aspergillus versicolor*, the most commonly found sterigmatocystin-producing mold. The method used in this survey (39) had been modified to achieve a 5 ng/g detection limit; the highest level found was 600 ng/g. Most of the toxin was confined to the thin plastic coating (40), which can be easily removed without damaging the cheese. A screening method for detecting sterigmatocystin-contaminated rind has been developed (41). When informed of these findings the FDA examined 100 samples of moldy cheese rind from a commercial cheese ripening operation in the United States. No *A. versicolor* was detected.

The Associate Referee recommends continuation of the "wait and see" attitude of previous years, and the Referee concurs.

Tree Nuts.—Vincent P. DiProssimo (FDA, New York, NY) reports progress in incorporating the economies of the column chromatography step (**26.A05**) of the cottonseed method with the extraction procedures of the CB method (**26.026-26.031**) toward achievement of an economical, general commodity method. He recommends continued study toward this end, and the Referee concurs.

Trichothecenes.—Robert M. Eppley (FDA, Washington, DC) reports that in 1980 Canada Agriculture and Canada Health and Welfare detected deoxynivalenol (DON, vomitoxin) in white winter wheat from Ontario and red spring wheat from Quebec (27, 42). DON has considerably less acute toxicity than T-2 toxin or diacetoxyscirpenol (DAS), but little is known about the chronic and subacute effects of the toxin. However, the widespread occurrence of DON has forced the development of a number of methods for its detection in cereal crops (27, 42-45). Most of these methods (27, 42, 44) use GLC as the preferred determinative step, with conversion of the compound to either the trimethyl silyl (TMS) ether or the heptafluorobutyrate ester for volatilization and detection by electron capture and/or mass spectrometric single ion monitoring. None of these methods is entirely satisfactory for routine work.

GLC of their TMS ethers has also been used for

the determination of DAS and T-2 toxin (**46**). By using an electron capture detector a limit of determination of 0.9 ng/g for T-2 toxin and 0.3 ng/g for DAS could be achieved, compared to 250 and 150 ng/g, respectively, for the 2 mycotoxins by flame ionization detection.

Use of TLC procedures continues. Although the limit of determination by TLC is not as low as with GLC, TLC is better suited for screening a large number of samples for the many different possible derivatives of the trichothecenes known to be produced by various molds. A recently published TLC procedure (47) can be used to detect many of the known *Fusarium*-produced mycotoxins, including 7 trichothecenes plus butenolide, moniliformin, and zearalenone. Detection of a specific mycotoxin can be followed by a quantitative method using GLC of the TMS derivative of that toxin.

Because of the generally unsatisfactory state of methodology for the trichothecenes and the difficulties encountered by analysts in obtaining reference standards, a workshop to develop solutions to these problems has been organized for the 1981 Annual Meeting of AOAC.

Isolation and identification of previously undescribed trichothecenes continues with the characterization of 3,15-dihydroxy-12,13-epoxytrichothec-9-ene-8-one (48) as a product from a *Fusarium* culture extract used for the preparatory isolation of DON. This new compound comigrates with DON on silica gel TLC plates. Another study (49) has resulted in the isolation and identification of 25 trichothecene derivatives produced by one strain of *Mycothecium verrucaria*. Many of these derivatives had not been characterized previously. Since many of the *Fusarium* species produce a number of different trichothecene derivatives as well as other mycotoxins, the multiplicity of mycotoxins produced by these molds should always be taken into consideration in assessing the significance of any natural contamination.

The Associate Referee recommends implementation of the recommendations that resulted from the workshop on trichothecene methodology, including the procurement of reference standards for distribution to analysts working on these recommendations or attempting to apply the recommended methods. The Referee concurs. The Associate Referee will report in full detail in the July issue.

Zearalenone.—Glenn A. Bennett (USDA, NRRC, Peoria, IL) reports that the International Standards Organization (ISO) completed a collaborative study (E. Nouat, Association Francaise de Normilisation, private communication) of a method (50) for zearalenone in corn and in mixed feed. The study format was far short of a design that could be accepted by the AOAC: One sample each of naturally contaminated corn and feed were provided each laboratory in sufficient quantity for 3 separate analyses of each sample. The open replicates bias the determination of repeatability; with no added contaminant there can be no determination of accuracy; and the only valid statistic, reproducibility, cannot be assessed over a range of concentrations. In this study, reproducibility for the corn sample, at a mean of 734 ng/g, was 38% and for the feed sample, at a mean of 219 ng/g, was 57%, comparable to the reproducibility observed for the AOAC method for zearalenone in corn (**26.124-26.132**) at these levels.

Three more methods for zearalenone, in which HPLC is used with a fluorescence detector, have been published (45, 51, 52); the first 2 methods apply to mixed feeds, the third to rat urine and liver.

A review (53) has been prepared of research at Canadian government and university laboratories on zearalenone (and other *Fusarium* toxins) and its relation to livestock problems. In other research on screening corn hybrids for resistance to infection by *Fusarium graminearum*, a consistent field infection method has been devised (54), and in a related study (55), 2 morphologically distinct strains of *F. graminearum*, with marked differences in virulence and zearalenone production, have been found co-occurring in infected ears of corn.

In studies of zearalenone metabolism by livestock (56–58) both zearalenone and its metabolite, β-zearalenol, were found in the milk of lactating animals (sows and cows); the transmission of zearalenol and zearalenone to milk from zearalenone in the feed of a sow at 40 μg/g induced signs of estrogenism in the suckling piglets (56). Most of the zearalenone administered to swine and cattle could be accounted for in the excreta, resulting in the recommendation that analyses of feces or urine might give more information on zearalenone exposure for animals exhibiting hyperestrogenism than analyses of the feed. The transmission to bovine milk (about 5% of the amount ingested) from the highest levels of zearalenone encountered in feed grains could not be considered a reasonable cause for concern (58). The mechanism of zearalenone metabolism has been studied in rats (59); α-zearalenol was found as the major metabolite.

Further studies on the estrogenic activity of zearalenone show an inhibition of implantation in sows (60) and of spermatogenesis in boars (61) and guinea fowl (62).

The Associate Referee recommends collaborative study of a modification of the Ware and Thorpe method (63) for zearalenone and zearalenol, development of a rapid screening method for zearalenone or zearalenol in feces and urine, and a continuation of studies on resistance of corn hybrids to infection by *Fusarium graminearum* and zearalenone production. The Referee concurs.

Recommendations

(1) Adopt as official final action the method (**26.111-26.116**) for patulin in apple juice.

(2) Adopt as official first action the Stubblefield-Shotwell method for aflatoxins B_1 and M_1 in liver, the van Egmond-Stubblefield method for confirmation of identity of aflatoxins B_1 and M_1 in liver, and the method for confirmation of identity of aflatoxin M_1 in dairy products (**26.A15**) as applicable to M_1 in liver.

(3) Complete the collaborative study of 2 methods for aflatoxin in peanut butter in which HPLC is used for the determinative step.

(4) Initiate collaborative studies of a method for aflatoxins in mixed feeds, of the Thorpe method for ochratoxin A in grains, of the Thorpe method for penicillic acid in corn and beans, and of the modification of the Ware-Thorpe method for zearalenone and zearalenol in grains.

(5) Implement the recommendations resulting from the workshop on trichothecene methodology.

(6) Continue study on all other topics.

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Report on Oils and Fats

DAVID FIRESTONE
Food and Drug Administration, Division of Chemical Technology, Washington, DC 20204

Antioxidants.—B. D. Page has conducted a collaborative study of the high pressure liquid chromatographic (HPLC) method for determination of antioxidants in oils and fats (1). Samples and instructions were sent to 13 laboratories over a 9-month period. Results received from 7 of the laboratories have been subjected to statistical evaluation, and recommendations await completion of the evaluation.

Cyclopropenoid Fatty Acids.—G. S. Fisher is planning a collaborative study of the capillary column GC method for cyclopropene fatty acids. This procedure involves on-column injection of cottonseed or other methyl esters.

Emulsifiers.—H. Brüschweiler is continuing an investigation of methodology involving HPLC, TLC, and GC for determination of different types of emulsifiers. The Associate Referee has also initiated a joint study between AOAC and IUPAC (Commission on Oils, Fats and Derivatives) of a GC procedure for several emulsifiers. The procedure involves separation of emulsifiers

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from the oil by column chromatography as described in method 28.133-28.138. The emulsifier fraction is hydrolyzed in alcoholic KOH solution and the components are silylated and analyzed by either packed or capillary column GC. The emulsifiers determined in the current collaborative study are monoglycerides, diacetyl tartaric acid monoglycerides, and sorbitan monostearates. Eight samples (including 4 standards) were sent to collaborators. Results received so far from 5 collaborators are in close agreement and results are awaited from the other laboratories. It is anticipated that the methodology will also be investigated for determination of additional emulsifiers such as propylene glycol mono- and diesters, polyglycerol esters, lactylated esters of mono- and diglycerides, lactylated esters of propylene glycol monoester, lactylated fatty acid esters, sorbitan esters, ethoxylated sorbitan esters, and succinylated monoglycerides. The procedure is, of course, unsuitable for polyethers that cannot be volatilized for detection by GC.

Chromatographic Methods.—W. G. Doeden, Jr., Research Manager, Analytical Chemistry, Swift and Co., Oak Brook, IL, has been appointed Associate Referee. Dr. Doeden is currently evaluating methodology for future study involving HPLC and capillary and packed column chromatography.

A joint AOCS-AOAC collaborative study of the GC method (2, 3) for determining trans-unsaturation in fats and oils has been carried out successfully. The study leader, L. Gildenberg, Colgate-Palmolive Co., Piscataway, NJ, is completing an evaluation of the collaborative results. Poor recovery of polyunsaturated esters relative to saturated or monoenoic components in stainless steel columns packed with 15% OV-275 was recently reported by Walker (4). This problem was not observed by the collaborators (7 of 13 collaborators reported using stainless steel columns). The method will be recommended for adoption upon publication of the collaborative results.

A European Economic Community method for specific determination of erucic acid in oils and fats (5) was studied collaboratively by the IUPAC Commission on Oils, Fats and Derivatives (6, 7). This method involves fractionation of the fatty acid methyl esters at −25°C on TLC plates containing silver nitrate, isolation of the erucic acid (13-*cis*-docosenoic acid) fraction, and quantitation by GC using methyl tetracosanoate as an internal standard. Statistical analysis of results from nine laboratories indicated coefficients of variation (CV) ranging from 5.5% (14.7% erucic acid) to 24% (1.6% erucic acid). The method will be recommended for adoption upon publication of the collaborative results.

Lower Fatty Acids.—G. Bigalli has completed a study of quantitation of lower fatty acids. The study demonstrated that GC conditions exert a great influence on the results obtained and should be very specific for determination of methyl butyrate and methyl caproate. The Associate Referee plans to initiate collaborative study of a specific method for lower fatty acids in the near future.

Marine Oils.—R. G. Ackman is continuing the study of methodology for analysis and identification of fish oils including the use of fused silica wall-coated open-tubular columns for GC determination of fatty acid composition.

Olive Oil Adulteration.—E. Fedeli is evaluating various methods for analysis of olive oils and other vegetable oils.

Oxidized Fats.—A. E. Waltking is continuing study of HPLC procedures for oxidation products (polymers) in vegetable oils. The Guhr and Waibel method for oxidation products in frying fats (8) has been granted interim first action status, and a communication has been prepared which describes the method and presents the results of IUPAC collaborative study (9). It is recommended that the interim first action method be adopted as official first action.

Pork Fats in Other Fats.—L. El-Sayed has completed work on methodology for detection of pork in canned meat and in animal and vegetable fats, and the methodology, also applicable to detection of lard in hydrogenated vegetable oils, is ready for collaborative study.

Spectrophotometric Methods.—A. J. Sheppard is continuing work on extension of the enzymatic method for *cis,cis*-methylene interrupted polyunsaturated fatty acids (PUFA) to analysis of PUFA in various foods.

Sterols and Tocopherols.—H. T. Slover has completed a precollaborative study of a GC method for determination of tocopherols and sterols in vegetable oils. The method involves saponification of the sample in capped tubes (heating in aqueous KOH for 8 min at 80°C in the presence of pyrogallol), extraction of the unsaponifiable matter, formation of the trimethylsilyl ethers of both tocopherols and sterols, and GC on a SP2100 fused silica capillary column 12 m or longer × 0.2 mm (5,7-dimethyltocol is used as an internal standard). The Associate Referee is planning a joint AOAC-AOCS-IUPAC-ISO collaborative study of the method.

Water Content.—R. Bernetti has carried out 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/SC11, N99). Four samples (refined and bleached soybean oil, crude soybean oil, vegetable oil deodorizer distillate, and soybean acid oil) were submitted to 22 laboratories. Repeatability was excellent at all of the laboratories. However, a sampling problem with the more heterogeneous acid oil sample (3% water content) caused wider than expected variation between laboratories, although reproducibility was good with the other 3 samples. The Associate Referee recommends revision of the text of the method to incorporate additional experimental details. Also, sampling techniques for handling acid oil and related samples with relatively high water content will be investigated before additional collaborative study.

Commission on Oils, Fats and Derivatives, Applied Chemistry (IUPAC).—The General Referee attended the annual meeting of the IUPAC Commission on Oils, Fats and Derivatives on August 26–28, 1981, in Leuven, Belgium. Professor C. Paquot completed four productive years as Chairman of the Commission. The new Chairman is D. Firestone. The Commission, which began its activities involving standardization of analytical methods for oil and fat products in 1930 as the International Commission for the Study of Fat Products, is the source of uniform methods frequently adopted by other national or international organizations, including ISO and the Codex Alimentarius Commission.

The Commission reviewed 25 projects, including methodology for glycerines and alkaline soaps, erucic acid, solid content of fats by NMR techniques, polycyclic aromatic hydrocarbons, industrial lecithin products, total sterols, erythrodiol in grapeseed and olive oils, mineral oil residues, tocopherols, chlorinated pesticides in wool wax, linoleic acid content of margarines, emulsifiers, heated fats, plastic polymers and plastic monomers in fats, triglyceride composition by GC, thiobarbituric acid value, and solvent residues in oils and oilseed cakes. Project work was completed on the following methods, which were adopted after successful collaborative study: tocopherols in oils, fats, and margarines by TLC fractionation and colorimetric or GC determination; *cis,cis*-linoleic acid in oils and margarines by the lipoxidase enzymatic method combined with GC; oxidation products in heated fats by the Guhr and Waibel chromatographic procedure; polyethylene-type polymers in fats by gravimetric and infrared spectrophotometric procedures; erucic acid by TLC-GC; and solid content of fats by NMR techniques. Part 3 of the first supplement of the Commission's *Standard Methods for the Analysis of Oils, Fats and Derivatives* was published recently (10). Part 3 includes methods for determining *cis,cis*-methylene interrupted polyunsaturated fatty acids (enzymatic method) and polyethylene-type polymers (gravimetric and infrared spectrophotometric procedures).

Recommendations

(1) Adopt as official final action the official first action method for isolated trans isomers **28.075–28.080**.

(2) Adopt as official final action the official first action method for 1-monoglycerides **28.139–28.147**.

(3) Adopt as official first action the interim first action method for oxidation products.

(4) Continue study on all other topics.

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Report on Processed Vegetable Products

THOMAS R. MULVANEY
Food and Drug Administration, Division of Food Technology, Washington, DC 20204

Fibrous Material in Frozen Green Beans.—Associate Referee George W. Varseveld indicated that he received no adverse comments on this method, which was adopted as official final action in 1980. Interested persons are invited to comment on this topic.

pH Determination in Acidified Foods.—A collaborative study of a proposed method for determining pH of acidified foods was conducted in 1979 and the method was adopted official first action, **32.B01-32.B08,** in 1980. Since no adverse comments regarding the method have been received during the past year, Associate Referee Frederick E. Boland recommends that the method be adopted official final action. He also recommends that study continue on this topic.

Sodium Chloride.—Associate Referee Wallace S. Brammell recommends that the following sodium chloride methods be declared surplus: **32.023,** Method I; and **32.024,** Method II (rapid method). These methods have been superseded by the official final action method, **32.025–32.030,** Method III (potentiometric method). The latter method is much faster and more accurate than those older methods, and it is doubtful that Methods I and II would be used much in the future. Continued study on this topic is anticipated.

Water Activity Determination.—Associate Referee William H. Stroup reported that a quality assurance sample, containing a salt slush and a sweetened condensed milk, was sent to each of 18 FDA district laboratories for water activity determinations by the Hair Hygrometer instrument. AOAC method **32.004-32.009,** water activity, was used to determine water activity values. From this quality assurance sample, the mean water activity value for the salt slush was 0.928, while the mean water activity value for the food was 0.853. Analysis of the data gathered suggested that the response of the Hair Hygrometer was sufficiently linear to use a least squares regression line of the calibration data to calculate water activity of unknown samples. The Associate Referee recommends that study be continued.

Other Topic.—George W. Varseveld reported that recent research at Oregon State University has shown that process time required to satisfy sterilization requirement for green beans packaged in institutional size flexible retort pouch (30 × 38 × 4 cm) varies with both the amount of entrapped air and the amount of liquid medium contained in the sealed pouch. This work points up the need for a reliable method to determine volume of residual entrapped air in the pouch after sealing.

The method is of interest for its value in establishing safe processes for vegetables sterilized in the pouch under varying conditions of static or rotary processing, orientation, and degree of constraints of the pouch during the process. Further research and development of a suitable method will be undertaken as funding becomes available. Sponsorship is needed for this funding.

Recommendations

(1) Adopt as official final action the pH determination of acidified foods, **32.B01-32.B08.**

(2) Declare the following sodium chloride methods as surplus: **32.023,** Method I, and **32.024,** Method II (rapid method).

(3) Initiate the new topic Determination of the Volume of Entrapped Air in the Flexible Retort Pouch; appoint an Associate Referee.

(4) Continue study on all topics.

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Report on Seafood Toxins

EDWARD P. RAGELIS
Division of Chemistry and Physics, Food and Drug Administration, Washington, DC 20204

In addition to paralytic shellfish poison (PSP) which, in 1981, became a part of the newly created General Referee topic, "Seafood Toxins," we have added two new associate refereeships to this program, namely, ciguatera poisoning (ciguatoxin) and pufferfish poisoning (tetrodotoxin). Since these latter two topics may not be familiar to many, they will be discussed in greater detail; each is presented in order of relevance to human health.

Ciguatoxin – Biochemical Methods

The Associate Referee on this topic is Prof. Yoshitsugi Hokama, School of Medicine, University of Hawaii at Manoa, Honolulu, Hawaii.

Introduction.—Ciguatera is a neurological disease produced in humans from ingesting the flesh or viscera of any of a large variety of reef-associated fishes containing ciguatoxin, believed to be the cause of ciguatera. It is separate and distinct from human illnesses due to spoilage of seafood from improper handling and/or processing. Cooking, smoking, drying, salting, or freezing does not appear to destroy the toxin in the fish flesh. The victim usually recovers from ciguatera within a few days (death occasionally occurs), but symptoms may last for several weeks, months, or possibly years.

Background.—Ciguatera poisoning (1-3) is widespread throughout the tropical and subtropical areas of the world and has been present in various regions of the Caribbean and Pacific for centuries. In general, outbreaks of ciguatera poisoning through the appearance of toxic fish are sporadic and unpredictable both in geographic distribution and time. Moreover, of the more than 400 species implicated, not all of the fish of the same species caught at the same time in the same place are toxic; only a few miles may separate ciguateric and safe fish of a given species.

In Florida and the Caribbean, the barracuda, the grouper, and the snapper are the fish most often implicated in the disease; in the Hawaiian region, the kahala (amberjack) and the ulua (jackfish) are the most common offenders. The number of cases of ciguatera in the U. S. and its territories may well average over 2000 per year; data recently accumulated from a 3-year study of Southern Florida indicate an average of 1300 cases per year (D. P. deSylva, University of Miami, Coral Gables, FL, private communication, September 2, 1981). Moreover, 1.6-4.4% of the local population of St. Thomas, U.S. Virgin Islands are annually afflicted with ciguatera representing approximately 940 cases among 60,000 persons (4). In Hawaii, over a period of 80 years (1900-1979), at least 600 people have been affected, according to the Hawaii State Department of Health.

Origin and Nature of Ciguatoxin.—In spite of the long history of ciguatera, the origin or identity of ciguatoxin is still not completely known. It is believed that the toxin(s) originates through the algae in the coral environment and then is transmitted through the food chain of fishes and thereby contracted by humans. Generally, the larger fishes of a species are involved, especially the carnivores which feed on reef fishes.

The toxin is lipid-soluble; it accumulates in the fish tissue and has a long biological half-life. At the AOAC Spring Workshop on Seafood Toxins in Ottawa, Canada, May 14, 1981, chaired by the Referee, Prof. Paul Scheuer (Chemistry Department, University of Hawaii at Manoa, Honolulu, Hawaii) reported that ciguatoxin possesses a molecular weight of 1111.1 daltons with structural features associated with ionophores. These features closely resemble those of okadaic acid, a highly toxic complex derivative of a C_{38} fatty acid, isolated from marine sponges, whose structure has been recently determined by X-ray diffraction (5).

The source of ciguatera in the Pacific is suspected to be a microscopic organism (dinoflagellate), *Gambierdiscus toxicus*, which appears to spawn and flourish following major disturbances and destruction of coral reef natural or man-contrived, e.g., dredging and construction (6, 7). At the workshop, Prof. Donald Tindall (Southern Illinois University, Carbondale, IL), Prof. Joseph McMillan (College of the Virgin Islands, St. Thomas, U.S. Virgin Islands), and Prof. Norman Doorenbos (Southern Illinois

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University, Carbondale, IL) reported that this organism is present in the Caribbean and is toxigenic. A tentative observation is that isolates from wild and cultured cells of *G. toxicus* appear to elaborate largely maitotoxin and lesser amounts of ciguatoxin. In addition, other dinoflagellates collected in the Caribbean, namely, *Prorocentrum lima*, *Prorocentrum rhathyma*, *Amphidinium klebsi*, and *Scrippiella trochoidea*, and blue-green algae, *Lyngbya* sp., are being investigated for ciguatoxigenic activity and toxin production. It was further reported that at least 60 bacteria isolates, collected from different regions in the Caribbean, are also being studied.

Detection of Ciguatoxin.—Presently, there is no validated quantitative method for the detection of ciguatoxin, largely because of the scarcity and unknown structure of the toxin. A variety of bioassays ranging from the use of cats, mongooses, mice, brine shrimp, and guinea-pig atria have been used for the detection of ciguatoxin (8–12). None of these have achieved the specificity, sensitivity, and practicality necessary for quantitative routine testing, although the procedure of Miyahara et al. (12) does appear to differentiate maitotoxin from ciguatoxin.

Currently, the radioimmunoassay developed at the University of Hawaii (13) is being investigated extensively in the screening of amberjacks (*Seriola dumerili*) and examination of fishes from clinically defined ciguatera poisoning outbreaks, both in Hawaii and in the Caribbean. To date the procedure has proved to be superior to the bioassays. A longitudinal study with commercial *Seriola dumerili* demonstrated that the RIA procedure was effective in keeping *large* ciguatoxic kahala or amberjacks from the market; there were no reported cases of ciguatera poisoning associated with this program. However, the RIA procedure lacks practicality in *routine screening* of fishes for the protection of the consumer. Therefore the Associate Referee is pursuing the development of a solid phase enzyme-linked immunosorbent assay (ELISA) for the detection of ciguatoxin. He believes that this approach will yield an assay method which will be inexpensive, rapid, and sensitive. The Referee concurs.

The Referee notes that in addition to the above methodology being developed for the detection of ciguatoxin, other procedures are being pursued which include the use of immunogenic and tissue cell cultures (Dr. H. Hugh Fudenberg, Medical University of South Carolina, Charleston, SC) and a nerve-muscle screening method (Profs. Donald Miller and Donald Tindall, Southern Illinois University, Carbondale, IL).

Shellfish Poison

Associate Referee William L. Childress (FDA, Boston, MA) reports that the initial results of the IUPAC collaborative study of his modification (14) of the fluorimetric assay for the detection of saxitoxin (STX) reported by Bates, Kostriken, and Rapoport (15) reveal that the method as described still has several shortcomings. Most of the collaborators, including himself, were unable to achieve reproducible quantitative recoveries of PSP standard (STX) carried through the method.

The Associate Referee, after studying the critical areas cited by the collaborators, has drawn several conclusions.

1. The pH must be carefully controlled throughout the procedure. It is absolutely essential that the acidity of the ion-exchange column (Bio-Rex 70, sodium form) be maintained at pH 5.0. This is best achieved by preparing the column according to Bates et al. (15). Using this method of resin preparation, the Associate Referee reports recoveries of STX to be far more consistent, in the 85–90% range.

2. The concentration of sodium hydroxide in the oxidation step appears to be critical; the fluorescence response can vary as the sodium hydroxide concentration is changed, even by a small amount. This problem is caused by the fact that samples eluting from the column have a different pH from the solutions (standard) not put through the column, even though the medium, 0.5N H_2SO_4, is the same. Two possible remedies are to add the sodium hydroxide to a pH of 13 or put the standard through the column. The other variables in the step, namely, reaction time and hydrogen peroxide concentration, appear to be less critical, though he reports that reproducibility was improved by adding at least 0.1 mL of the hydrogen peroxide reagent, rather than the "2 drops" called for in the method.

3. The temperature at which the fluorescence is read appears to be a significant factor. If at all possible, the cell should be thermostated.

Because of the foregoing problems, the IUPAC Commission decided to discontinue the collaborative study until the problems encountered with the procedure have been resolved. The Referee concurs.

The Referee notes that part of the Associate Referee's comments were presented at the AOAC Spring Workshop on Seafood Toxins in Ottawa this past May. At the workshop, Dr. Robert Bose (Dept. of Food Science, University of British Columbia, Canada) who is similarly investigat-

ing the fluorimetric procedure, reported that he can achieve quantitative isolation and recovery of paralytic shellfish toxins on a specially prepared column of CM-cellulose (in place of Bio-Rex 70) by elution with 0.2N HCl. His procedure and results will be published shortly. The Referee recommends that use of this resin be investigated as a possible replacement for Bio-Rex 70.

Sherwood Hall (University of Alaska, Fairbanks, Alaska) reported at the workshop that 4 new PSP toxins (designated B_1, B_2, C_1, and C_2) of relatively low toxicity can be isolated from clones of *Protogonyaulax* sp. (from Porpoise Islands, Alaska) by extraction with 1M aqueous acetic acid (16). Upon exposure to low pH, these "masked" toxins are converted, respectively, to saxitoxin, neosaxitoxin, gonyautoxin-II, and gonyautoxin-III (results submitted for publication). The Referee notes that these toxins have yet to be found in shellfish; if they were present, we may need to rethink the safety of the quarantine limit of 80 μg toxin per 100 g shellfish meats.

It was agreed by the participants of the workshop that more studies need to be done to optimize the oxidation step, since not all of the known PSP toxins give a "measurable fluorescence" under the present set of alkaline/peroxide conditions. The Referee concurs. The Associate Referee reports that in studying the oxidation of neosaxitoxin and gonyautoxins I to IV (supplied by Sherwood Hall, University of Alaska, Fairbanks, Alaska) with 0.1% aqueous KIO_4 some reaction is observed for all of these toxins, but only gonyautoxins II and III show strong fluorescence. Other conditions, including peroxide alone and buffered periodate, yield similar results. Interestingly, when these toxins were reacted with 1% $NaIO_4$ at pH 9.5, followed by treatment with fluorescamine, the strongest responses were observed for neosaxitoxin and gonyautoxin-IV.

The Referee suggests another approach to this analytical problem, namely, to transform the paralytic shellfish toxins to saxitoxin. Prof. Shimizu has shown (17) that the toxins in scallop homogenates undergo reductive transformation, eventually leading to saxitoxin as the final product.

The Referee notes that other approaches actively being pursued for the analysis of PSP toxins include differential pulse polarography (Raymond Gajan, FDA, Washington, DC); an enzyme immunoassay system (Dr. Patrick Guire, Bio-Metric Systems, Inc., Minneapolis, MN); and a biochemical binding exchange assay with tritium-labelled saxitoxin (Dr. Richard Rogart, Harvard Medical School, Children's Hospital Medical Center, Boston, MA).

Tetrodotoxin

The Associate Referee on this topic is Prof. Yuzuru Shimizu, College of Pharmacy, University of Rhode Island, Kingston, RI.

Introduction.—Puffers have been recognized as deadly toxic fish for thousands of years. Nevertheless some ethnic groups indulge in these otherwise extremely delicate fish. For example, Japanese esteem the fish (called *Fugu* in Japanese) so much that they are one of the most expensive fish in the market and are commercially aquacultured. If properly prepared, the fish are supposedly safe for human consumption, but still a significant number of people die from the poisoning every year in Japan. Outside of Japan, in the recent trend to exploit unutilized fish, puffer fish often come to the market mixed with other innocuos fish or are processed together with other trash fish. This certainly presents some problems and requires our preparedness for unexpected events.

The chemical structure of the toxic principle, tetrodotoxin, has been determined. However, besides the chemistry, only very limited knowledge is available concerning such important subjects as the biogenetic origin of the toxin, the extent of toxin distribution, analytical methods, especially physico-chemical assays, and detoxification.

Background.—In 1910, tetrodotoxin was first isolated and purified from the eggs of puffer fish (18), but it was not until 1950 that crystalline amounts of the toxin were obtained (19). The structure of tetrodotoxin was simultaneously confirmed and reported in 1964 by 4 independent groups (20). It is a low molecular weight ($C_{11}H_{17}N_3O_8$) ortho ester having a guanidinium group and a polyoxygenated ring system.

Tetrodotoxin is a very fast-acting toxin which blocks the sodium channels in the crucial excitable membranes. The action is almost identical with that of paralytic shellfish poisons. There is no known effective treatment for the poisoning.

Biogenetic Origin of Tetrodotoxin.—Biogenesis of tetrodotoxin is surrounded by mystery. First of all, the occurrence of tetrodotoxin is not limited to the puffer family, Tetrodontidae. Tetrodotoxin is now found in several organisms, e.g., the goby fish, *Gobius criniger;* the newt, *Taricha* spp.; the frog, *Atelopus chiriquiensis;* the octopus, *Octopus maculosus;* and most recently the snail, *Babylonia japonica.* It seems very likely that this

distribution of tetrodotoxin will expand to more animals. Another intriguing fact is that the toxin content varies conspicuously from individual to individual. This is true with all the tetrodotoxin-containing animals. For example, *Babylonia japonica* are good eating snails and only those from a particular area are toxic. The most striking fact is that *Fugu rubripes*, one of the most virulent puffers, completely lacks the toxicity when raised in aquaculture.

In view of this anomalous occurrence it is quite possible that there is an unexpected source of the toxin. Therefore, it is extremely important to investigate the distribution of the toxic fish. We have already discovered that *Spheroides maculatus*, which was reported to be toxic in Florida, was not toxic in northern waters.

The chemical origin of the toxin is also not known. Our experiments using *Taricha* spp. resulted in no uptake of common precursors into the toxin, indicating the absence of the *de novo* synthesis in the newt in captivity.

Assay Methods for Tetrodotoxin.—Tetrodotoxin, like paralytic shelfish toxins, has been assayed by using mice (21–25). Other biological methods have been reported based on the measurement of the action potential of frog (26, 27) and guinea pig (25) nerves incubated with tetrodotoxin. However, with these procedures, it is very difficult to obtain reproducibility or objectivity in results (28).

A chemical method for the direct analysis of tetrodotoxin is still unavailable. The toxin itself is uncharacteristic in that it does not contain a chromophore or other functional groups useful for the development of a rapid sensitive spectroscopic method. However, a few methods have been reported. These depend on the use of strong alkali to decompose tetrodotoxin to aminoquinazolines, specifically, 2-amino-6-hydroxymethyl-8-hydroxyquinazoline (C_9 base) whose fluorescence intensity (excitation, 370 nm; emission, 495 nm) is measured (29, 30) or detected by gas chromatography as trimethylsilylated (TMS) derivatives (28). Ellen King of FDA's Bureau of Foods has investigated these methods and found much variability in sensitivity and reproducibility; further studies need to be done. The Referee concurs.

Another approach is to prepare the antibody of tetrodotoxin, and to devise an immunoassay system. Tetrodotoxin with many functional groups should be derivatized easily to form appropriate antigens. The Associate Referee believes that this approach should be actively pursued. The Referee concurs.

Recommendation

It is recommended that study continue on all topics.

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AOAC Regional Section Meetings

AOAC Midwest Regional Section Meeting, June 2–3, 1982, Ames, IA. For more information, contact H. Michael Stahr, Iowa State University, 515/294-1950

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AOAC Pacific Northwest Regional Section Meeting, June 16–17, 1982, Olympia, WA. For more information, contact H. Michael Wehr, Oregon Department of Agriculture, 503/378-3793

■

AOAC Northeast Regional Section Meeting, June 22–23, 1982, Syracuse, NY. For more information, contact Audrey Gardner, NY State Agriculture Experiment Station, 315/787-2281

GENERAL REFEREE REPORTS: COMMITTEE D

Report on Alcoholic Beverages

RANDOLPH H. DYER
U.S. Department of the Treasury, Bureau of Alcohol, Tobacco and Firearms, 1401 Research Blvd, Rockville, MD 20850

Alcohol Content by Oscillating U-Tube Density Meter.—A second collaborative study comparing the density meter and the pycnometer for determining proof of alcoholic beverages was submitted by D. H. Strunk (J. E. Seagram & Sons) for evaluation. Compared with the pycnometer, the density meter procedure is simpler, requires less time and a smaller sample, and gives more reproducible results. The Associate Referee recommends adoption of the method, and the General Referee concurs.

Color Intensity for Distilled Alcoholic Beverage Products.—A collaborative study to determine the color intensity for distilled alcoholic beverage products was submitted by D. H. Strunk for evaluation. The method offers advantages over the current AOAC method for determining the color of whisky and possibly other similarly colored, aged alcoholic products. The Associate Referee recommends adoption of the method, and the General Referee concurs.

Ethanol in Wine by GLC.—Associate Referee Arthur Caputi of E & J Gallo Winery reports that a collaborative study is underway.

Malic Acid in Wine.—Robert Dowrie of Almaden Vineyards has been appointed Associate Referee.

Malt Beverages and Brewing Materials.—After many years of outstanding effort applied to the task of Associate Referee for Malt Beverages and Brewing Materials and also AOAC-ASBC (American Society of Brewing Chemists) Liaison Officer, Dwight B. West has retired. His dedication contributed greatly toward a better AOAC. The General Referee and the entire AOAC sincerely appreciate his work and hope that his retirement years are rich and rewarding. Anthony J. Cutaia of the Stroh Brewery has been appointed Associate Referee and AOAC-ASBC Liaison Officer.

Gordon Pilone of the Christian Brothers Winery has indicated he is initiating a new collaborative study of the alcohol determination in wine by dichromate oxidation.

As indicated in last year's General Referee report, the U.S. National Bureau of Standards now has available a wine reference standard. It may be purchased by sending $90 for NBS Wine Reference Standard No. 1590 to National Bureau of Standards, Chemistry Building, Room B 311, Washington, DC 20234.

Recommendations

(1) Adopt as official first action the method for determining alcohol content by oscillating U-tube density meter (*J. Assoc. Off. Anal. Chem.* **65,** 218–223 (1982)).

(2) Adopt as official first action the method for determining color intensity for distilled alcoholic beverage products (*J. Assoc. Off. Anal. Chem.* **65,** 224–226 (1982)).

(3) Adopt as official first action the barley extract determination (*J. Assoc. Off. Anal. Chem.* **64,** 1140 (1981)).

(4) Adopt as official final action the method for moisture in barley, **10.B01.**

(5) Adopt as official first action the Celite separation screening method for determining *N*-nitrosamines in beer and the distillation confirmatory method.

(6) Carry out a collaborative study of determining ethanol by GLC, and by dichromate oxidation.

(7) Continue study on all topics.

This report of the General Referee was presented at the 95th Annual Meeting of the AOAC, Oct. 19–22, 1981, 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 for detailed recommendations, this issue.

Section numbers refer to "Changes in Methods," *J. Assoc. Off. Anal. Chem.* (1981) **64,** 501–540.

Report on Cacao Products

JAY C. MUSSER
214 Marietta Ave, Mount Joy, PA 17552

The HPLC determination of caffeine and theobromine in chocolate and cacao products (**13.A05-13.A07**), and the HPLC determination of fructose, glucose, lactose, and maltose in milk chocolate (**13.A01**) were adopted official final action.

The Associate Referee on Moisture in Cacao Products continues study on the Karl Fischer method (**13.003-13.004**) for application to chocolate liquor.

The Associate Referee on Shell in Cacao Products, Micro Methods continues study of a component to be used as indicator of shell contamination in liquor.

Recommendation

Continue study on all topics.

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Report on Cereal Foods

DORIS BAKER
Agriculture Research Service, Nutrition Composition Laboratory, Beltsville, MD 20705

Phytates.—Associate Referee Barbara Harland has developed a method that she recommends for further study.

Iron.—James Martin, FDA, Atlanta Center for Nutrient Analysis, has studied method **14.012** for iron and believes time could be saved in the procedure by using fewer points on the standard curve. An evaluation by a mini-collaborative study could result in an alternative procedure for routine analysis of samples not known to cause problems.

Starch.—The enzyme, Rhozyme-S, used in method **14.075-14.080** has been discontinued by Rohm and Haas. Because of the unavailability of this enzyme and the fact that method **14.081-14.082** can be used as a replacement, method **14.075-14.080** should be deleted. The Associate Referee concurs. Robin Saunders has asked to be released as Associate Referee for Starch Methods. A new Associate Referee has been found, Bert D'Appolonia, North Dakota State University.

Protein Factors.—The Nitrogen-to-Protein Conversion Factor Committee met on December 17, 1980. They concluded that accurate factors for converting nitrogen to protein do not exist, and made the following recommendations:

1. All analyses report the percentage nitrogen in the sample.
2. If the percentage protein is reported, the nitrogen-to-protein conversion factor used shall be specified in the report.
3. If desired, the traditional and customary nitrogen-to-protein conversion factors may be used as listed in **14.063** in the 12th edition of *Official Methods of Analysis*, or **14.068** of the 13th edition, modified to read as follows:
   "**2.057,** Protein = nitrogen × 6.25 except for wheat and its product in which protein equals N × 5.7."

Other traditional and customary factors are 5.18 for almonds; 5.46 for peanuts and brazil nuts; 5.30

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for tree nuts and coconut; 6.38 for dairy products.

Modification of **14.068** was approved interim official first action in March 1980. Committee D gave official first action approval at the October 1980 meeting, and the 6.25 factor approved in October will appear in "Changes in Methods" in March 1981. It should be made clear that this "change" is actually a reversion to the factors as published in the 12th Edition of *Official Methods of Analysis*.

Recommendations

(1) Study the modified method for phytate analysis using an ion-exchange procedure.

(2) Delete methods **14.075-14.080** for starch.

(3) Adopt the Ad Hoc Committee report on the nitrogen-to-protein conversion factor.

Report on Fruit and Fruit Products

FREDERICK E. BOLAND
Food and Drug Administration, Division of Food Technology, Washington, DC 20204

Adulteration of Orange Juice by Pulpwash and Dilution.—Associate Referee D. R. Petrus has completed a collaborative study on spectral characteristics of Florida orange juice and orange pulpwash. The Referee has reviewed this collaborative study and has several reservations, among which are the following:

The laboratory results reported by each collaborator were adjusted by the Associate Referee to compensate for instrument differences. Apparently, individual collaborators did not standardize their spectrophotometers before analyzing the samples. This fact introduces an error into the data reported by each collaborator. The method should contain a statement that each individual analyst should standardize his spectrophotometer before running any samples.

Individual collaborators were requested to classify the samples into one of four cases. These results should have been reported to demonstrate how the collaborators interpreted the spectral data.

Equations for predicting % total citrus materia. (% TCM), % orange juice (% OJ), and % pulpwash (% PW) sometimes yield answers above 100% and less than zero. If % TCM for a sample is greater than 100%, OJ and PW are arbitrarily adjusted so that % TCM = 100%. Any values less than 0% are assumed to be zero. For example, Collaborator 6 reports 111% TCM for Sample 2, but 76% OJ and 24% PW. These results don't balance with the equation: % TCM = % OJ + % PW. When one considers the natural variation in orange juice composition, why should one juice which is 11% high (111% TCM) be made to equal 100% while another juice (90% TCM) is considered adulterated?

The Associate Referee has developed 10 equations for calculating % TCM, etc. After an analyst has classified a sample into one of four cases, he then refers to the proper case to determine which equations to use. The use of this number of equations is time consuming and could introduce errors, and the question arises as to whether the same ends could be accomplished by a smaller number of equations.

The data base used by the Associate Referee in developing his method and equations represents Florida orange juice and pulpwash. Before his method is approved, the validity of the Florida data base with regard to other orange juice samples (California, Texas, Arizona, and foreign) has to be determined.

In view of the above reservations, the Referee recommends further study.

The Associate Referee has done some good work in collecting spectral data on Florida orange juice and pulpwash and there is a need for methodology of this type. If certain discrepancies could be eliminated and/or certain limits applied to the method (e.g., only for Florida orange juice, or only for Texas orange juice, etc.), the result could be helpful to both the citrus industry and regulatory agencies.

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Fruit Acids.—Associate Referee E. D. Coppola believes that high pressure liquid chromatography (HPLC) is the most promising approach to separate the different acids in fruits. He has had some experience with HPLC using a radial compression system, which is similar to a reverse phase C_{18} column. Using this procedure, he has been able to separate the most important fruit acids. The Referee recommends continued study.

The topics Fruit Juices, Identification and Characterization; and Iso-Ascorbic Acid (Erythorbic Acid-Antioxidant) in Fruit Purees are new and Associate Referees should be appointed. The topic Dimethylpolysiloxane in Fruit Juices should be discontinued.

Orange Juice Content.—The study on the authentication of orange juice has continued this past year. Forty-two different chemical and physical tests were applied to early-, mid- and late-season samples of California naval orange juice concentrate and pulpwash. Analysis of variance showed many significant compositional differences due to season of harvest and product type. Various proposed statistical methods of predicting juice authenticity were tried and evaluated. The most promising parameters and statistical approaches are currently being evaluated on a similar set of California Valencia orange juices. It is recommended that the study on orange juice content be continued.

$^{13}C/^{12}C$ Isotope Ratio Analysis for Detection of Adulteration of Apple Juice with Corn Sugars.—This subject has been transferred to the new topic Stable Carbon Isotope Ratio Analysis under Sugar and Sugar Products.

Recommendations

(1) Discontinue the topics $^{13}C/^{12}C$ Isotope Ratio Analysis for Detection of Adulteration of Apple Juice with Corn Sugar; and Dimethylpolysiloxane in Fruit Juices.

(2) Appoint Associate Referees on the following topics: Fruit Juices, Identification and Characterization; and Iso-Ascorbic Acid (Erythorbic Acid-Antioxidant) in Fruit Purees.

(3) Continue study on all other topics.

Report on Preservatives and Artificial Sweeteners

WILLIAM S. ADAMS
Food and Drug Administration, 585 Commercial St, Boston, MA 02109

Benzoates, Saccharin, and Caffeine (High Pressure Liquid Chromatography).—Associate Referee B. Woodward and others (1, 2) previously reported on 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 preliminary studies to determine the applicability of the methods to fruit juices as well as soda beverages. Sorbic acid was included in the study in addition to benzoates, saccharin, and caffeine. Modifications in the method included a radial compression system for HPLC and provided a more rapid analysis with greater sensitivity. The results appear to be promising. It is recommended that the study be continued.

Formaldehyde in Olives.—Associate Referee R. J. Reina conducted a recovery study for formaldehyde in olives at a level of 10.25 ppm by using the following procedure: The drained olives were blended in a Waring blender to a paste-like consistency, and 100 g of this composite was spiked with formaldehyde at 10.25 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 and eluted with a mixture of $CHCl_3$, hexane, and ethyl ether to separate the hydrazone derivative from interfering oils. The eluate containing the derivatives was evaporated to dryness, dissolved in hexane, and diluted to a specific volume. A portion of this solution was further cleaned up

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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 acetonitrile and water, and a UV detector at 254 nm. Recovery was 99.4%. It is recommended that the method be further investigated to determine its applicability at the 1 ppm level.

Meats, Ground, Screening Method for Chemical Preservatives and Added Blood.—Associate Referee J. J. Maxstadt (3) continued the study of the official first action method for chemical preservatives in ground meats, **20.A01-20.A05,** to improve the procedure for added blood. During the past year, a limited amount of within-laboratory evaluation was made on the procedure. A second collaborative study is planned. It is recommended that the study be continued.

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. The preservatives included *p*-hydroxybenzoic acid, methyl paraben, ethyl paraben, propyl paraben, butyl paraben, salicylic acid, potassium sorbate, benzoic acid, and dehydroacetic acid. Thin layer chromatography was used as the determinative step. The method appears promising and yields consistent qualitative results. Although no work was performed on the method during the past year due to other commitments, a collaborative study is planned. It is recommended that the study be continued.

Saccharin in Foods by Differential Pulse Polarography.—W. Holak, Associate Referee, and B. Krinitz (4) have previously reported on a successful collaborative study for the determination of saccharin by differential pulse polarography. The method was adopted official first action, **20.A06-20.A10.** No adverse comments were received during the past year. It is recommended that the method be adopted official final action.

o-Toluenesulfonamide in Saccharin.—B. Stavric and R. D. Klassen, Associate Referees, previously reported on a collaborative study of the quantitative determination of *o*-toluenesulfonamide (*o*-TS) in commercial saccharin samples (5). A second collaborative study was planned for *o*-TS in acid saccharin; however, due to other commitments they will be unable to conduct this study. It is recommended that the study be discontinued.

Recommendations

(1) Appoint Associate Referees for the topics Benzoates and Hydroxybenzoates in Foods; Preservatives (Quantitative Methods).

(2) Continue official first action status of method **12.051-12.053** for determination of sodium saccharin, sodium benzoate, and caffeine by HPLC; continue study for applicability to other compounds and for quantitating potassium sorbate.

(3) Continue official first action status of method for chemical preservatives in ground meat, **20.A01-20.A05;** continue study of the method for added blood.

(4) Adopt as official final action the method for saccharin in foods by differential pulse polarography, **20.A06-20.A10.**

(5) Discontinue topic *o*-Toluenesulfonamide in Saccharin.

(6) Continue study on all other topics.

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Report on Sugars and Sugar Products

ARTHUR R. JOHNSON
Food and Drug Administration, Division of Food Technology, Washington, DC 20204

New Associate Referees were appointed for Maple Saps and Syrups, Stable Carbon Isotope Ratio Analysis, Sugars in Cereal, and Enzymatic Methods since the last meeting. Collaborative studies have been presented for the determination of corn syrups in fruit juices, for the determination of minor saccharides in corn sugars, and for the determination of sugars in cereals. Studies are projected for the determination of adulteration in maple syrups and the determination of sugars by instrumental enzymatic methods.

Chromatographic Methods.—Associate Referee Michael Gray reports progress in assembling information on chromatographic methods of analysis for carbohydrates with regard to future collaborative studies. He recommends continued study.

Color, Turbidity, and Reflectance—Visual Appearance.—Associate Referee Carpenter reports progress in his efforts to revise the AOAC method for the determination of color in sugars to more closely reflect the provisions of ICUMSA methods.

Corn Syrups and Corn Sugars.—Associate Referee Bernetti reports that work is in progress at the Augustana Research Foundation, Augustana College, Rock Island, Illinois under the sponsorship of the Analytical Procedures Committee of the Corn Refiners Association. This study is a companion to earlier published studies on the relationship of refractive index to dry substance (1). The evaluation and publication of the information will result in the preparation of new tables which will be recommended to replace **31.09** and **31.10** now appearing in the 13th edition of *Official Methods of Analysis*.

The Associate Referee has presented data from a second collaborative study to indicate that potentiometric end points taken to pH 7 are preferable to the "faint pink" end point at pH 8.3 in the current method for acidity, **31.217**.

Dr. Bernetti presented the results of a collaborative study of the high pressure liquid chromatographic method for the determination of minor saccharides in starch conversion products. This work expands the scope of the official first action method, **31.228-31.236,** saccharides by liquid chromatography (2). He will recommend, in an interim action, the addition of 2 new paragraphs after **31.236**, covering standardization, sample preparation, and calculations relative to high dextrose-type samples. In addition, he will recommend that commercial, prepacked columns equivalent to resins named in **31.230**(b) be used rather than laboratory-packed columns.

Dry Substance.—Associate Referee Dowling stated that the sugar industry is developing new data for the 100° Pol standard for sugars and for refractive indices for various sugar syrups. These data are not yet available for publication.

Enzymatic Methods.—Marc Mason has recently been appointed Associate Referee. He plans to investigate instrumental enzymatic methods for determination of sugars in various foods.

Honey—Associate Referee J. W. White, Jr reports progress on the carbon isotope ratio examination of citrus nectars and citrus honeys.

Maple Sap and Syrup.—Maria Franca Morselli reports the initiation of a collaborative study on the application of the stable carbon isotope–mass spectrometric procedure to the detection of adulteration in maple syrups. She also reports progress with additional recommendations for the detection of syrup adulteration.

Stable Isotope Ratio Analysis.—L. W. Doner, Associate Referee, and D. D. Bills have presented a collaborative study on a method to detect high fructose corn syrup (HFCS) in orange juices by a stable carbon isotope ratio–mass spectrometric method. The $^{13}C/^{12}C$ ratios in natural orange juices are sufficiently uniform and different from those in HFCS so that adulteration can be detected.

Natural orange juices have an average value of −24.5‰ while mixtures of juice and HFCS show intermediate values. Seven collaborators correctly identified one pure orange juice and 4 orange juice–HFCS mixtures containing from 25 to 70% orange juice. Data are presented to show

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that juice samples with $\delta\ ^{13}C$ values less negative than −22.1‰, 4 standard deviations from the mean for pure juices, can, with a high degree of confidence, be classified as adulterated. (Samples with a value more negative than −22.1‰ must be considered to be unadulterated.)

The Associate Referee recommends adoption as official first action of the stable isotope ratio-mass spectrometric procedure for the detection of high fructose corn syrup in orange juices.

Standardization of Sugar Methods of Analysis.—Associate Referee Whitney Newton reports that he is re-evaluating the widely used methods for sugar analysis before making recommendations for adoption as AOAC methods of analysis; he recommends continued study.

Sugars in Cereals.—Associate Referee Lucian Zygmunt has presented a collaborative study on the determination of mono- and disaccharides in pre-sweetened breakfast cereals by using a modified cleanup procedure with the high pressure liquid chromatography procedure for sugars in chocolate (3). The study data indicate precision comparable to the AOAC HPLC procedure for chocolate and to the AOAC Lane-Eynon copper reduction method (4). The value of the HPLC method becomes obvious for the analysis of sugars in breakfast cereals when considering possible ingredient sources and vitamin premixes that may be used. He recommended adoption as official first action of the modified HPLC method for the determination of sugars in cereals as presented.

Sugar and Sugar Cane.—The Associate Referee reports progress on the analysis of both sugars and dextrans in sugar cane juices using Aminex ion exchange resin columns.

Associate Referees for Sugars, Reducing; and Weighing, Taring, and Sampling report no new developments during the past year.

Recommendations

(1) Adopt as official first action the collaboratively studied mass spectrometric stable carbon isotope procedure for determination of high fructose corn syrup in orange juice.

(2) Adopt as official first action the high pressure liquid chromatographic method for determination of sugars in presweetened cereals.

(3) Continue study on all topics.

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Report on Vitamins and Other Nutrients

MIKE J. DEUTSCH
Food and Drug Administration, Division of Nutrition, Washington, DC 20204

The activities of the association's 95th Annual Meeting included a symposium titled "Infant Formula Regulation and Infant Food Problems." In addition, 11 papers dealing with methodology were presented at the sectional session on vitamins and other nutrients. Frits J. Mulder, former Associate Referee, was given a special award by the AOAC for his perserverance in the development of the official chemical assay for vitamin D in foods, feeds, and pharmaceuticals, and for his extended world-wide efforts of better acquainting scientists of the goals of the Association and in promoting use of its methodology.

David Egberg of General Mills, Minneapolis, MN resigned as Associate Referee for automated nutrient analysis and Jonathon DeVries of that organization replaced him.

Recommendations

(1) Adopt as official final action the first action semiautomated method for riboflavin in food products, **43.B01–43.B04**.

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(2) Adopt as official final action the first action semiautomated method for niacin and niacinamide in food products, **43.B05-43.B08**.

(3) Adopt as official final action the first action HPLC method for vitamin D in fortified milk and milk powder, **43.B09-43.B15**.

(4) Adopt as official first action the HPLC method for vitamin D in pet foods and animal feeds.

(5) Adopt as official first action the screening in vitro C-PER/DC-PER assays for predicting PER as reported by the investigators and with the concurrence of the Associate Referee.

(6) Continue study on all other topics.

GENERAL REFEREE REPORTS: COMMITTEE E

Report on Carbamate Pesticides, Fumigants, and Miscellaneous

ROBERT W. STORHERR
Environmental Protection Agency, Chemical and Biological Investigations Branch, Beltsville, MD 20705

All interlaboratory studies planned for this past year were postponed, for several reasons: method not ready, specialized equipment not available, or no interest in the subject. By this time next year, at least one of 3 interlaboratory studies may be concluded. In particular, the Referee is hopeful that Richard Krause of FDA will find enough laboratories with the specialized HPLC equipment to carry on his carbamate study. Also that R. W. Young, Virginia Polytechnic Institute, will finalize his on-column GLC silylation derivative method for carbamates and prepare an interlaboratory study.

Due to the pressure of other workloads, most Associate Referees report no progress this year.

Recommendations

(1) *Carbamate Insecticides, Gas Liquid Chromatographic Methods.*—Write up the method for the silylated derivatives of carbamates for GLC and submit it to a rigorous interlaboratory test after review by the General Referee.

(2) *Carbamate Insecticides, Liquid Chromatographic Methods.*—Initiate a collaborative study of the Associate Referee's high performance liquid chromatographic method with post-column fluorescent derivative formation for determining *N*-methyl carbamate insecticides 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 reported by Scudamore and Heuser (*Pestic. Sci.* (1971) **2,** 80–91) for determining ethylene oxide, ethylene chlorohydrin, and ethylene bromohydrin in foods.

(5) *Fenvalerate.*—Initiate study of analytical methods for determining residues of fenvalerate in foods.

(6) *Fumigants.*—Adopt as official final action the official first action gas-liquid chromatographic method for determining chloroform, carbon tetrachloride, trichloroethylene, and 1,2-dibromoethane in wheat and corn grain, **29.056-29.057;** appoint Associate Referee and initiate study to extend **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.*—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.*—Continue study.

(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) for determining the total residue of intact phosphine and phosphine derived from residual aluminum phosphide in wheat.

(10) *Resmethrin.*—Continue study.

(11) *Sodium Monofluoroacetate.*—Continue study of the method described by the Associate Referee in 1980, with gas-liquid chromatography of fluoracetic acid, underivatized, for determining residues of sodium monofluoroacetate in food.

This report of the General Referee was presented at the 95th Annual Meeting of the AOAC, Oct. 19–22, 1981, at Washington, DC.

The recommendations of the General Referee except No. 6 were approved by Committee E and were adopted by the Association. See the report of the committee for detailed recommendations, this issue.

Section numbers refer to *Official Methods of Analysis* (1980) 13th Ed.

Report on Fungicides, Herbicides, and Plant Growth Regulators

W. HARVEY NEWSOME
Health and Welfare Canada, Food Research Division, Health Protection Branch, Ottawa, Ontario, Canada K1A 0L2

Three Associate Referee reports were received. There was one resignation, James F. Lawrence, Associate Referee for Anilazine and *s*-Triazines.

Associate Referees are required (*1*) to conduct a collaborative study of the HPLC method for anilazine described by Lawrence and Panopio (*J. Assoc. Off. Anal. Chem.* **63,** 1300 (1980); (*2*) to develop a method for benomyl, methyl benzimidazole carbamate, and thiabendazole in crops on which these compounds are registered; (*3*) to develop and test methods for captan, captafol, folpet, and their metabolites, and carbamate herbicides; (*4*) to study methods for the determination of dinitro aryl herbicides; (*5*) to develop and test a method capable of determining ethylenebis(dithiocarbamates), and dimethyl dithiocarbamates as separate entities in food; (*6*) to develop HPLC or GLC methods for maleic hydrazide in food crops; (*7*) to develop an HPLC method for *o*-phenylphenol in foods; (*8*) to develop a GLC or HPLC method for succinic acid 2,2-dimethylhydrazide; (*9*) to develop and test methods for thiocarbamate herbicides in foods; (*10*) to develop and test a method for trifluralin in crops. Associate Referees are also needed for the topics Chlorothalonil; and *s*-Triazines.

Chlorophenoxy Alkyl Acids.—Associate Referee Allan Smith reports that a method has been developed for 2,4-dichlorophenoxyacetic acid in green wheat, straw, and wheat grain. The method involves an alkaline extraction from the green plant material, partitioning into ether/hexane, and cleanup by further acid-base extraction. The acid is methylated with boron trifluoride/methanol and the methyl ester is cleaned up on a 5% deactivated Florisil column. The ester must be eluted from the column within 10 min to prevent losses due to hydrolysis. The determinative step specifies electron capture GLC. By this method, recoveries at the 1 ppm level averaged 94%. Sample blanks averaged 0.03 ppm. Further study is recommended on ruggedness and statistical design.

Chlorophenoxy Alkyl Acids in Water and Waste Water.—The GLC method outlined in *J. Assoc. Off. Anal. Chem.* **64,** 394 (1981) should be collaboratively studied.

Diquat and Paraquat.—Associate Referee Harry McLeod reports that a GLC method based on that of King (*J. Agric. Food Chem.* **26,** 1460 (1978)) has been selected for the determination of diquat and paraquat in foods. In this procedure, the pyridinium salt is reduced with sodium borohydride and the resulting amine is extracted and partitioned into chloroform. The chloroform extract is concentrated and the amine is determined by GLC on an OV-17 column with an N/P detector. Recoveries of diquat from potatoes fortified from 0.06 to 10 ppm averaged 92%. Further work is being conducted to remove interferences found in some samples of substrate and to extend the method to paraquat.

1-Naphthaleneacetic Acid and 1-Naphthaleneacetamide.—Collaborators are needed by Associate Referee William P. Cochrane to study the HPLC method for naphthaleneacetic acid and naphthaleneacetamide in apples. The method is similar to that described in *J. Assoc. Off. Anal. Chem.* **62,** 100 (1979).

Pentachlorophenol.—Associate Referee Arnold Borsetti reports that a slight modification of the method described for pentachlorophenol (*J. Agric. Food Chem.* **28,** 710 (1980)) has permitted extension of the method to eggs. The modification involved replacement of the hexane final extraction solvent with a solution of hexane-toluene (4 + 1). By the modified method, recovery data were generated with eggs, gelatin, fish, and shellfish and averaged 105% overall. The Associate Referee recommends that seafood be analyzed fresh or frozen, since permitting frozen samples to warm to room temperature results in high recoveries. Limited inter- and intralaboratory trials are beginning.

Substituted Ureas.—Collaborators are needed to study the GLC method proposed by the Associate Referee for urea herbicides. The method is based on that described by Baunok and Geissbuehler (*Bull. Environ. Contam. Toxicol.* **3,** 7 (1968)).

Recommendation

Continue study on all topics as outlined above.

This report of the General Referee was presented at the 95th Annual Meeting of the AOAC, Oct. 19–22, 1981, at Washington, DC.

The recommendation of General Referee was approved by Committee E and was accepted by the Association. See the report of the committee for detailed recommendations, this issue.

Report on Metals and Other Elements

KENNETH W. BOYER
Food and Drug Administration, Division of Chemical Technology, Washington, DC 20204

Atomic Absorption Spectroscopy (AAS).—Milan Ihnat, Associate Referee, is continuing the comprehensive assessment of atomic absorption spectrophotometry (AAS) method in *Official Methods of Analysis* with the intention of consolidating the methods to the maximum extent possible into one general analytical scheme for all food commodities. The analytical technique of acid digestion flame AAS was studied to delineate parameters leading to precise and accurate results for several metals in plant and animal matrix reference materials. Factors considered were digestion technique, spectrometric measurement procedure, pure analyte and matrix matched standards and nonatomic absorption by sample matrix, and protocols for dilution of sample and standard preparations. The protocols adopted for preparation and measurement were applied to the National Bureau of Standards Reference Materials Orchard Leaves, Tomato Leaves, Bovine Liver, and a proposed Standard Reference Material Citrus Leaves to give precise and accurate data for Ca, K, Mg, Na, Cu, Fe, Mn, and Zn. The Associate Referee will continue the comprehensive assessment of AOAC AAS methods in the coming year.

Carbon Rod Atomization.—Robert Dabeka, Associate Referee, reports that collaborative study of a graphite-furnace AAS method for Pb in canned milk and infant formula was conducted with 9 participating laboratories. The study was not successful because the improper use of background correction by a majority of the collaborators resulted in an unacceptably high variability in the accuracy of the results obtained. However, during a week-long workshop conducted in the Associate Referee's laboratory, 7 analysts with diverse backgrounds were trained in contamination control and AAS background correction techniques. These analysts then analyzed the collaborative study samples and achieved excellent results.

Cadmium and Lead in Earthenware.—Benjamin Krinitz, Associate Referee, reports that the International Standards Organization (ISO) has officially approved methods of analysis that are essentially the same as AOAC **25.031-25.034** for determining extractable Cd and Pb from ceramic ware used as food contact surfaces.

John Gould, Division of Chemical Technology, FDA, has successfully completed an ISO collaborative study of the proposed ISO hot leach method, which uses a hot plate as the heat source, for leachable Pb and Cd from cookware. Sixteen collaborators, equally distributed between U.S. and non-U.S. laboratories, participated in the study. The ISO will consider adoption of this method as an official ISO method at their meeting in 1982. If it is adopted by the ISO, the method will be recommended for adoption as an official AOAC method at a later date.

Emission Spectrochemical Methods.—Fred Fricke, Associate Referee, reports that a collaborative study designed by Ronald Suddendorf for multielemental analysis of infant formula is in progress. The method consists of a nitric-perchloric acid digestion with inductively coupled plasma emission spectroscopy (ICP) as the determinative step. Of the 8 participating laboratories, 4 have completed the study

The Associate Referee is currently planning a collaborative study for multielemental analysis of raw agricultural crops by ICP. This study will be carried out when the workload permits and when sufficient collaborators with ICP capability are identified.

Fluorine (Vacant).—A collaborative study of a method for determination of fluoride in foods by microdiffusion and fluoride-specific electrode was conducted by R. W. Dabeka and A. D. McKenzie, Food Research Division, Health and Welfare, Canada with only partial success. The results of the study were submitted to this General Referee for consideration for adoption as official first action. In the study 12 collaborators each analyzed 12 freeze-dried study samples and a practice sample. The study samples consisted of 3 foods (whole milk, strained peas, and strained pears) at 3 levels (unfortified and 2 levels of fortification) with 1 blind duplicate for each food. Statistical analysis of the study results

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showed that the method performed well for milk and peas, but that low recoveries (54–65%) were obtained for pears. Thus it appears that the method may be matrix-dependent for achieving satisfactory results. In addition, a fourth food, strained chicken with broth, was prepared for inclusion in the study, but had to be rejected because of inhomogeneity with respect to fluoride. The sample size (0.1 g dry weight) appears to be a limiting factor for nonhomogeneous samples. Several recommendations for improvements in the method were made as a result of the study.

Hydride Generating Techniques.—Stephen Capar, Associate Referee, reports no progress for the year because of heavy involvement in the collaborative study for Cd and Pb in foods.

Mercury.—Ronald Suddendorf, Associate Referee, has continued work on rapid total mercury generation and determination using an apparatus described at the 1979 AOAC meeting. Initial studies have concentrated on interferences from selenium or tellurium in mercury vapor generation. Once these interferences have been fully studied, a collaborative study using this apparatus will be conducted. In the area of methyl mercury, the collaborative study of the Watts method mentioned in last year's report was not successfully completed. Because of problems related to packing and conditioning of the GC column, only 4 of the 12 collaborators completed the study without any difficulties. The problem areas have been thoroughly studied and resolved by James O'Reilly during a 1-year appointment at FDA. It is anticipated that a collaborative study of the method will be re-issued later in 1981.

Polarography.—Raymond Gajan, Associate Referee, has completed collaborative study of a rugged dry-ash voltammetric method, using potassium sulfate as an ash aid, for determination of lead and cadmium in foods. The study was issued as a cooperative effort by FDA, National Marine Fisheries Service, and National Food Processors Association. Before the collaborative study, the method was extensively tested by 6 laboratories. Twenty laboratories participated in the collaborative study by analyzing a practice sample and 12 study samples each (blind duplicates of 6 samples selected from 6 commodities each fortified at a low and a high level for Pb and Cd). The low fortification levels ranged from 0.03 to 0.08 ppm for Cd and from 0.05 to 0.15 ppm for Pb. The high levels ranged from 0.12 to 0.28 ppm for Cd and from 0.24 to 0.45 ppm for Pb. Statistical analysis of the data indicated that all but 3 of the laboratories completed the study satisfactorily. The data from 1 laboratory were rejected because they had not followed the specified instructions. The Pb data from 2 other laboratories were rejected because they obtained reagent blanks for Pb that were 4-fold higher than specified. Comparison of the levels found by the collaborators with reference values determined by the 3 issuing laboratories indicated an agreement range of 85–114% for Cd and 78–109% for Pb over all commodities and levels. Interlaboratory precision ranged from 9 to 35% standard deviation for Cd and 13 to 38% for Pb over all commodities and levels.

The success of the study was attributed in part to the timely completion and assignment of experienced analysts by the collaborating laboratories.

Voltammetric Methods.—Eric Zink, Associate Referee, is developing a method for catecholamines in blood plasma. It is suspected that blood catecholamine levels are related to blood lead poisoning and may possibly be used as an indicator of subclinical lead poisoning. Current efforts are focused on developing a reliable procedure for routine analysis of catechols at normal levels in plasma using an electrochemical detector for HPLC.

Associate Referees on all other topics reported no progress this year.

Recommendations

(1) Continue the comprehensive assessment of AAS methods by the Associate Referee for AAS.

(2) Surplus the official first action rapid screening method for Pb and Cd in earthenware, **25.035-25.037.**

(3) Collaboratively study the fluoride method by Dabeka and McKenzie again after the improvements cited above have been incorporated.

(4) Establish a new Associate Refereeship on Methyl Mercury in Fish and Shellfish to collaboratively study the Watts method as modified by J. O'Reilly for methyl mercury in fish and shellfish.

(5) Adopt as official first action the dry ash anodic stripping voltammetry method studied collaboratively by Gajan et al. for all foods except fats and oils, and continue study of the method to include fats and oils.

(6) Continue official first action status for Sn in foods, **25.136-25.138.**

(7) Continue official first action status for determination of Pb in milk and fruit juices by

anodic stripping voltammetry, **25.080-25.082.**

(8) Discontinue the Associate Refereeship on Contamination Control.

(9) Continue work on all other topics.

(10) The guidelines for designating a collaborative study as an "AOAC" collaborative study are unclear. The *Handbook of the AOAC*, 4th Ed., states on page 13: "The Associate Referee is encouraged to consult with the General Referee in his choice of a method if it is not clear which method should be collaborated upon." On page 36, the Associate Referee is instructed to "send the write-up of the method to your General Referee for review and comment before sending to collaborators . . . you may start the collaborative study as soon as the General Referee concurs." Although the second quote implies that approval by the General Referee is necessary before a collaborative study is issued, in practice this requirement is frequently ignored. This results in issuance of poorly designed collaborative studies and the consequent loss of time and resources. To standardize the conduct of collaborative studies, the following recommendations are made:

(*a*) Clearly specify in the *Handbook of the AOAC* (and in other AOAC publications, such as *The Referee*) that approval by the General Referee of the method to be collaborated and of the instructions to collaborators is *required* before the study is designated as an "AOAC collaborative study."

(*b*) Likewise, specify that the Associate Referee must obtain approval of the statistical design for a proposed collaborative study from the appropriate Committee statistical consultant before submission of the proposed study to the General Referee.

Report on Multiresidue Methods (Interlaboratory Studies)

PAUL E. CORNELIUSSEN
Food and Drug Administration, Division of Chemical Technology, Washington, DC 20204

Five Associate Referees have reported on the status of work on their topics. An Associate Referee has recently been assigned to a new sixth topic, Pollutant Phenols in Fish. The topic Pesticides in Meat and Meat Products remains vacant.

Comprehensive Multiresidue Methodology.—J. Froberg's ongoing objective has been to conduct a collaborative study on the method of Luke et al. (*J. Assoc. Off. Anal. Chem.* (1975) **58,** 1020-1026). The GLC columns which have been effective in the chromatography of polar pesticides have been prepared from stabilized DEGS. Difficulty in obtaining reproducible responses from batch to batch of DEGS packing has prevented collaborative study.

The originally published method has been modified considerably by the authors through the years, and the Associate Referee now wishes to undertake a collaborative study of the method with all modifications included. One of the more significant modifications proposed by the Associate Referee is the use of a standard additions approach to offset the quantitation problems resulting from enhanced GLC responses when sample extracts are present rather than clean standard solutions.

The General Referee recommends that the method be subjected to appropriate ruggedness and interlaboratory testing before formal collaborative study.

Gas-Liquid Chromatography (Alkaline Precolumn).—G. Miller expects during 1982 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 show effectiveness in actual food analyses.

Multielement Determination After Closed System Digestion.—W. Holak's collaboratively studied closed system nitric acid digestion method was adopted 2 years ago as official first action for

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Section numbers refer to *Official Methods of Analysis* (1980) 13th Ed., and to "Changes in Methods," *J. Assoc. Off. Anal. Chem.* (1980) **63,** 374-423.

determining arsenic, cadmium, lead, selenium, and zinc in foods (*J. Assoc. Off. Anal. Chem.* (1980) **63,** 485–495). The method has since been expanded by the Associate Referee to cover copper, chromium, and nickel.

The extension of the method to these additional elements incorporates some minor procedural changes which should be verified through collaborative study. The total method including all modifications should be published in the *Journal of AOAC* if collaborative study is successful because the revised method would replace the existing method.

The General Referee recommends that this topic be transferred to the General Refereeship on Metals and Other Elements for continued study as outlined in this report.

Organophosphorus Pesticide Residues.—Associate Referee R. Laski last year anticipated conducting a collaborative study on the method of Watts and Storherr (*J. Assoc. Off. Anal. Chem.* (1969) **52,** 522–526) as modified by the National Food Institute of Denmark (*Analyst* (1977) **102,** 865–868). That modification involves the substitution of a toluene–acetone mixture for the acetonitrile–benzene mixture in the charcoal column elution. The revised method underwent a successful interlaboratory trial (not a formal collaborative study) including 6 organophosphorus pesticides in varying combinations on 6 different crops.

The revised method would have 2 distinct advantages over the carbon column cleanup method, **29.039–29.043.** First, use of the toxic chemical benzene is eliminated. Second, acetonitrile is not used in the extraction of charcoal column elution steps. Elimination of acetonitrile will be advantageous because it has been noted that traces of acetonitrile remaining in extracts from the procedure of Watts and Storherr cause tailing responses on nitrogen-selective GLC detectors. That problem places limitation on the potential for expansion of the method to include numerous nitrogen-containing pesticides.

Unfortunately, the Associate Referee has determined that the cost of the evaporation equipment needed for the revised method prohibits obtaining enough laboratories for a collaborative study or ultimate wide use of the revised method. Accordingly, it is recommended that study be continued on this topic with a view toward expansion of the original method to cover additional chemicals as possible.

Pesticides in Meat 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 **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 for meats in the future.

Pollutant Phenols in Fish.—Associate Referee Larry Smith has been appointed to this recently established topic. He has developed a tentative procedure involving a dual column chromatographic operation to recover purified phenolic residues, derivatization, and electron capture GLC determinative step. A wide range of phenolic and other acidic residues are recoverable by this approach. At this time, the Associate Referee wishes to obtain interlaboratory evaluation of the procedure, with a future collaborative study if the interlaboratory evaluation is successful. The General Referee concurs with this course of continued study.

Whole Blood.—Associate Referee H. M. Stahr has gathered comparative recovery data between the original method (*J. Assoc. Off. Anal. Chem.* (1973) **56,** 1173–1177), which employs sulfuric acid treatment of blood followed by extraction with acetone–hexane, and a later method (*J. Assoc. Off. Anal. Chem.* (1980) **63,** 965–969) which is similar but does not include sulfuric acid treatment. The former method was studied collaboratively, and results reported in the 1973 publication were variable enough to prevent official adoption at that time. The latter method, based on tentative interlaboratory trials, appears to provide recoveries for most of the organochlorine pesticides about equal to the former method and much better for mirex (which was recovered at about 60% in the former method).

At this time the Associate Referee would like to have the original (collaborated) method considered for official first action adoption for chlorinated pesticides except mirex. He believes that data from 5 of the 12 laboratories in the original collaborative study can be discarded because of technical considerations, leaving 7 laboratories with relative standard deviations on the order of 20%. The General Referee, however, recommends that study be continued with the aim of collaborative study of the more recent method, which not only will provide coverage for mirex but also has potential for other classes of pesticides in future extension.

The Associate Referee wishes to emphasize that he has available freeze-dried calves' blood containing lindane and mirex. Because this

blood is stable, it should be extremely valuable to laboratories wishing to evaluate their own analyses of blood or compare method performance.

Recommendations

(1) Continue official first action status of the multielement determination after closed system digestion, **25.A01-25.A05.**

(2) Transfer the topic Multielement Determination After Closed System Digestion to the General Referee on Metals and Other Elements.

(3) Continue study on all other topics as outlined in this report.

Report on Organochlorine Pesticides

BERNADETTE M. McMAHON
Food and Drug Administration, Division of Chemical Technology, Washington, DC 20204

Associate Referees are appointed for 10 topics. The topics of Dicofol and Root-Absorbed Residues are vacant. A brief summary of the status and recommendations for each topic follows.

Chlordane.—Wilbur Saxton, FDA, Seattle, has been appointed as Associate Referee. He has searched the literature in an initial effort to define the terminal residues of chlordane in various substrates. This search will be supplemented by analyses of samples contaminated with (metabolized) chlordane during the coming year; appropriate samples are being requested from other laboratories. The Associate Referee also plans to study the application of capillary column GLC to the determination of chlordane residues. It is recommended that these studies continue.

Chlorinated Dioxins (David Firestone, FDA, Washington).—Work is continuing on development and evaluation of cleanup procedures suitable for the low level (parts per trillion) examination of foods for the presence of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). Work is continuing in the Associate Referee's laboratory on development and validation of a procedure to determine TCDD in fish; the method uses multi-column high performance liquid chromatography (HPLC) for cleanup of fish extracts before analysis by electron capture/capillary gas chromatography. The presence of TCDD is confirmed by capillary column gas chromatography/mass spectrometry. Work on this topic also includes preparation of reference standards for dioxins and cooperative efforts among government and industry laboratories active in the determination of TCDD. Thirteen laboratories in the United States and Canada participated in a round-robin study to check the performance of laboratories conducting analyses for TCDD. It is recommended that this work be continued.

Chlorobenzilate, Chloropropylate, and Bromopropylate (Roy Brosdal, FDA, Chicago).—The Associate Referee has studied in detail the application of 2 methods to the analysis of citrus fruit for chlorobenzilate, chloropropylate, and bromopropylate: **29.001-29.018** and the Luke procedure (*J. Assoc. Off. Anal. Chem.* (1975) **58,** 1020-1026) including the Florisil column cleanup and electron capture (EC) GLC determination. Both methods gave adequate recoveries at levels as low as 2.5 ppm, one-half the level at which the 5 ppm tolerances on citrus are set.

Because the tolerances for these chemicals in fatty foods are below the level of quantitation for method **29.001-29.018,** attempts have been made to incorporate a derivatization procedure to enhance EC-GLC sensitivity. Derivatives of the chemicals can be formed by reaction with trifluoroacetic anhydride, but the coextractives remaining in the final solution from the method for fatty foods, **29.001-29.018,** interfered with the formation of the derivatives. A better cleanup procedure is needed before such a derivatization step can be made a practical part of the method.

The Associate Referee has changed jobs and will be unable to pursue further laboratory in-

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vestigations on this topic. He intends to prepare a report of his work before resigning the refereeship. It is recommended that this report be completed, and that a new Associate Referee be sought to replace the current one when he resigns.

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).—A miniaturized method for the determination of Kepone, mirex, and Kepone metabolites in finfish and shellfish has been investigated by the Associate Referee. The method utilizes 35% water-acetonitrile as the extractant, a small Florisil column for cleanup, dihydrokepone as an internal standard, and OV-17 and OV-105 liquid phases for packed column GLC determination. Attempts to chromatograph Kepone on a fused silica capillary column have not been successful because of the incompatibility of the benzene solvent with this system. The Associate Referee plans to continue refining and testing this method with other chemicals likely to be found in fish. Recovery experiments will also be performed by other analysts in the Associate Referee's laboratory. It is recommended that the Associate Referee continue to study methods for Kepone. It is also recommended that a comparison be made of results obtained by the method currently under investigation and by the oleum procedure previously tested, in order to evaluate the extraction capabilities of the new method before further development.

Low Moisture–High Fat Samples (Leon Sawyer, FDA, Minneapolis).—At the 1981 meeting the Associate Referee presented a description of a method for extracting the oils and pesticide residues from oilseeds. This method is intended as an alternative to the fat extraction techniques of **29.012,** to be followed by the cleanup procedures of **29.014** and **29.015** or **29.029–29.034.** The method has been tested by many FDA laboratories and has been found effective. It is recommended that the method be subjected to collaborative study to expand the applicability of method **29.001–29.018** to oilseeds and some related low moisture-high fat products.

Multiresidue Methodology, Miniaturization (Ronald Erney, FDA, Detroit).—Samples for a collaborative study of a miniaturized version of **29.001–29.018** (modification of *J. Assoc. Off. Anal. Chem.* (1974) **57,** 576–579) have been prepared and sent to 7 collaborators. It is recommended that, after completion of the study and evaluation of the results, the Associate Referee prepare and submit a report on which the Committee can take interim action.

Photochemical Derivatization for Confirmation of Residue Identity (Paul Ward, FDA, Atlanta).—As recommended last year, the Associate Referee has adapted his published procedure on photolytic derivatization (*J. Assoc. Off. Anal. Chem.* (1977) **60,** 673–678) to include use of a commercially available photochemical reactor as replacement for the homemade equipment originally specified. The greater strength of the UV sources in the commercial reactor has cut optimum exposure times in half and the commercial reactor is therefore recommended by the Associate Referee. Standard solutions of 56 organochlorine pesticides known to be recovered through method **29.001–29.018** were treated by this procedure; of these, 28 formed "good" derivatives (i.e., derivatives causing a GLC response at least 10% the size of that caused by the parent compound), 11 formed poor derivatives, 9 were degraded by UV light, and 8 were unaffected by UV light. The technique of UV photolytic derivatization seems to be nonquantitative in most cases because complete conversion of the parent to the photoproduct is seldom achieved. In addition, most photoproducts are also affected by the UV light and break down further as they are irradiated; the degree of breakdown is expected to be affected by varying amounts and kinds of sample co-extractives. At best, usefulness of this method is expected to be limited to qualitative confirmation, with some quantitative application for those pesticides which form very stable derivatives.

It is recommended that work continue as follows: (1) determination of the extent to which sample co-extractives affect the photolytic derivatization of chemicals which form "good" derivatives, by performing the reactions in the presence of sample extracts cleaned up by the methods **29.001–29.018** and **29.029–29.034;** and (2) determination of the identity of the derivatives formed from any compound for which this confirmation procedure appears suitable.

Polychlorinated Biphenyls (Leon Sawyer, FDA, Minneapolis).—The Associate Referee reports that the alternative (individual peak) quantitation technique for PCBs has continued to gain acceptance as more analysts become familiar with it and as more data-handling instruments are programmed to perform the necessary calculations with ease. The original technique in which PCB residues are quantitated against commercial

Aroclor mixtures is still valuable, however, in those cases of direct contamination where the residue and Aroclor reference material give identical chromatographic responses. It is therefore recommended that the first action status of method **29.001-29.018,** including both quantitation techniques, as it applies to polychlorinated biphenyls in poultry fat, fish, and dairy products, be changed to official final action. (Note: Sections of this method describing TLC have not been collaboratively studied with PCBs and are therefore not included in this official status.)

Root-Absorbed Residues, Extraction Procedures (vacant)—The former Associate Referee, Charles Parfitt, has resigned. 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 procedure 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 procedure.

Tetradifon, Endosulfan, Tetrasul (Lawrence Mitchell, FDA, Atlanta).—No work was done on the project this year. It is recommended that the Associate Referee complete the intralaboratory trials using **29.029-29.034** to determine tetradifon, endosulfan I, II, and sulfate, and tetrasul in the 11 nonfatty foods not yet tested, so that the results can be used to validate the method for all foods now covered by **29.001-29.018.**

Toxaphene (Larry Lane, Mississippi State University).—The Associate Referee has studied the applicability of capillary column GLC with ^{63}Ni-EC detector to the quantitation of toxaphene. Toxaphene standards have initially been separated into 15 portions by thin layer chromatography and the components scraped from the TLC plate and then examined by capillary GLC. It has been found possible to separate most toxaphene components from one another in this way. It is recommended that work on this topic continue, with emphasis on the determination of the terminal residues of toxaphene and the best means by which they can be quantitated.

Recommendations

(1) Appoint Associate Referees on Dicofol and on Root-Absorbed Residues.

(2) Complete the evaluation of the currently ongoing collaborative study on miniaturization of multiresidue methodology and submit recommendation for interim action.

(3) Collaboratively study the method developed for oilseeds and some related low moisture-high fat samples.

(4) Adopt as official final action the current official first action methods for hexachlorobenzene and mirex in adipose tissue (**29.A01-29.A04,** *J. Assoc. Off. Anal. Chem.* **63,** 396 (1980)), for polychlorinated biphenyls in poultry fat, fish, and dairy products (**29.001-29.018,** including both quantitation techniques), and for polychlorinated biphenyls in paper and paperboard, **29.035-29.038.**

(5) Continue the ongoing work on chlordane; chlorinated dioxins, chlorobenzilate, chloropropylate, and bromopropylate; Kepone; photochemical derivatization; tetradifon, endosulfan, and tetrasul; and toxaphene.

Report on Organophosphorus Pesticides

KEITH A. McCULLY
Health and Welfare Canada, Health Protection Branch, Ottawa, Ontario, Canada K1A 1B7

During the past year the General Referee has continued to monitor the literature for publications on the determination of organophosphorus pesticide residues. Geen et al. (1) described a method for the determination of acephate and methamidophos in fish, sediment, and insects. Following extraction with acetonitrile, the extracts were deproteinized with a coagulating solution (an aqueous solution of ammonium chloride, sodium chloride, and phosphoric acid) and further cleaned up on a charcoal column. Residues were eluted with acetone and ethanol and determined by gas-liquid chromatography (GLC) with flame photometric detection. Saner and Gilbert (2) compared a methylene chloride liquid/liquid extraction and Sep-Pak C_{18} cartridge adsorption technique for the determination of chlorpyrifos in water, using reverse phase high performance liquid chromatography (HPLC). The presence of a large adsorbed fraction of the chlorpyrifos in the environmental water samples resulted in lower residue concentrations being determined by the methylene chloride extraction. Inman et al. (3) described procedures for the determination of chlorpyrifos and its metabolite, 3,5,6-trichloro-2-pyridinol in mint hay and oil. After extraction with hexane–2-propanol (4 + 1), chlorpyrifos residues were cleaned up on a silica gel column and quantitated by GLC with flame photometric detection. The metabolite was separated from the extraction solvent by liquid-liquid partitioning with aqueous sodium carbonate followed by cleanup on an acid alumina column. Following derivatization with *N,O*-bis(trimethylsilyl)acetamide, the residue was determined by GLC with electron capture detection. Airborne DEF residues were collected by high volume sampling through XAD-4 resin (4). Residues were extracted from the resin with ethyl ether and determined by GLC with flame photometric detection. Dialifor residues were determined in grapes by GLC with flame photometric detection following chloroform extraction and cleanup on Florisil (5). Florisil provided separation of dialifor from its oxygen analog; 6% (v/v) ethyl ether in benzene eluted the parent compound and 12.5% (v/v) acetone in benzene eluted the oxygen analog. Volpé and Mallet (6) reported the use of Amberlite XAD-4 and XAD-7 resins to recover fenitrothion and 5 possible metabolites from water. Fenitrothion, aminofenitrothion, fenitrooxon, and *S*-methylfenitrothion recoveries, using either XAD-4 or XAD-7, were over 90%. However, formylfenitrothion and hydroxymethylfenitrothion were incompletely recovered under the same conditions.

George (7) developed a method for residues of fensulfothion and its 3 metabolites. Residues were extracted with dichloromethane followed by cleanup with Nuchar C190N and oxidation with potassium permanganate. Fensulfothion and its sulfone were oxidized to their respective oxygen analogs which were determined by GLC with a nitrogen-phosphorus detector. Greenhalgh and Read (8) determined fensulfothion and fensulfothion sulfone residues in carrots, radishes, and rutabagas by GLC with a nitrogen-phosphorus detector following ethyl acetate extraction and cleanup on silica gel with acetone elution. Residues of fensulfothion and fensulfothion sulfone in rutabagas declined on storage of samples at 5°C for 90 days. In carrots, which contained higher residue levels, residues were only slightly reduced on storage.

The modified spectrophotometric method for the determination of malathion based on a bismuth complex was further modified (9). The modification involved a ligand exchange reaction in which the bismuth-dimethyldithiophosphate complex was transformed quantitatively to a bismuth-dithizone complex. The orange-yellow solution absorbed at 495 nm.

Winterlin et al. (10) reported a method for the determination of methidathion and its monoxone metabolite in safflower seed, meal, and oil by GLC with flame photometric detection. Following acetonitrile extraction, methidathion residues were cleaned up by partitioning into petroleum ether and chromatography on Florisil. Methidathion monoxone was extracted from the aqueous acetonitrile with chloroform and further cleaned up on Florisil. Iwata et al. (11) determined phenthoate residues in citrus fruit by GLC

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with a nitrogen-phosphorus detector following acetone extraction and liquid-liquid partitioning with benzene. The acetone blending extraction procedure used had an efficiency of 81% when compared with exhaustive Soxhlet extraction with methanol-chloroform (13 + 87). Phenthoate residues were stable in chopped samples in frozen storage and in acetone extracts over the 5-week period studied. Residues of phorate and its 5 oxidation products were determined in lettuce by GLC with alkali-flame ionization detection (12). Following extraction with dichloromethane-methanol (9 + 1), residues were cleaned up and fractionated on a silica gel column. Phorate, phorate sulfone, and phorate oxygen analog were eluted with cylohexane-acetone (80 + 20); phorate sulfoxide and phorate oxygen analog sulfone were eluted with cyclohexane-acetone (60 + 40); and finally phorate oxygen analog sulfoxide was eluted with acetone.

Chapman and Harris (13) reported a method for the determination of terbufos, terbufos sulfoxide, and terbufos sulfone residues in soil by GLC with alkali flame ionization detection. Following extraction with acetone and chloroform, the extracts were chromatographed on aluminum oxide to fractionate the residues. Terbufos was eluted with hexane, terbufos sulfone with benzene, and terbufos sulfoxide with chloroform. The terbufos sulfoxide fraction was oxidized by potassium permanganate to the sulfone to facilitate the GLC determination.

Carson (14) modified the method of Storherr et al. (15) which has AOAC official final action status for parathion, paraoxon, carbophenothion, carbophenothion oxygen analog, and EPN in nonfatty foods to permit its use in analyzing the residues of nonfatty food total diet composites. The modifications were: use of the entire filtered sample extract, concentration of solutions by rotary evaporation, and use of 9 g adsorbent. These permitted the determination of residues at the parts per billion level. In addition to the 5 compounds listed above, recovery data were presented for fensulfothion, monocrotophos, malathion, fonofos, fonofos oxygen analog, ethion, ronnel, dimethoate, DEF, diazinon, and phorate. An interlaboratory method trial of the potatoes and legumes composites fortified at 5–13 ppb with malathion, parathion, paraoxon, and monocrotophos was successfully carried out.

Ambrus et al. (16–18) published a series of 3 papers on a general method for the determination of pesticide residues in samples of plant origin, soil, and water. Acetone extraction followed by partitioning into methylene chloride was used. Samples were divided into 8 groups according to the extraction process and cleaned up on mixed adsorbent (active carbon, magnesia, diatomaceous earth), alumina, or silica gel columns. Elution characteristics of these columns were presented. TLC screening with several modes of detection and GLC with thermionic and electron capture detection with column packings of various polarities were used for determination. Over 50 organophosphorus compounds including some oxygen analog metabolites were included in these studies. Delventhal (19) described a multiresidue method that included 28 organophosphorus compounds. Samples were extracted with acetone–dichloromethane (1 + 1) and cleanup was on Florisil with fractionation into 2 fractions.

A method for the cleanup of foods and feeds for multiresidue analysis by gel permeation chromatography was published (20). Samples were extracted with acetone and co-extracted sample constituents were removed by gel permeation chromatography on a polystyrene gel (Bio-Beads S-X3) with ethyl acetate–cyclohexane (1 + 1) as eluting solvent. Further cleanup may be achieved on a deactivated silica gel column. Data were presented for the elution of 32 organophosphorus compounds from the gel chromatography. The elution pattern for 21 of these compounds on the silica gel column was also given. Hopper (21) developed a gel permeation chromatography system for the cleanup of fatty foods. Using ORPVA-2000 cross-linked polyvinyl acetate gel and methylene chloride–acetone (30 + 70) eluting solvent, elution volumes for 26 organophosphorus compounds were given. Thier (22) published the results of a ring test carried out in 18 laboratories on the determination of bromophos, parathion, and ethion in tomato powder. Gel chromatography was one of the 2 methods used in the study.

Desmarchelier (23) reported that 24 h extraction in acetone, methanol, or ethanol of methacrifos, fenitrothion, and pirimiphosmethyl on barley, oats, sorghum, rice in husk, malt, and polished, milled, and cooked rice resulted in recoveries ranging between 89 and 100%. Using the above solvents, complete recovery for dichlorvos was obtained for rice and rice products. A study of the application of graphitized carbon black to the extraction of trace organic pollutants from water included the organophosphorus pesticides disulfoton, malathion, parathion, and ronnel (24). Hexane–diethyl ether (1 + 1) was used to recover these compounds from the graphitized carbon black.

McDougall (25) recovered chlorpyrifos, bromophosethyl, and ethion from beef fat by using a modification of the assisted distillation technique (26) which is a modification of the sweep-codistillation technique.

Bargnoux et al. (27) employed lyophilization as a preconcentration technique in the determination of parathion, malathion, diazinon, ronnel, and dichlorvos in water. Lyophilization stabilized the samples, permitting extended storage, and yielded a concentration suitable for quantitative analysis.

Ercegovich et al. (28) developed a radioimmunoassay for parathion. Specific antibodies were developed in rabbits by using a bovine serum albumin conjugate. The method used either ^{3}H- or ^{14}C-labeled parathion as a tracer. The lower limit of detection in blood plasma and lettuce without any cleanup of the sample extract corresponded to 0.1 ppm parathion.

A brief summary of each Associate Referee topic follows:

Confirmation Procedures.—The Associate Referee was unable to work on the assignment this year. He plans to re-evaluate the pentafluorobenzyl bromide derivatization procedure and initiate a collaborative study.

High Fat Samples.—The Associate Referee reported no progress during the past year. He plans to evaluate available gel systems as to their applicability to organophosphorus pesticide residues in high fat samples.

Sweep Codistillation.—The Associate Referee reports the status of the subject is unchanged from last year. Satisfactory commercial units are still not available.

Thin Layer Chromatography.—The Associate Referee reports the status of the subject is unchanged from last year. He plans to carry out an interlaboratory study.

Other Topics.—The topics Extraction Procedures; General Method for Organochlorine and Organophosphorus Pesticides; Soils; and Water are vacant.

Recommendations

(1) Collaboratively study the Coburn and Chau (29, 30) confirmation procedure for organophosphorus pesticides recovered from water by a suitable method such as that of Ripley et al. (31).

(2) Appoint an Associate Referee to study the efficiency of extraction procedures for residues of organophosphorus pesticides.

(3) Continue studies to evaluate the applicability of the AOAC general multiresidues method to additional organophosphorus pesticides.

(4) Continue studies to evaluate the gel permeation chromatographic cleanup procedure on high fat samples for organophosphorus pesticide residues and when satisfactorily validated carry out a collaborative study.

(5) Appoint an Associate Referee for soils to develop multiresidue extraction and cleanup procedures for organophosphorus pesticides.

(6) Evaluate any commercial sweep-codistillation apparatus that becomes available and, if satisfactory, initiate a collaborative study on some organophosphorus pesticides in fatty foods.

(7) Carry out an interlaboratory study to evaluate the quantitative thin layer chromatographic approach and continue to evaluate and refine cleanup and enzyme detection procedures as applied to quantitative thin layer chromatography.

(8) Discontinue the topic Organophosphorus Pesticides in Water with further work to be carried out under direction of the General Referee for Water.

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Report on Water

A. S. Y. CHAU
Inland Waters Directorate, Water Quality Branch, Box 5050, Burlington, Ontario, Canada LTR 5A6

The new General Referee for Water sent a letter to each Associate Referee and followed up with a phone call to obtain current progress.

Chemical Pollutants in Aquatic Biota.—Associate Referee D. Stalling will continue to study the application of GPC (gel permeation chromatography-automatic) to cleanup of high fat samples of animal tissue for analysis of pesticide residues; chlorophenols in aquatic biota; and analysis of toxaphene by GLC and GC/MS negative ion.

Chlorinated Solvents in Water.—An Associate Referee is needed to develop and collaboratively study methods for water and wastewater.

Organophosphorus Pesticides in Water.—Associate Referee Patricia Smith plans to initiate a collaborative study on the EPA method for water and wastewater.

Triazine Herbicides in Water.—Appoint an Associate Referee to select methods and to initiate collaborative study for determining triazine herbicides in water and wastewater.

Recommendation

Continue study on all topics as outlined above.

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GENERAL REFEREE REPORTS: COMMITTEE F

Report on Analytical Mycology of Foods and Drugs

STANLEY M. CICHOWICZ
Food and Drug Administration, Division of Microbiology, Washington, DC 20204

The Associate Referee on Standardization of Plant Tissue Concentrations for Mold Counting has collaboratively studied a method for preparing nectars, purees, and pastes for mold counting. At present there is no method for standardizing tissue concentrations for various product forms of a fruit or vegetable. The source of mold is the comminuted fruit or vegetable tissue, which can vary in concentration. Fruits such as guavas, mangos, peaches, and pears are found in the form of nectars, purees, and preserves. In the proposed method, the product is centrifuged under standard conditions (**44.082**) and rediluted to a prescribed ratio of sediment to stabilizing solution.

The Associate Referee for *Geotrichum* Mold in Canned Fruits, Vegetables, and Fruit Juices has recommended a method of determining *Geotrichum* mold in comminuted fruit and vegetables. The official first action method (**44.079**) for whole or coarsely chopped products relies on a series of sieving steps to separate product from packing medium and mold mycelium fragments from packing liquid. With a comminuted product such as a nectar or puree, separating and concentrating the mold by sieving is impossible because the plant tissue exists in the same size range as the mycelial fragments. In the proposed method, the tissue concentration is standardized by centrifugation (**44.082**), and then the preparation is stained with crystal violet and counted.

An important training aid in teaching the Howard mold-counting technique, **44.096**, is a Howard cell, **44.002**(o)(2), a cover glass with built-in disc grid, which is a thin piece of plastic film sandwiched between 2 pieces of glass. The grid pattern on the film is composed of 5 rows of 5 circles 1.382 mm in diameter. In the past these grids have been mechanically stamped out. The resulting 1.382 mm diameter cutouts have rough or irregular edges and vary in diameter. The only other alternative has been to purchase precision-made cover glasses at a high cost, $25–$40 for the first one. The problem is compounded because large numbers are needed for workshops, and the coverslips are easily broken.

A technique has been developed to produce these grids in the laboratory. For owners of a copy stand and standard 35 mm camera with a micro lens who routinely process their own transparencies, the only additional expense is the film. A grid pattern is made using white circles (i.e., $\frac{3}{8}$ in. Prestype, or equivalent) on a black paper background. The alignment and spacing of the original sheet of transfer circles is used. The grid pattern is then photographed using a standard fine grain color reversal film. The density of grain may be decreased by over-exposing one stop. The film is then commercially processed, but not inserted in cardboard mounts.

A critical step in the process is obtaining a circle 1.382 mm in diameter on the film. The best method for obtaining the correct image size on the film is to use the following formula:

$$D = mF + 2F + F/m$$

where $m = 1.382/n$; n = diameter of grid circles (mm); F = focal length of lens (mm); and D = film plane-to-subject distance (mm).

Most cameras have the film plane marked on the top; a universal designation is a small circle with a line through it, the line being parallel to the film plane. For photographing, mount the camera on a copy stand and adjust the camera height to the nearest millimeter.

The film grids obtained can be cut up with scissors and mounted between 2 No. 2 glass coverslips, 22 × 22 mm. This will give a thin cover glass. A more durable cover glass can be made by using a standard Howard cover glass as a base with a 22 × 22 mm No. 2 cover glass over the film. A third alternative would be to cut a standard 1 × 3 in. microscope slide in half with a diamond or tungsten carbide stylus cutter, and

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Section numbers refer to *Official Methods of Analysis* (1980) 13th Edition.

use one half as the base with a 22 X 22 mm cover glass on top of the film.

The best mounting liquid is clear nail polish. To mount the grid film, put 1 or 2 drops of polish on the glass base and place the grid film on the liquid polish. Place 1 or 2 more drops of polish on top of the grid film and place that glass cover on top. Gently press down on the film-glass sandwich so that the nail polish comes to the edge. Estimating the correct amount of nail polish may require several trials.

It is recommended that these grids be used only in the earliest stages of training where the beginning mold counter is learning to recognize mold and to count positive fields. The training grid will degrade the microscope image and should never be used for routine counting.

Recommendations

(1) Adopt as official first action the method for Howard mold count of fruit nectars, purees, and pastes described by the Associate Referee.

(2) Adopt as official first action the method for *Geotrichum* mold in comminuted fruits and vegetables described by the Associate Referee.

(3) Continue study on all other topics.

Report on Disinfectants

RETO ENGLER
Environmental Protection Agency, Registration Division, Washington, DC 20460

Tubercocidal Test.—Associate Referee J. M. Ascenzi reports that studies to improve the reliability and reproducibility of the tubercocidal test are still ongoing. However, no test method has been developed which is ready to be submitted to collaborative testing.

Use-Dilution Test.—Associate Referee G. Walter reports that activities in his area have been held in abeyance. However, a means of improving the test will be considered, and suggestions by users, based on laboratory experience, are solicited

Sporicidal Tests.—T. Wendt, Surg.kos, has been studying means to improve the sporicidal testing. He should be appointed Associate Referee for those tests.

Bacteriostatic Activity of Textile Additives.—L. B Arnold and L. Smith have been suggested as Co-Associate Referees for testing the bacteriostatic activity of textile additives. A quantitative assay for evaluating textile additives is under development.

Virucidal Tests.—Associate Referee C. McDuff intends to pursue research to determine whether drying of viruses onto hard surfaces is a desirable step in the virucidal tests. Preliminary studies will be undertaken to determine the susceptibility to disinfectant of several viruses in the dry and liquid state.

Recommendation

Continue studies on all topics.

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Report on Extraneous Materials in Foods and Drugs

JOHN S. GECAN
Food and Drug Administration, Division of Microbiology, Washington, DC 20204

Collaborative studies were successful on methods for the determination of internal insect infestation of wheat (*J. Assoc. Off. Anal. Chem.* **64**, 1408–1410 (1981)); for the extraction of filth from rice flour, rice paper, and extruded rice products; and for the application of the solvent economizer apparatus to the method for filth in ground turmeric.

Collaborative studies will be initiated on methods for filth in botanicals.

New Associate Referees were appointed to the following existing topics: Vertebrate Excreta, Chemical Identification Test (formerly Mammalian Excreta, Chemical Confirmation Test); Soluble Insect and Other Animal Filth; Methods for Urine Detection (formerly Urine TLC); and Spices.

Discontinuation of the following topics is recommended: Cacao Bean Products, Ground; Dairy Products; Fruit Products; Defatting Solvent Economizer, Application to Filth Methods; Rice and Rice Products; and Tea.

Methods development research will continue on the following existing topics: Automated Filth Analysis; Filth in Botanicals; Brine Extraction Techniques; Ready-to-Eat Breakfast Cereals; Filth in Chocolate Products; Cocoa Powder and Press Cake; Canned Fish; Filth in Food Supplement Tablets; Insect Excreta in Flour; Processed Meats; Filth in Dried Mushrooms; Particulates in Large-Volume Parenterals; Mammalian Excreta Fragments in Milled Food Products; Rye Bread; Water-Insoluble Inorganic Residue in Peanut Butter; Adulteration of Botanical Drugs by Foreign Plant Materials; Mites in Stored Foods; Isolation of Extraneous Filth from Dehydrated Vegetable Products; Mite Contamination Profiles and Characterization of Damage to Foods; and Filth in Mole.

Associate Referees are needed for the following topics: Asbestos Measurements in Foods and Drugs, Filth in Canned Mushrooms, and Canned and Dehydrated Soups.

A new Associate Refereeship was established for Fecal Sterols.

Recommendations

(1) Adopt as official first action the use of the solvent economizer apparatus described by the Associate Referee as an alternative defatting procedure for ground turmeric; discontinue topic.

(2) Adopt as official first action the method for the determination of internal insect infestation of wheat.

(3) Adopt as official first action the method for filth in rice flour, rice paper, and extruded rice products described by the Associate Referee; discontinue topic.

(4) In addition to those mentioned above, discontinue the following topics: Cacao Bean Products, Ground; Dairy Products; Fruit Products; and Tea.

(5) Appoint new Associate Referees on the following topics: Fecal Sterols; Methods for Urine Detection (formerly Urine TLC); Mite Contamination Profiles and Characterization of Damage to Foods; Soluble Insect and Other Animal Filth; Spices, and Vertebrate Excreta, Chemical Identification Test (Formerly Mammalian Excreta, Chemical Confirmation Test).

(6) Continue study on all other topics.

This report of the General Referee was presented at the 95th Annual Meeting of the AOAC, Oct. 19–22, 1981, at Washington, DC.

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Report on Microbiological Methods

ARVEY C. SANDERS
Food and Drug Administration, Division of Microbiology, Washington, DC 20204

Collaborative Studies

Bacillus cereus.—Fourteen laboratories collaborated in the examination of 8 unknown *Bacillus* cultures representing 3 biotypes of the *B. cereus*, using a new method for identification. All collaborators correctly identified the cultures as *B. cereus* (biotype 1), *B. cereus* var. *mycoides* (biotype 2), or *B. thuringiensis* (biotype 3). These unknown cultures were coded with an identification number and letter combination to assure anonymity thoughout the study. In addition, one collaborator examined 5 strains of mammalian pathogen *Bacillus anthracis*, and determined that the new method is suitable for differentiating it from the other biotypes of *B. cereus*. Accordingly, the Associate Referee recommends that the official final action method for the enumeration of *B. cereus* in foods (**46.A10-46.A14**) be revised official first action to include the additional differential test for the identification of other *Bacillus* species biotypes. The General Referee concurs.

Escherichia coli and Yersinia species.—Six laboratories collaborated in a study to evaluate 2 methods for recognition of the invasive potential of *E. coli* and *Yersinia* species. HeLa monolayer cultures prepared in antibiotic-free minimal essential medium–fetal bovine serum were compared with HeLa monolayer cultures prepared with 0.2% bovine albumin fraction V dissolved in Earle's buffered salts. These preparations are referred to as the JAOAC(I) and the BAM(II) methods, respectively. All laboratories correctly detected invasiveness of *Yersinia* sp. by both methods; all participants correctly characterized invasiveness of *E. coli* by the JAOAC method while 5 of 6 did so using the BAM procedure. However, the JAOAC method was more reproducible than the BAM method. No significant difference in repeatability was observed between the methods for the invasiveness of *E. coli*. Results must be confirmed in an animal model system. The Associate Referee recommends that the JAOAC(I) method be adopted as official first action for the detection of *E. coli* invasiveness. The BAM(II) method has been under further study and may prove to be satisfactory for the detection of *Yersinia* species invasiveness. The General Referee concurs in the adoption of method JAOAC(I) for *E. coli* invasiveness.

Somatic Cell Count.—The rolling ball viscometer (RBV) has been collaboratively studied and evaluated in 3 laboratories, along with currently approved instrumental methods for measuring somatic cells in milk. In addition to the Wisconsin Mastitis Test (WMT), the participating laboratories used the Coulter counter, the DNA filter, and the Fossomatic methods. All of the collaborators preferred the instrumental methods to use of the WMT or RBV. The Associate Referee recommends further study and the General Referee concurs.

Cosmetics.—Ten laboratories participated in a comparative study of 3 rapid identification biochemical test kits and conventional tubed-media systems for the characterization of *Enterobacteriaceae*. The Associate Referee recommends that one of the test kits be adopted official first action as an alternative to the conventional tubed media. The General Referee does not concur in this recommendation because conventional tubed media presented only 67% accuracy in identification; further study with emphasis on the adequate preparation of stock cultures and isolatory substrates is recommended.

IMVIC Agar Plate Rapid Method.—Nineteen collaborators tested 20 cultures by agar plate (Powers & Latt, *Appl. Environ. Microbiol.* **34**, 274–279 (1977)) and reference methods. The test cultures were *Escherichia coli* (12), *Klebsiella pneumoniae* (6), *Enterobacter cloacae* (2), and *E. aerogenes* (1). Due to multiple assays in some laboratories, the number of assays varied between 20 and 24. Problems in correct readings of the results occurred in the citrate and MR test. False positive citrate reactions occurred when the agar in the section turned blue due to leakage of alkaline components from adjacent sections in the "X" plates. The MR reactions were often weak and easily misread as negative. The Associate Referee suggests that the leakage problem

This report of the General Referee was presented at the 95th Annual Meeting of the AOAC, Oct. 19–22, 1981, at Washington, DC.

The recommendations of the General Referee were approved by Committee F, except recommendation 1, and were adopted by the Association. See the report of the committee for detailed recommendations 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).

could be solved by using multi-well tissue culture plates instead of "X" plates. This might also solve the weak MR reactions by having less agar which could increase the concentration of acidic products, and thus intensify the MR color reaction. The Associate Referee recommends further study and the General Referee concurs.

Associate Referee Reports

Escherichia coli and Coliform Bacteria.—Previous studies resulted in the acceptance of the A-1-M procedure as an official method for the recovery of fecal coliform indicator organisms from seawater. Applicability of the A-1-M method for the detection of shellfish contamination is currently under collaborative study.

Salmonella.—The Associate Referee recommends revision of **46.058(b)**, presumptive positive reactions, to clarify directions for the treatment of typical or suspicious triple sugar iron (TSI) agar cultures. This section is subject to some interpretation when a typical TSI reaction is obtained and the lysine iron agar reaction is not typical for *Salmonella*. The revision directs analysis of TSI culture as a presumptive positive *Salmonella* culture.

Parasitology.—A collaborative study of a method for the recovery of *Ascaris* and *Trichuris* eggs has been completed. This study compared the efficacy of the original Nacconol–ether centrifugation method with the zinc sulfur flotation procedure (Lindquist) following Nacconol–ether centrifugation. The study will be evaluated in 1982.

Recommendations

(1) Revise the official final action method for the enumeration of *Bacillus cereus* in foods (**46.A10–46.A14**) to include additional differential tests for the identification of other *Bacillus* species biotypes.

(2) Adopt as official first action the method for detection of *Escherichia coli* invasiveness using monolayer HeLa cells prepared in antibiotic-free minimal essential medium with fetal bovine serum as described by the Associate Referee.

(3) Adopt as official final action the revised first action method for the detection of *Salmonella* species in edible casein and milk chocolate, **46.054–46.067**.

(4) Adopt as official final action the official first action revision of method **46.054–46.067** for the detection of *Salmonella* to delete the combination of brilliant green, *Salmonella–Shigella* (SS), and bismuth sulfite agars, and replace them by a combination of bismuth sulfite, xylose lysine desoxycholate, and Hektoen enteric agars.

(5) Editorially revise **46.058(b)**, presumptive positive reactions, to clarify directions for treatment of typical or suspicious triple sugar iron (TSI) agar cultures. This section is subject to some interpretation when a typical TSI reaction is obtained and the lysine iron agar is not typical for Salmonella. The following revision directs analysis of the TSI culture as a presumptive positive Salmonella culture:

(**b**) *Presumptive reactions.*—Incubate TSI and LIA slants at 35° for 24 ± 2 h and 48 ± 2 h, resp. Cap tubes loosely to maintain aerobic conditions while incubating slants to prevent excessive H_2S production. *Salmonella* cultures typically have alk. (red) slant and acid (yellow) butt, with or without H_2S (blackening of agar) in TSI agar. In LIA, *Salmonella* cultures typically have alk. (purple) reaction in butt. Consider only a distinct yellow coloration in butt of tube as an acid (neg.) reaction. Do not eliminate cultures that produce discoloration in butt solely on this basis. Most *Salmonella* cultures produce H_2S in LIA. Retain all presumptive pos. *Salmonella* cultures on TSI (alk. slant and acid butt) agar for biochem. and serological tests whether or not corresponding LIA reaction is pos. (alk. butt) or neg. (acid butt). Do not exclude a TSI culture that appears to be non-*Salmonella* if the reaction in LIA is typical (alk. butt) for *Salmonella*. Treat these cultures as presumptive pos. and submit them to further examination. LIA is useful in detection of *S. arizonae* and atypical *Salmonella* strains that utilize lactose and/or sucrose. Discard only apparent non-*Salmonella* TSI agar cultures (acid slant and acid butt) if corresponding LIA reactions are not typical (acid butt) for *Salmonella*. Test retained presumptive pos. TSI agar cultures as directed in **46.058**(c) to det. if they are *Salmonella* sp., **46.062**(e)(*1*), or *S. arizonae* organisms, **46.062**(e)(*2*).

If TSI slants fail to give typical *Salmonella* reactions, pick addnl suspicious colonies from selective medium plate not given presumptive pos. culture and inoculate TSI and LIA slants as in (**a**).

(**6**) Continue official first action status of the following methods: examination of frozen, chilled, precooked, or prepared foods (**46.013–46.016**); surface plating method for *S. aureus* (**46.075–46.076**); thermophilic bacterial spores in sugars (**46.026–46.030**); virus in ground beef (**46.120–46.122**).

(7) Discontinue the topic *Shigella*.

(8) Initiate the topic Detection of Helium Leaks in Canned Foods.

(9) Continue study on all other topics.

GENERAL REFEREE REPORTS: COMMITTEE G

Report on Antibiotics

STANLEY E. KATZ
Rutgers University, Cook College, Department of Biochemistry and Microbiology, New Brunswick, NJ 08903

This past year has been one of considerable activity. A number of collaborative studies have been completed: qualitative and quantitative determination of β-lactam antibiotic residues in milk, and bacitracin in premixes and mixed feeds. Both have been problem areas.

β-Lactam residues in milk are of considerable importance. Regardless of the divergent opinions concerning the epidemiological significance of such residues, it is a fact that residues are illegal. Hypersensitivity reactions notwithstanding, residues in milk pose significant problems for the production of milk products.

Analytical methodology is required to meet the wide diversity of needs. No single laboratory procedure can meet the needs of both industry and regulatory agencies, needs which vary from laboratory procedures to procedures to be used at the dairy as tank trucks unload.

There were 5 collaborative studies on β-lactam residues this year. The results of these studies will be summarized under the headings of qualitative and quantitative procedures.

β-Lactam Qualitative Procedures

1. *Delvotest Systems.*—Two systems, the multitest and the ampule test, were studied collaboratively. Both procedures are based on the same principle, namely, the test organism, *Bacillus stearothermophilus* var. *calidolactus*, produces acid which changes the indicator from purple to yellow. In the presence of inhibitors, the purple remains.

a. *Ampule Procedure.*—This procedure uses ampules seeded with the organism in solid medium, nutrient tablets, and a syringe and tips for dispensing the milk. Samples are compared against penicillinase-treated samples. Incubation time is 2½ h. The study appeared to have the capability of detecting between 0.005 and 0.007 unit penicillin/mL milk. The concentration used in the study did not span the entire range for all products, although the 0.007 unit/mL level appeared to be detected consistently. The General Referee concurs with the Associate Referee that this procedure be adopted official first action. The General Referee recommends that a level of 0.007 unit/mL be the lower limit of detectability used, applicable to all products with the exception of chocolate products.

b. *Multitest Procedure.*—This procedure uses plates containing 96 cups each. Each cup contains the assay organism in solid medium containing the bromocresol purple indicator. Nutrient tablets are added from similarly designated plates. The incubation period is 2¾ h. Samples of milk are compared against penicillinase-treated samples. The data indicated that 0.004–0.007 unit penicillin/mL milk was the lower limit of detection for different products. The Associate Referee recommended adoption as official first action. The General Referee concurs but recommends that the lower limit of detection be 0.007 unit/mL; this level can be detected consistently in a wide variety of products.

With the exception of chocolate products, where the color change is obscured, these 2 methods should be adopted official first action.

2. *Disc Assay Procedure.*—This procedure is designated as an alternative to the present official first action qualitative disc method. The procedure is based on a pad-plate technique using *B. stearothermophilus* as the assay organism and is capable of determining the presence of 0.008 unit penicillin/mL milk. The test requires 3–4 h for incubation at 55°C, or 2–3 h for incubation at 64°C. Zones greater than 14 mm indicate the presence of an inhibitory substance; zones less than 14 mm are considered negative. Samples are confirmed with a penicillinase treatment. The Associate Referee recommended adoption as official first action. The General Referee concurs.

3. *Charm Test.*—The 15-min assay system, commercially known as the Charm Test, is based

This report of the General Referee was presented at the 95th Annual Meeting of the AOAC, Oct. 19–22, 1981, 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 for detailed recommendations.

on the specific, irreversible affinity of penicillin and other β-lactam antibiotics for select enzyme sites on the cell wall of microorganisms. The assay measures the quantity of ^{14}C-labeled penicillin added to milk and measures the degree of binding to the sites. The degree of binding is correlated to the amount of β-lactam present in milk.

The results of the collaborative study indicated no significant differences between the proportion of positive milk samples, milk containing 0.01 unit penicillin/mL, and negative milk samples. Using a control point which is at least 3 standard deviations from the 0.01 unit/mL mean, 100% of the samples were detected correctly. Using a control point that would be the lower limit of the 95% confidence limit, 95% of the samples were detected correctly. The Associate Referee recommended that this method be adopted official first action. The General Referee concurs for the qualitative detection of penicillin levels no lower than 0.01 unit/mL.

β-Lactam Quantitative Procedures

The study of a quantitative estimate of β-lactam residues in raw milk used *B. stearothermophilus* ATCC No. 10149 as the test organism and a disc or pad-plate system. Standards and unknown milk samples were applied to the discs with 90 μL pipets, and plates were incubated at 64 ± 2°C for 2¾ h.

The results indicated that 51% of the samples were quantifiable at 0.016 unit/mL concentration, 69% at 0.017 unit/mL, 96% at 0.018 unit/mL, and essentially 100% above 0.018 unit/mL. Concentrations of 0.003 unit/mL different from the reference concentration were distinguishable at the 95% confidence limits. The Associate Referees recommend acceptance as official first action.

The General Referee concurs, with a level of 0.016 unit penicillin activity as the lower limit of quantitation. The reference level of 0.016 unit/mL is relatively insensitive, but the procedure fills a niche and a need for a rapid quantitative procedure.

Bacitracin

This year there were 3 collaborative studies on determination of bacitracin in premixes and mixed feeds.

1. *HPLC Procedure for Bacitracin in Premixes.*—The HPLC procedure studied is a new approach to a refractory problem. Bacitracin is composed of at least 3 bioactive components. The procedure separates and measures all 3 components. The method recovered 96.3–115.8% of the MD bacitracin activity, with an average value of 101.0 ± 4.6%. Recoveries obtained for Zn bacitracin ranged from 96.8 to 111.2%, with an average value of 103.1 ± 3.6%. These recoveries were obtained in premixes ranging from 10 to 50 g/lb.

The Associate Referee recommends adoption as official first action. The General Referee concurs, recognizing the fact that the combination of collaborators and samples, namely 54, was slightly below the recommended number of 60. The overall consistency of results is sufficiently good to offset this modest problem.

2. *Microbiological Assay for Bactracin in Premixes.*—The microbiological assay is a cylinder-plate system. Recoveries obtained from MD bacitracin premixes ranged from 95 to 106.1%, with an average recovery of 101.2 ± 4.2%. Recoveries obtained from Zn bacitracin premixes ranged from 96.7 to 113.0%, with an average recovery of 103.1 ± 4.8%. Recoveries were obtained in premixes ranging from 10 to 50 g/ton.

The Associate Referee recommends adoption as official first action. The General Referee concurs, and emphasizes that equivalent results were obtained by both the HPLC and microbiological procedures on the same samples, that bacitracin premixes are biomass products and do not have a known concentration of antibiotic and, hence, only method recoveries are possible.

The results obtained from 2 different assay systems agree remarkably well. It is the opinion of the General Referee that 2 reproducible systems are now available for the assay of bacitracin activity in premix materials.

3. *Microbiological Assay for Bacitracin in Mixed Feeds.*—The microbiological assay procedure, collaboratively studied, was based on the cylinder-plate technique. The extraction/cleanup procedures were quite complicated. The results reflected poor recoveries and considerable variation. The Associate Referee recommends continued study and that the procedure not be considered for official action. The General Referee concurs.

Report on Color Additives

KEITH S. HEINE
Food and Drug Administration, Division of Color Technology, Washington, DC 20204

The Associate Referee on Atomic Absorption in Color Analysis, L. Moten, reports that in his continuing study of methods for determination of trace elements in color additives exempt from certification he has developed a method for detection of lead in iron oxide pigments, as low as 5 ppm.

The Associate Referee on High Pressure Liquid Chromatography (HPLC), E. Cox, has submitted a collaborative study on the HPLC determination of intermediates and reaction by-products in FD&C Yellow No. 5 and has recommended that the method be adopted official first action. The Associate Referee has also submitted a paper on a reverse phase HPLC method for the determination of intermediates, reaction by-products, and subsidiary colors in FD&C Yellow No. 6.

Also under this topic two papers have been contributed by Calvey and Goldberg: Automated HPLC Determination of Intermediates/Side Reaction Products in FD&C Red No. 3, and High Performance Liquid Chromatographic Determination of Subsidiary Colors in FD&C Red No. 3.

The Associate Referee for Color in Other Foods, N. Adamo, reports that he is working on a collaborative study involving colors in specific categories of foods in an attempt to make the study more statistically acceptable.

The Associate Referee for Colors in Candy and Beverages, M. Young, reports preliminary success in the separation of FD&C colors from commercial products, and plans further work to refine the method and apply it to natural and EEC colors.

The Associate Referee for Inorganic Salts, W. Brammell, reports some promising results on determining iodide in FD&C Red No. 3 with iodide ion-selective electrodes and hopes to develop an improved method for this determination.

The Associate Referee for Intermediates, Uncombined, in Other Certifiable Colors, P. Bulhack, has asked to be relieved of this assignment because of other AOAC duties.

Recommendations

(1) On the basis of routine use by the Division of Color Technology for the past year, adopt as official final action the method for HPLC determination of intermediates and reaction by-products in FD&C Red No. 40 (**34.B01-34.B06**).

(2) Adopt as official first action the HPLC determination of intermediates and reaction by-products in FD&C Yellow No. 5.

(3) Discontinue the topic Intermediates, Uncombined, in Other Certifiable Colors.

(4) Continue study on all other topics.

This report of the General Referee was presented at the 95th Annual Meeting of the AOAC, Oct. 10–22, 1981, 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 for detailed recommendations.

Report on Cosmetics

RONALD L. YATES
Food and Drug Administration, Division of Cosmetics Technology, Washington, DC 20204

Essential Oils and Fragrance Materials, Composition.—Associate Referee Harris H. Wisneski has completed work on the development of an analytical method for the determination of safrole (1,2-methylenedioxy-4-allylbenzene) in fragrances. The proposed procedure uses combined high pressure liquid chromatography/fluorometry as the respective separation and

detection techniques. Recoveries ranged from 98 to 102% with a mean of 100%. Standard deviation was ±0.8%.

No Associate Referee reports were received on the remaining topics.

Recommendations

(1) Continue official first action status of the method for soluble zirconium, **35.019-35.023,** and the method for water and alcohol, **35.001-35.006.**

(2) Continue study on all other topics.

This report of the General Referee was presented at the 95th Annual Meeting of the AOAC, Oct. 19-22, 1981, at Washington, DC.

The recommendations of the General Referee were approved by Committee G and were accepted by the Association. See the report of the committee for detailed recommendations.

Report on Drugs in Feeds

RODNEY J. NOEL
Office of Indiana State Chemist, Department of Biochemistry, Purdue University, West Lafayette, IN 47907

Amprolium.—Associate Referee Kathleen Eaves is studying a high performance liquid chromatographic procedure. A ruggedness test is planned for the near future with a possible collaborative study planned for next summer.

Arprinocid.—Associate Referee David W. Fink has reported on a collaborative study. Sixteen laboratories analyzed pairs of samples of arprinocid in the concentration range 10.1-16.2%. Each collaborator ran 16 samples, 8 on different days. Arprinocid is extracted with $CHCl_3$, transferred into 0.1N HCl, and measured at 258 nm after liquid-liquid partitioning. Means ranged from 96.9 to 101% of the true concentration and were not statistically different ($P > 0.1$) from the true values. Systematic standard deviations of the 4 unit blocks (s_b) were each 0.4% arprinocid, and analysis of variance shows that laboratory bias is not statistically significant ($P < 0.05$). There is no statistically significant difference in either accuracy ($P < 0.5$) or precision ($P < 0.05$) between days. Ruggedness test confirms that none of the 7 procedural variations which were selected for study has a significant effect on the analyses. The Associate Referee recommends that the method be adopted official first action.

Carbadox.—Associate Referee Mark A. Litchman is continuing work on a high performance liquid chromatographic method.

Dimetridazole.—Associate Referee Larry Frahm recommends that method **42.063** remain official first action.

Ethopabate.—Associate Referee Kathleen Eaves has developed a high performance liquid chromatographic method and plans a possible collaborative study next summer.

Furazolidone and Nitrofurazone.—Associate Referee Robert L. Smallidge plans a collaborative study this fall for furazolidone, using high performance liquid chromatography.

Nifursol.—Associate Referee Glenn M. George recommends that method **42.098** remain official first action.

Pyrantel Tartrate.—Associate Referee James Braswell is continuing to develop a high performance liquid chromatographic method.

Roxarsone.—Associate Referee Glenn M. George has reported on a method specifying atomic absorption spectroscopy with a graphite furnace. Work on a high performance liquid chromatographic method is also progressing.

Sulfa Drug Residues in Animal Feeds.—Associate Referee Robert K. Munns is preparing a collaborative study for a rapid method of determining sulfamethazine residues in feeds.

No other reports were received.

Recommendations

(1) Adopt the arprinocid method official first action.

(2) Continue study on arsanilic acid and appoint a new Associate Referee.

(3) Continue official first action status of **42.063,** 1,2-dimethyl-5-nitroimidazole.

(4) Continue official first action status of **42.098,** nifursol.

(5) Continue study on all other topics.

This report of the General Referee was presented at the 95th Annual Meeting of the AOAC, Oct. 19-22, 1981, 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 for detailed recommendations.

Section numbers refer to *Official Methods of Analysis* (1980) 13th Ed.

Report on Toxicological Tests

SAMUEL I. SHIBKO
Food and Drug Administration, Division of Toxicology, Washington, DC 20204

AOAC no longer has Associate Referees in the following areas: LD_{50} Test; Aspiration Test; Rabbit Eye Irritation Test; Skin Irritation Test; Skin Sensitization Test. The situation is reflective of the difficulty in developing collaborative studies involving whole-animal studies. A similar situation also exists in tests involving in vitro systems. A major cause appears to be the lack of funds to support such studies. Where regulatory agencies have decided to fund studies to validate techniques, e.g., tests in behavioral toxicology, such studies are being carried out without the involvement of AOAC. This raises the question of the role of AOAC in developing collaborative toxicity studies, which was discussed at a roundtable discussion at the 94th annual meeting of AOAC. A previously unpublished report developed by representatives at the meeting is incorporated into this report. The recommendations to AOAC include (1) development of quality assurance of existing methods, rather than standardization of methods, and (2) development of a consensus body to discuss quality assurance rather than develop refereeships for toxicity tests. These recommendations represent a major departure from the normal AOAC procedures and need to be given serious consideration by the committees concerned with method development.

Cell Culture Enzyme Induction Bioassay System.—(June Bradlaw). Results have been submitted of a pre-collaborative study carried out by Thomas W. Sawyer (Department of Chemistry, University of Guelph, Guelph, Ontario, Canada), as part of the requirements for a Ph.D thesis on the biological activity of PCB congeners. Results showed that the cell culture enzyme induction method can be followed from the written procedure, and provides a precision and sensitivity similar to previously reported findings (Bradlaw, J. A., & Casterline, J. L., Jr. (1979) *J. Assoc. Off. Anal. Chem.* **62**, 904–916). No additional collaborative studies are planned at this time.

In Vitro Mutagenic Assay using L5178Y/TK$^+$/-Mouse Lymphoma Cells.—(Kenneth Palmer). Initiation of a collaborative study has been discussed with investigators that are actively performing the L5178Y/TK$^+$/-assay. There is little interest because of the cost. If AOAC is able to obtain funds to support a collaborative study, then it is likely that other laboratories will participate.

NTP (National Toxicology Program) sponsored a study to validate the L5178Y/TK$^+$/-assay (not involving AOAC). However, there is concern about the usefulness of the study, primarily because of the criteria used to accept data from the 2 laboratories in the study.

Ames Test.—(Virginia Dunkel). Four laboratories participated in a collaborative study to evaluate the reproducibility of the Ames *Salmonella* plate incorporation assay; 50 different chemicals have been tested. Preliminary evaluation of the data indicates good agreement among laboratories.

Role of AOAC in Developing Collaborative Studies

The need for evaluation of biochemical effects and pathophysiology of chemicals and naturally occurring compounds has resulted in the rapid growth of toxicological testing. If toxicological evaluation is to be done in a scientifically rational and cost-effective manner, the reliability of the test methods as well as the manner in which the tests are used should be carefully defined. A preliminary discussion of those matters took place at a roundtable discussion during the 94th annual meeting of AOAC.

The following representatives of major toxicological professional societies, and other groups involved in toxicity studies, participated in the discussion: V. Morgenroth, Interagency Regulatory Liaison Group; J. Dean, National Toxicology Program; W. Hayes, American Society of Pharmacology and Experimental Therapeutics; E. Pfitzer, Society of Toxicology; B. Butterworth, Chemical Industry Institute of Toxicology; S. I. Shibko, T. Balaaz, and J. O'Rangers, AOAC.

It was the consensus of all participants that better standardization of existing test procedures is needed and efforts in this direction would be helpful. But specific standardization procedures must be implemented extremely carefully, with full and open communication between all interested parties, and with the scientific commonly having the opportunity to evaluate and comment on standard proposals. Not only must the standardization process be flexible but it must

be international in scope. Because toxicology is a relatively new and rapidly moving discipline, there is some concern that standardization could have an inhibitory effect on creative research. This is a very critical point, since inhibition of research would have far more serious long-range consequences would lack of standardization. Initial efforts should be directed at quality assurance of existing procedures being used for safety evaluation. This clearly recognizes that research investigations must be free of a priori methodological constraints if good science is to be achieved.

Recommendations

(1) AOAC, with competent scientific advice, should develop a list of quality assurance concerns relative to toxicological testing and then invite articles for publication in the *Journal*.

(2) AOAC should provide a forum for discussion of toxicological quality assurance matters.

(3) At this time AOAC should not develop toxicological refereeships, but should encourage or participate in a consensus body to discuss quality assurance problems.

This report of the General Referee was presented at the 95th Annual Meeting of the AOAC, Oct. 19–22, at Washington, DC.

The recommendations of the General Referee were approved by Committee G and were accepted by the Association. See the report of the committee for detailed recommendations, this issue.

TRANSACTIONS OF THE ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS

The ninety-fifth annual meeting of the Association of Official Analytical Chemists was held at the Marriott Motor Hotel, Twin Bridges, Washington, DC, on October 19, 20, 21, and 22, 1981. The following reports, along with the actions of the Association, were given at the business meeting held Thursday, October 22, 1981, Helen L. Reynolds presiding.

Report of the Official Methods Board

ELMER GEORGE, JR, *Chairman*
NY State Department of Agriculture and Markets, Food Laboratory, Albany, NY 12235

Other members: R. L. Burnelle, K. Helrich, J. C. Kissinger, H. P. Moore, A. Romano, Jr, H. M. Wehr, D. N. Willett

The 95th Annual Meeting of the AOAC marks the first meeting of the Official Methods Board since its creation in 1980. Richard L. Burnelle and Anthony Romano did not attend. Evelyn Sarnoff represented Anthony Romano.

Nominations were received for the best Associate Referee Report of the year: Vernon J. Meinen, Committee A; Walter Holak, Committee B; Nrishinha P. Sen, Committee C; Peter S. Vora, Committee D; Raymond J. Gajan, Committee E; Russell G. Dent, Committee F; and David W. Fink, Committee G. Raymond J. Gajan was chosen as the winner. The award was given by President Helen L. Reynolds at the General Session, October 19, 1981.

The association received 40 reports of collaborative studies conducted during the year and gave interim first action status to 9 reports. Thirty-two collaborative study reports were submitted last year. An average of 48 reports/year has been considered for the last decade.

Proposed Board functions occupied a major portion of the first committee agenda. Members were requested to give consideration to the proposals and make further suggestions during the next two months, with final recommendations submitted to the Board of Directors by January 1982.

The Official Methods Board reviewed a recommendation from Kenneth W. Boyer for standardizing the procedure for calculating percent recovery of analytes. The recommendation was amended and circulated for comments among attendees at the combined meeting of the Official Methods Board, Committees A-G, and General Referees. No further changes were made in the amended copy. It is recommended that the Board of Directors approve the amended procedure and publish it in *The Referee*.

The Official Methods Board also concurs with the intent of the recommendations of the Ad Hoc Nitrogen-to-Protein Conversion Factor Committee. Committees A, C, and D, which will be affected by the proposed action, presented their recommendations to the Association for adoption.

Committees F and G were requested to prepare recommendations to the Association on standardization of microbiological media ingredients and the need for establishment of generic equivalents of proprietary names of such ingredients.

The Chairman sent the customary letters to all Associate and General Referees in January and June outlining responsibilities and providing a schedule for submitting reports.

Accepted.

Report of Committee A on Recommendations for Official Methods

HOWARD P. MOORE (State Department of Agriculture, Feed and Fertilizer Section, Reynoldsburg, OH 43068), *Chairman;* ALAN R. HANKS (Texas A & M University, Agricultural Analytical Services, College Station, TX 77843); GUNTER ZWEIG (University of California at Berkeley, School of Public Health, Berkeley, CA 94720); RICHARD H. COLLIER (Purdue University, Department of Biochemistry, West Lafayette, IN 47907), *Secretary;* and EDWIN M. GLOCKER (14697 Roxbury Rd, Glenelg, MD 21737), *Statistical Consultant*

The Committee wishes to emphasize the importance of inclusion of samples representative of large lots of material in collaborative studies. When analytes and sample matrix are sufficiently stable, the use of such samples in these studies will enable the materials to be used as Reference Materials or possibly Certified Reference Materials. We recommend that, when planning collaborative studies, Associate Referees in all areas of AOAC contact Robert Alvarez, General Referee on Reference Materials and Standard Solutions, to determine whether a Reference Material is available or whether a material which could serve as the basis for a new Reference Material may be included in the collaborative study.

A number of Associate Referees are needed to fill vacancies and establish new topics. Of particular significance is the vacancy in Crude Fat in Pet Foods. A number of problems continue in this area. Persons interested in serving in this capacity are urged to contact the General Referee for Feeds, Clyde Jones, Colorado Department of Agriculture.

Committee A concurs with the recommendation of the Nitrogen-to-Protein Conversion Factor Committee.

Committee A concurs with the recommendation that a uniform procedure for the determination of analyte recovery be adopted. Committee A further suggests that the language of such a procedure should provide that the concentration of added analyte should be at least equal to the concentration of analyte in the unfortified sample *and* should be in the range of concentration expected in samples to be analyzed.

FEEDS

(1) *Amino Acid Analysis in Mixed Feeds:* Appoint Associate Referee and continue study.

*(2) *Fat, Crude, in Pet Foods:* (a) Editorially modify the subtitle of the methods for crude fat or ether extract (**7.055–7.060**) to delete the word "entirely" in its two occurences. The modified subtitle reads as follows: Use method **7.056** or **7.057** for mixed feeds other than (*1*) baked and/or expanded, (*2*) dried milk products, or (*3*) contg urea. (b) Appoint Associate Referee and continue study.

*(3) *Fiber, Crude:* (a) Adopt as official first action the substitution of ceramic fiber for asbestos fiber, as described by the Associate Referee (*J. Assoc. Off. Anal. Chem.* **65,** 265–269 (1982)).

(4) *Fiber, Crude, in Milk Replacers:* Continue study.

(5) *Infrared Reflectance Techniques in Mixed Feeds:* Appoint Associate Referee and continue study.

(6) *Minerals:* Continue study.

(7) *Non-Nutritive Residues:* Continue study.

(8) *Protein, Crude:* Continue study.

(9) *Sampling:* Continue study.

(10) *Water (Karl Fischer Method):* Continue study.

FERTILIZERS

(1) *Biuret in Urea and Mixed Fertilizers:* Continue study.

*(2) *Boron:* (a) Adopt as official first action the azomethine H colorimetric method as proposed by the Associate Referee (*J. Assoc. Off. Anal. Chem.* **65,** 234–237 (1982)). (b) Continue study.

(3) *Calcium and Magnesium:* Continue study.

(4) *Copper:* Continue study.

(5) *Elemental Analysis of Liming Materials:* Continue study.

(6) *Free and Total Water:* Continue study.

(7) *Iron:* Continue study.

(8) *Molybdenum:* Appoint Associate Referee and continue study.

(9) *Nitrogen:* (a) Continue official first action status of the following methods:

* 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 Ed., and "Changes in Methods," *J. Assoc. Off. Anal. Chem.* (1980) **63,** 374–423; (1981) **64,** 501–540.

water-insoluble nitrogen, Method II (**2.073-2.074**); modified comprehensive nitrogen (**2.061-2.062**). (b) Continue study.

(10) *Phosphorus:* Continue study.
(11) *Potash:* Continue study.
(12) *Sampling and Preparation of Sample:* Continue study.
(13) *Slow-Release Mixed Fertilizers:* Continue study.
(14) *Sodium:* Continue study.
(15) *Soil and Plant Amendment Ingredients:* Continue study.
(16) *Sulfur:* (a) Continue official first action status of the following method: sulfur (**2.A01**). (b) Continue study.
(17) *Water-Soluble Methylene Ureas:* Continue study.
(18) *Zinc:* Initiate 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:* Continue study.
(6) *Flammable Substances in Pressurized Containers:* Continue study.
(7) *Flash Point of Solids and Semisolids:* Continue study.
(8) *Formaldehyde:* Continue study.
(9) *Hazardous Components in Resin Systems:* Continue study.
(10) *Pentachlorophenol in Toy Paints:* Continue study.
(11) *Petroleum Distillates in Mixtures:* Continue study.
(12) *Selenium:* Continue study.
(13) *Toxic Metals in Paints:* Continue study.
(14) *Turpentine:* Continue study.
(15) *Viscosity of Liquids:* Continue study.
(16) *Other Topics:* (a) Continue official first action status of the method for volatile denaturants in alcoholic products (**5.012-5.013**). (b) 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:* (a) Adopt as official first action the interim official first action gas-liquid chromatographic method (*J. Assoc. Off. Anal. Chem.* **64,** 1315–1318 (1981)). (b) Continue study.

PESTICIDE FORMULATIONS: FUNGICIDES AND DISINFECTANTS

(1) *Benomyl:* Continue study.
*(2) *Captan:* (a) Adopt as official final action the official first action gas-liquid chromatographic (**6.215-6.219**) and the high performance liquid chromatographic methods (**6.A09-6.A14**). (b) Continue study.
(3) *Carboxin:* Continue study.
(4) *Chlorothalonil:* Continue study.
*(5) *Copper Naphthenate:* (a) Adopt as official final action the official first action titrimetric and electrolytic methods (**6.065-6.066**). (b) Continue study.
(6) *Dinocap:* Continue study.
(7) *Dithiocarbamate Fungicides:* Continue study.
(8) *Folpet:* Continue study.
(9) *Oxycarboxin:* Continue study.
*(10) *Pentachloronitrobenzene:* (a) Adopt as official first action the gas-liquid chromatographic method described by the Associate Referee (*J. Assoc. Off. Anal. Chem.* **65,** 110–114 (1982)). (b) Continue study.
(11) *o-Phenylphenol:* Continue study.
*(12) *Triphenyltin:* (a) Adopt as official final action the official first action potentiometric titration method (**6.436-6.439**).

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):* (a) Delete the official first action method for sampling of pressurized cans (**6.002**). (b) 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 for water-soluble copper in water-insoluble copper fungicides (**6.B01-6.B08**).

PESTICIDE FORMULATIONS: HALOGENATED INSECTICIDES

(1) *Benzene Hexachloride and Lindane:* (a) Continue official first action status of the benzene hexachloride radioactive tracer method (**6.202**). (b) Continue study.
*(2) *Chlordane:* (a) Declare as surplus the following methods: AG chlordane (**6.232-6.235**), AG chlordane in granular formulations—infrared method (**6.236-6.240**), heptachlor in AG chlordane—GLC method (**6.241-6.244**), and colorimetric method for chlordane (**6.223-6.227**). (b) Continue study.
(3) *Chlordecone:* Discontinue topic.
(4) *Chlordimeform:* Continue study.
(5) *Dicofol (1,1-Bis(chlorophenyl)-2,2,2-trichloroethanol):* (a) Continue official first action status of the hydrolyzable chloride method (**6.283-6.288**). (b) Continue study.
(6) *Endosulfan:* Continue study.
(7) *Fenvalerate:* Continue study.
*(8) *Heptachlor:* (a) Declare as surplus the gas-liquid chromatographic method for heptachlor in AG chlordane (**6.241-6.244**). (b) Continue study.
(9) *Methoxychlor:* Continue study.
(10) *Perthane:* Continue study.
*(11) *Tetradifon:* (a) Adopt as official final action the official first action CIPAC-AOAC gas-liquid chromatographic method (**6.B09-6.B14**). (b) Continue study.
(12) *Toxaphene:* Continue study.
(13) *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-chlorophenoxyacetic 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) *Diphacinone:* Continue study.
(4) *Pentachlorophenol:* Continue study.
(5) *Picloram:* Continue study.
(6) *Plant Growth Regulators:* Continue study.
(7) *2,3,6-Trichlorobenzoic Acid:* 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:* Continue study.
(5) *Bromacil and Lenacil:* Continue study.
(6) *Chloroxuron:* Continue study.
(7) *Dimethyl Tetrachloroterephthalate:* Continue study.
(8) *Dinitramine:* Discontinue topic.
(9) *Dinoseb:* Continue study.
(10) *Diuron:* Continue study.
(11) *S-Ethyl Dipropylthiocarbamate:* Continue study.
(12) *Fluchloralin:* Continue study.
(13) *Fluometuron:* Continue study.
(14) *Linuron:* Continue study.
*(15) *Methazole:* (a) Adopt as official first action the interim official first action infrared method for methazole (*J. Assoc. Off. Anal. Chem.* **64**, 1185–1186 (1981)). Continue study.
(16) *Monuron:* Continue study.
(17) *Oryzalin (3,5-Dinitro-N,N-(dipropyl)sulfanilamide):* Continue study.
(18) *Paraquat:* Continue study.
(19) *Penoxalin:* Continue study.
(20) *Profluralin:* Continue study.
(21) *Siduron:* Continue study.
(22) *Thiocarbamate Herbicides:* (a) Continue official first action status of the gas-liquid chromatographic method for thiocarbamate herbicides (**6.426-6.430**). (b) Continue study.

(23) *Trifluralin and N-Butyl-N-ethyl-α,α,α-trifluoro-2,6-dinitro-p-toluidine (Benefin®):* (a) Continue official first action status of the method for trifluralin (**6.314**). (b) Continue study.

PESTICIDE FORMULATIONS: HERBICIDES III

(1) *Alachlor and Propachlor:* Continue study.
(2) *Amitrol:* Continue study.
(3) *Atrazine:* Include under Triazine Herbicides.
(4) *Bentazone:* Continue study.
(5) *Bromoxynil:* Continue study.
(6) *Cacodylic Acid:* Continue study.
(7) *Cyanazine (Bladex®):* Continue study.
(8) *Dalapon:* Continue study.
(9) *Dichlobenil:* Continue study.
(10) *Disodium Methane Arsenate:* Continue study.
(11) *Glyphosate:* Continue study.
(12) *Metalochlor:* Continue study.
(13) *Metribuzin (4-Amino-6-(1,1-dimethylethyl)-3-(methylthio)-1-2,4-triazin-5(4H)one):* Continue study.
(14) *Monosodium Methane Arsenate:* Continue study.
(15) *Propanil (3′,4′-Dichloropropionanilide):* Continue study.
(16) *Terbuthylazine:* (a) Continue official first action status of the gas-liquid chromatographic method (**6.B20-6.B27**). (b) Continue study.
(17) *Triazine Herbicides:* Continue study.

PESTICIDE FORMULATIONS: INORGANIC PESTICIDES

(1) *Aluminum Phosphide:* Continue study.
(2) *Sodium Chlorate:* Continue study.

PESTICIDE FORMULATIONS: ORGANOTHIOPHOSPHORUS 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:* (a) Adopt as official final action the official first action method (**6.B15-6.B19**). (b) Continue study.
(4) *Coumaphos:* Continue study.
(5) *Demeton:* Continue study.
(6) *Demeton-S-Methyl:* Continue study.
(7) *Diazinon (O,O-Diethyl O-(2-Isopropyl-6-methyl-4-pyrimidinyl) Phosphorodithioate):* (a) Continue official first action status of the gas-liquid chromatographic method for diazinon (**6.331**). (b) Continue study.
(8) *Dimethoate:* Continue study.
(9) *Dioxathion:* Continue study.
(10) *Disulfoton:* Continue study.
*(11) *Encapsulated Organophosphorus Pesticides:* (a) Adopt as official final action the official first action gas-liquid chromatographic method for encapsulated ethyl and methyl parathion (**6.409-6.414,** as modified, *J. Assoc. Off. Anal. Chem.* **63,** 380 (1980)). (b) Adopt as official first action the gas-liquid chromatographic method for diazinon described by the Associate Referee (*J. Assoc. Off. Anal. Chem.* **65,** 115–118 (1982)). (c) 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:* Continue study.
(17) *Fenthion:* Continue study.
(18) *Fonophos:* Continue study
(19) *Malathion:* Continue study.
(20) *Methidathion (Supracide®):* Continue study.
(21) *Parathion and Methyl Parathion:* (a) 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 the high performance liquid chromatographic (**6.384-6.387**) methods for parathion, and the gas-liquid chromatographic (**6.400-6.404**) and the high performance liquid chromatographic (**6.405-6.408**) methods for methyl parathion. (b) Continue study.
(22) *Phorate:* Continue study.
*(23) *Temephos:* (a) Adopt as official first action the high performance liquid chromatographic method (CIPAC provisional method). (b) Continue study.

PESTICIDE FORMULATIONS: OTHER INSECTICIDES, SYNERGISTS, AND INSECT REPELLANTS

(1) *Allethrin:* (a) Continue official first action status of the gas-liquid chromatographic method for technical allethrin (**6.149-6.154**). (b) Continue study.

(2) *2,3,4,5-Bis(2-butylene)tetrahydro-2-furfural (MGK Repellant 11®)*: Continue study.
(3) *Dipropyl Isocinchomeronate (MGK Repellant 326®)*: Continue study.
(4) *Fumigants:* (a) Continue official first action status of the gas-liquid chromatographic method for fumigant mixtures (**6.143-6.148**). (b) Initiate a study for sulfuryl fluoride under this topic. (c) Continue study.
(5) *Nicotine:* Continue study.
(6) *N-Octyl Bicycloheptenedicarboximide (MGK 264®)*: Discontinue topic.
(7) *Permethrin:* Continue study.
*(8) *Piperonyl Butoxide and Pyrethrins:* (a) Adopt as official first action the gas-liquid chromatographic method described by the Associate Referee. (b) Continue study.
(9) *Resmethrin:* Continue study.
(10) *Rotenone and Other Rotenoids:* (a) Continue official first action status of the infrared method for rotenone (**6.163-6.164**) (b) Continue study.
(11) *Other Topics:* Continue official first action status of the UV method for sulfoxide (**6.419**).

PESTICIDE FORMULATIONS: OTHER ORGANOPHOSPHATE INSECTICIDES

(1) *Crotoxyphos:* Continue study.
(2) *Crufomate:* Continue study.
(3) *Dichlorvos (2,2-Dichlorovinyl Dimethyl Phosphate)*: (a) Continue official first action status of the infrared methods **6.324-6.327** and **6.328-6.330.** (b) 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:* Continue study.

PESTICIDE FORMULATIONS: RODENTICIDES AND MISCELLANEOUS PESTICIDES

(1) *Brodifacoum (Talon®)*: Continue study.
(2) *Chlorophacinone:* Continue study.
(3) *Diphacinone:* Continue study.
(4) *α-Naphthylthiourea:* (a) Continue official first action status of the surplus method (**6.139**). (b) Continue study.
(5) *N-3-Pyridyl-N'-p-nitrophenyl Urea (Vacor®)*: Continue study.
(6) *Rodenticides:* Discontinue topic.
(7) *Strychnine:* Continue study.
(8) *Warfarin:* Continue study.

PLANTS

(1) *Ashing Methods:* Continue study.
(2) *Atomic Absorption Methods:* (a) Continue official first action status of the atomic absorption method for calcium, copper, iron, magnesium, manganese, potassium, and zinc (**3.006-3.009**). (b) Continue study.
(3) *Boron:* Continue study.
(4) *Chromium:* Continue study.
(5) *Copper and Cobalt:* Continue study.
(6) *Emission Spectroscopy:* Continue study.
(7) *Fluoride:* (a) Continue official first action status of the potentiometric method (**3.077-3.082**) and the semi-automated method (**3.083-3.095**) for fluoride. (b) Continue study.
(8) *Nitrogen, Non-Protein:* Appoint Associate Referee and continue study.
(9) *Selenium:* Continue study.
(10) *Starch:* Continue study.
(11) *Sulfur:* Appoint Associate Referee and initiate topic.
(12) *Zinc:* 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:* (a) 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**). (b) Continue study.
(3) *Nicotine, Gas Chromatography:* (a) Continue official first action status of the method for nicotine on Cambridge filter pads (**3.158-3.161**). (b) Continue study.
(4) *Tar and Nicotine in Cigarette Smoke:* Continue study.

Report of Committee B on Recommendations for Official Methods

ANTHONY ROMANO, JR (Drug Enforcement Administration, Southeastern Regulatory Laboratory, Miami, FL 33166), *Chairman;* JOSEPH V. THOM (State Department of Health, Laboratory Services Program, Berkeley, CA 94704); EVELYN SARNOFF (Food and Drug Administration, 850 Third Ave, Brooklyn, NY 11232); JAMES B. KOTTEMANN (Food and Drug Administration, Division of Drug Chemistry, Washington, DC 20204), *Secretary;* CHANG S. LAO (Food and Drug Administration, 5600 Fishers Ln, Rockville, MD 20857), *Statistical Consultant*

DRUGS, ACIDIC AND NEUTRAL NITROGENOUS ORGANICS

(1) *Acetaminophen in Drug Mixtures:* Continue study.
(2) *Aspirin, Phenacetin, and Caffeine with Other Drugs:* Continue study.
(3) *Aspirin and Salicylic Acid in Aspirin Products (Semiautomated Analysis):* Continue study.
(4) *Barbiturates (General Methods):* Discontinue topic.
(5) *Barbiturates (Liquid Chromatography):* Discontinue topic.
(6) *Benzothiazine Derivatives:* Continue official first action status of the method for bendroflumethiazide (**37.168-37.174**); continue study.
(7) *Benzthiazide by HPLC:* Continue study.
(8) *Methyldopa:* Continue study.
*(9) *Nitroglycerin Tablets (Semiautomated Method):* Adopt as official final action the official first action method, **39.A01-39.A07**, for nitroglycerin in sublingual tablets; discontinue topic.
(10) *Primidone:* Continue study.
(11) *Probenecid:* Continue study.
(12) *Sulfonamides (Thin Layer Chromatography):* Continue study.
(13) *Thiazide Diuretics, Semiautomated Individual Dosage Units Analysis:* Continue study.
(14) *Thyroid and Thyroxine-Related Compounds:* Continue study.
*(15) *Thyroid by Differential Pulse Polarography:* Adopt as official first action the method reported by the Associate Referee.
(16) *Barbiturates:* Initiate topic combining all previous barbiturates studies and place under leadership of present Associate Referee for Barbiturates (Liquid Chromatography).

DRUGS, ALKALOIDS

(1) *Atropine in Morphine and Atropine Tablets and Injections:* Continue study.
(2) *Belladonna Alkaloids:* Continue study.
(3) *Colchicine in Tablets:* Continue study.
(4) *Curare Alkaloids:* Continue study.
*(5) *Ephedrine:* Adopt as official final action the official first action method, **38.A01-38.A05**.
(6) *Ergot Alkaloids:* Continue study.
(7) *Neostigmine:* Continue study.
*(8) *Physostigmine and Its Salts:* Adopt as official first action, for the solution dosage form only, the method described by the Associate Referee. Continue study of method for injectable and ointment dosage forms.
(9) *Pilocarpine:* Continue study.
(10) *Rauwolfia Alkaloids:* Continue study.
(11) *Rauwolfia serpentina:* Continue study.

DRUGS, MISCELLANEOUS

(1) *Benzoyl Peroxide:* Continue study.
*(2) *Chlorinated Hydrocarbons in Drugs:* Discontinue topic. Designate as surplus the official first action method (**36.013-36.017**) for chlorinated hydrocarbons.
(3) *Disulfiram:* Continue official first action status of method **36.B01-36.B04** for the determination of disulfiram in tablets.
(4) *Ethylene Oxide:* Continue study.
(5) *Fluoride:* Continue study.
(6) *Identification of Drugs (Mass Spectroscopy):* Continue study.
(7) *Medicinal Gases:* Continue study.
(8) *Menadiol Sodium Diphosphate Injection:* Continue study.
(9) *Mercurial Diuretics:* Continue study.
(10) *Mercury-Containing Drugs:* 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 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.* **63**, 374-423 (1980); **64**, 501-540 (1981).

(11) *Metals in Bulk Drug Powders:* Continue study.
(12) *Microcrystalline Tests:* Continue official first action status of the changes made in official final action tables **36:05** and **36:06**; continue study.
*(13) *Protein Nitrogen Units in Allergenic Extracts:* Adopt as official first action the interim first action precipitation method described by the Associate Referee (*J. Assoc. Off. Anal. Chem.* **64**, 1435–1438 (1981)).
(14) *Total Mercury in Biologicals:* Discontinue topic.

DRUGS, NARCOTIC AND DANGEROUS

(1) *Amphetamines in Mixtures:* Continue study.
(2) *Benzodiazepines:* Continue study.
(3) *Chemical Microscopy:* Continue study.
(4) *Cocaine:* Continue official first action status of method **40.002–40.005**; continue study.
(5) *Dimethyltryptamine (DMT), Diethyltryptamine (DET), and Dipropyltryptamine (DPT):* Continue study.
(6) *Heroin:* Continue study.
(7) *Lysergic Acid Diethylamide (LSD):* Continue study.
(8) *Marihuana and Synthetic Tetrahydrocannabinol (THC):* Continue study.
(9) *Methadone:* Continue study.
(10) *Methamphetamine:* Continue study.
(11) *Methaqualone Hydrochloride:* Continue study.
(12) *Methylphenidate Phenidine Hydrochloride:* Continue study.
(13) *Optical Chrystallographic Properties:* Continue study.
(14) *Phencyclidine Hydrochloride (PCP):* Continue official first action status of method **40.016–40.018**; continue study.
(15) *Other Topics:* Continue official first action status of method **38.122–38.126**, amphetamine spectrophotometric method.

DRUGS, NONALKALOID ORGANIC NITROGENOUS BASES

(1) *Aminacrine:* Continue study.
(2) *Antihistamines, Adrenergic Combinations by HPLC:* Continue official first action status of method **38.B01–38.B06** for determining pseudoephedrine hydrochloride and triprolidine hydrochloride or chlorpheniramine maleate in combination in syrups or tablets.
*(3) *Chlorpheniramine Maleate Tablets (Semiautomated Analysis):* Adopt as official final action the official first action method, **38.A06–38.A11**; discontinue topic.
(4) *Chlorpromazine:* Continue study.
(5) *Dicyclomine Capsules:* Continue study.
(6) *Epinephrine–Lidocaine Combinations:* Continue study.
(7) *Epinephrine and Related Compounds by HPLC-Electrochemical Detectors:* Continue study.
(8) *Homatropine Methyl Bromide in Tablets:* Continue study.
(9) *Phenethylamine Drugs—Semiautomated Individual Unit Analysis:* Continue study.
(10) *Pheniramine with Pyrilamine, Phenylpropanolamine, and Phenylephrine:* Continue study.
(11) *Phenothiazine Drugs, Identification by TLC:* Continue study.
(12) *Phenothiazines in Drugs:* Continue study.
(13) *Procaine and Related Local Anesthetics:* Discontinue topic.
(14) *Quaternary Ammonium Compounds:* Continue study.
(15) *Tropane Aminoxides:* Continue study.
*(16) *Other Topics:* (a) Delete the official first action method for amphetamine (**38.122–38.126**). (b) Adopt as official final action the following official first action methods: phenothiazine (**38.185–38.186**) and procainamide hydrochloride (**38.224–38.227**). (c) Editorially revise method **38.231–38.235**, fluorometric determination of quinacrine hydrochloride in pharmaceuticals, so that full baseline scans are obtained. Change **38.234** to read:

38.234 ***Determination***

Adjust spectrophotofluorometer to ca 80% fluorescence intensity (*F*) at 500 nm with std soln. Transfer ca 3 mL 0.1N HCl to clean 10 × 10 mm cell and record the blank scan between 350 and 650 nm. Repeat with std and sample solns. In each case, draw baseline from 350 to 650 nm. Det. %*F* at peak max. (ca 500 nm) of sample and std solns relative to 0.1N HCl blank.

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

DONALD N. WILLETT (State Department of Agriculture, Trade and Consumer Protection, Hill Farms Laboratory, 4702 University Ave, Madison, WI 53705), *Chairman;* D. EARLE COFFIN (Health and Welfare Canada, Food Research Laboratory, Tunney's Pasture, Ottawa, Ontario, Canada K1A 0L2); ALFRED D. CAMPBELL (Food and Drug Administration, Office of Science, Washington, DC 20204); ARTHUR R. JOHNSON (Food and Drug Administration, Division of Food Technology, Washington, DC 20204), *Secretary;* MICHAEL W. O'DONNELL (Food and Drug Administration, Division of Mathematics, Washington, DC 20204), *Statistical Consultant*

COFFEE AND TEA

(1) *Ash in Instant Tea:* Initiate collaborative study on ash and moisture in instant tea; continue study.

(2) *Caffeine:* Continue study.

(3) *Chlorogenic Acid:* Continue study.

(4) *Crude Fiber in Tea:* Appoint Associate Referee; continue study.

(5) *Moisture in Coffee and Tea:* Initiate collaborative study on ash and moisture in instant tea; continue study.

(6) *Solvent Residues in Decaffeinated Coffee:* Continue study.

(7) *Solvent Residues in Decaffeinated Tea:* Appoint Associate Referee; continue study.

(8) *Water Extract in Tea:* Continue study.

(9) *Other Topic:* Appoint Associate Referee for new topic Theophylline in Tea.

DAIRY PRODUCTS

(1) *Carrageenan in Milk and Milk Products:* Discontinue topic.

(2) *Casein and Caseinates:* Continue study.

(3) *Cheese Spreads, Gums In:* Discontinue topic.

(4) *Chocolate Milk, Fat Test:* Continue study.

(5) *Cryoscopy of Milk:* Continue study to resolve problems relative to converting degrees Hortvet to degrees Celsius.

(6) *Desserts, Frozen:* Discontinue topic.

(7) *Desserts, Frozen, Gums In:* Discontinue topic.

(8) *FAO and AOAC Methods, Comparison:* Discontinue topic.

(9) *Fat, Automated Methods:* Continue study.

(10) *Fat Content:* Discontinue topic.

(11) *Fat in Milk (AutoAnalyzer):* Continue study.

(12) *Fats, Foreign (Sterol Acetates):* Discontinue topic.

(13) *Infrared Milk Analyzer (IRMA):* Continue study.

(14) *Lactose in Dairy Products (Chromatographic Determination):* Continue study.

(15) *Lactose in Dairy Products (Enzymatic Determination):* Continue study.

(16) *Milk, Reconstituted:* Discontinue topic.

(17) *Moisture in Cheese (Karl Fischer Method):* Continue study.

(18) *Nitrates in Cheese:* Continue study.

(19) *Phosphatase, Rapid Method:* Continue study.

(20) *Phosphatase, Reactivated:* Continue study.
(21) *Phosphatase Test:* Discontinue topic.
(22) *Phosphorus:* Continue study.
(23) *Protein in Milk, Rapid Tests:* Continue study.
(24) *Protein Constituents in Processed Dairy Products:* Continue study.
(25) *Protein Reducing Substances Test:* Continue study.
(26) *Salt:* Discontinue topic.
(27) *Sampling, Fat and Moisture in Cheese:* Discontinue topic.
(28) *Solids-Not-Fat:* Continue study.
(29) *Titanium Dioxide in Cheese:* Discontinue topic.
(30) *Vapor Pressure Osmometry:* Continue study.
(31) *Other Topic:* Correct errors in Chapter 16, *Official Methods of Analysis*, changing **16.052** to read **16.056** and changing **16.053** to read **16.057** in the last sentence of the first paragraph of **16.085.**

DECOMPOSITION AND FILTH IN FOODS (CHEMICAL METHODS)

(1) *Amines in Seafoods:* Discontinue topic.
(2) *Ammonia in Dogfish:* Continue study.
(3) *Apple Products:* Discontinue topic.
(4) *Coprostanol:* Continue study.
(5) *Crabmeat:* Continue study.
(6) *Diacetyl in Citrus Products:* Continue study.
(7) *Eggs, Dried and Frozen:* Discontinue topic.
(8) *Ethanol in Seafoods:* Continue study.
(9) *Fish Products:* Discontinue topic.
(10) *Gas and Liquid Chromatography:* Continue study.
(11) *Nuts, Rancidity in:* Discontinue topic.
*(12) *Shellfish:* Incorporate the modifications as presented in the report by the Associate Referee into **18.075-18.078,** the GLC method for the determination of indole in shrimp; adopt as official final action the official first action HPLC method, **18.B01-18.B05,** for the determination of indole in shrimp; continue study.
(13) *TLC Identification of Indole in Shrimp:* Change title of topic to *TLC Identification of Amines in Fishery Products;* continue study.
(14) *Tomatoes:* Continue study.

EGGS AND EGG PRODUCTS

(1) *Color:* Continue study.
(2) *Fat:* Continue study.
(3) *Phosphorus:* Continue study.
(4) *Sterols (Gas Chromatography):* Continue study.
(5) *Total Solids:* Continue study.

ENZYMES

(1) *Amylase Activity in Cereal and Cereal Products:* Continue study.
(2) *Catalase in Frozen Vegetables:* Continue study.
(3) *Papain:* Continue study.
(4) *Peroxidase in Frozen Vegetables:* Continue study.
(5) *Proteolytic Enzymes in Treated Meats:* Appoint Associate Referee; continue study.
(6) *Rennet:* Continue study.

FISH AND OTHER MARINE PRODUCTS

(1) *Crabmeat Identification:* Continue study.
(2) *Drained Weight of Block Frozen, Raw, Peeled Shrimp:* Continue study.
(3) *Drip Fluid in Fish Fillets and Fish Fillet Blocks:* Continue study.
(4) *Fish Species Identification, Thin Layer Isoelectric Focusing:* Initiate additional collaborative study of the agarose gel isoelectric focusing method as investigated by the Associate Referee as an alternative method to the official polyacrylamide gel isoelectric focusing method; continue study.
(5) *Net Weight of Glazed Shrimp:* Discontinue topic.
(6) *Nitrites in Smoked Fish:* Initiate collaborative study of the proposed specific ion electrode method; continue study.

FOOD ADDITIVES

(1) *Anticaking Agents:* Appoint Associate Referee; continue study.
(2) *Antioxidants:* Continue study.
(3) *Brominated Oils:* Appoint Associate Referee; continue study.
(4) *Chloride Titrator:* Continue study.
(5) *Chlorobutanol in Milk:* Continue study.
(6) *Dichlorodifluoromethane in Frozen Foods:*

* 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 C were adopted by the Association. Section numbers refer to *Official Methods for Analysis* (1980) 13th Ed., and "Changes in Methods," *J. Assoc. Off. Anal. Chem.* (1980) **63,** 374–423; and (1981) **64,** 501–540.

Appoint Associate Referee; continue study.

(7) *Dilaurylthiodipropionate:* 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 Associate Referee; continue study.
(13) *Indirect Additives from Food Packaging:* Continue study.
(14) *Mineral Oil in Raisins:* Continue study.
(15) *Nitrates and Nitrites:* Appoint Associate Referee; study.
(16) *Nitrates (Selective Ion Electrode Titration):* Continue study.
*(17) *Nitrosamines:* Adopt as official first action the collaboratively studied method for the determination of *N*-nitrosodimethylamine in beer; 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:* Continue study.
(2) *Other Topic:* Appoint General Referee.

MEAT AND MEAT PRODUCTS

(1) *Ashing Methods:* Appoint Associate Referee; continue study.
(2) *Automated Methods:* Initiate collaborative study to evaluate block digestor; continue study.
(3) *Bone Content:* Appoint Associate Referee; continue study.
(4) *Chlorinated Hydrocarbons in Poultry:* Continue study.
(5) *Fat and Moisture Analysis, Rapid Methods:* Continue study.
(6) *Fluoride in Deboned Meat and Poultry:* Continue study.
(7) *Identification of Meats, Serological Tests:* Continue study.
(8) *Moisture, Automated Karl Fischer Titrator Method:* Continue study.
(9) *Nitrates and Nitrites:* Appoint Associate Referee; continue study.
*(10) *Nitrosamines in Bacon:* Adopt as official first action the interim official first action collaboratively studied mineral oil distillation–TEA method for the determination of nitrosamines in fried bacon; continue study.
(11) *Non-meat Proteins in Meat:* Appoint Associate Referee; continue study.
(12) *Peanut and Cottonseed Proteins in Meat Products:* Continue study.
(13) *Protein in Meat:* Continue official first action status of the crude protein methods, **24.027–24.037;** continue official first action status of the crude protein block digestion method, **24.B01–24.B03;** continue study.
(14) *Proximate Composition Relationships:* Appoint Associate Referee; continue study.
(15) *Sodium Caseinate in Meat Products:* Incorporate subject under Non-meat Proteins in Meat; discontinue topic.
(16) *Soy Proteins in Meat Products:* Continue study.
(17) *Sugars and Sugar Alcohols:* Continue study.
(18) *Temperature, Minimum Processing:* Continue study.
(19) *Other Topics:* Initiate the new topics Sodium and Potassium in Meat Products, Fat in Meat Products, and Specific Ion Electrode Applications for Meat and Meat Products and appoint Associate Referees; consolidate the topics Non-meat Proteins in Meat, Peanut and Cottonseed Proteins in Meat Products and Soy Proteins in Meat Products, under a coordinating committee for the determination and analysis of non-meat proteins.

MICROCHEMICAL METHODS

HPLC of Vegetable Material: Continue study.

MYCOTOXINS

*(1) *Aflatoxin M:* Adopt as official first action the method for the determination of aflatoxins B_1 and M_1 in liver and the method for confirmation of identities; the official first action method for the confirmation of aflatoxin M_1 identity in milk, **26.A15,** as applicable to M_1 in liver; continue official first action status of **26.A10–26.A14,** Method I, **26.090–26.094,** Method II, and **26.A15,** confirmation of identity, for aflatoxin M_1 in milk; continue study.
(2) *Aflatoxin Methods:* Continue official first action status of **26.026–26.031** and **26.032–26.036** for aflatoxins in peanut products; evaluate results of collabora-

tive study of 2 HPLC methods for aflatoxins in peanut products; continue study.

(3) *Alternaria Toxins:* Continue study.
(4) *Citrinin:* Continue study.
(5) *Ergot Alkaloids:* Continue study.
(6) *Grains:* Continue study.
(7) *Mixed Feeds:* Initiate collaborative study of the method for aflatoxins in mixed feeds; continue study of methods for trichothecenes in feeds.
(8) *Ochratoxins:* Initiate collaborative study of the modified Thorpe method for ochratoxin A in grains.
*(9) *Patulin:* Adopt as official final action the official first action method for patulin in apple juice, **26.111-26.116;** continue study.
(10) *Penicillic Acid:* Initiate collaborative study of the method for penicillic acid in corn and beans; continue study.
(11) *Sterigmatocystin:* Continue study.
(12) *Tree Nuts:* Continue study.
(13) *Trichothecenes:* Implement recommendations resulting from the workshop on trichothecene methodology; continue study.
(14) *Zearalenone:* Initiate collaborative study of a modification of the method for zearalenone and zearalenol in grain; continue study of a screening method applicable to animal excreta.

NUTS AND NUT PRODUCTS

(1) *Antioxidants:* Appoint Associate Referee; continue study.
(2) *Composition (Ash, Fat, Fiber, Protein, Water):* Appoint Associate Referee; continue study.
(3) *Moisture and Water Activity:* Appoint Associate Referee; initiate study of methods for determining water activity in nuts, especially in-shell pistachio nuts; continue study.
(4) *Oil, Hydrogenated, in Peanut Butter:* Appoint Associate Referee; continue study.

OILS AND FATS

(1) *Antioxidants:* Continue study.
(2) *Chromatographic Methods:* Continue study.
(3) *Cyclopropene Fatty Acids:* Continue study.
(4) *Emulsifiers:* Continue study.
(5) *Fats and Fatty Acids, Gas Chromatography:* Discontinue topic.
(6) *Karl Fischer Method for Water in Oils and Fats:* Continue study.
(7) *Lower Fatty Acids:* Continue study.
(8) *Marine Oils:* Continue study.
(9) *Olive Oil Adulteration:* Continue study.
*(10) *Oxidized Fats:* Adopt as official first action the interim official first action IUPAC method for the determination of oxidation products in frying fats; continue study.
(11) *Pork Fat in Other Fats:* Continue study.
(12) *Spectrophotometric Methods:* Continue study.
(13) *Sterols and Tocopherols:* Continue study.
*(14) *Other Topics:* Adopt as official final action the official first action method for isolated trans isomers, **28.075-28.080;** adopt as official final action the official first action method for 1-monoglycerides, **28.139-28.147.**

PROCESSED VEGETABLE PRODUCTS

(1) *Fibrous Material in Frozen Green Beans:* Continue study.
*(2) *pH Determination:* Adopt as official final action the official first action method, **32.B01-32.B08;** continue study.
*(3) *Sodium Chloride:* Declare as surplus, **32.023,** Method I, and **32.024,** Method II, Rapid Method, for the determination of sodium chloride as recommended by the Associate Referee; continue study.
(4) *Water Activity Determination:* Continue study.
(5) *Other Topic:* Initiate new topic, *Volume of Entrapped Air in Flexible Retort Pouches;* appoint Associate Referee.

SEAFOOD TOXINS

(1) *Ciguatoxins:* Continue study.
(2) *Paralytic Shellfish Poisoning: Immunoassay Method:* Continue study.
(3) *Shellfish Poisons:* Continue study.
(4) *Tetradotoxins:* Continue study.

Report of Committee D on Recommendations for Official Methods

JOHN C. KISSINGER (U.S. Department of Agriculture, Eastern Regional Research Center, 600 E Mermaid Lane, Philadelphia, PA 11918); *Chairman;* ELMER GEORGE, JR (Department of Agriculture and Markets, 1220 Washington Ave, Albany, NY 12235); DONALD PARRISH (Kansas State University, Department of Biochemistry, Manhattan, KS 66506); ROBERT MARTIN (Hershey Food Corp., Research Laboratory, Box 54, Hershey, PA 17033), BENJAMIN KRINITZ (Food and Drug Administration, 850 Third Ave, Brooklyn, NY 11232), *Secretary,* DENNIS RUGGLES (Food and Drug Administration, Division of Mathematics, Washington, DC 20204), *Statistical Consultant*

ALCOHOLIC BEVERAGES

(1) *Acetate in Wines and Fruit Juices (Enzymatic Assay):* Continue study.

*(2) *Alcoholic Content by Oscillating U-Tube Density Meter:* Adopt as official first action the density meter method for determining proof of alcoholic beverages as described by the Associate Referee (*J. Assoc. Off. Anal. Chem.* **65,** 218-223 (1982)); continue study.

(3) *β-Asarone:* Continue study.

(4) *Bromide Ion in Wine:* Continue study.

(5) *Carbon Dioxide in Wine:* Continue study.

(6) *Citric Acid in Wine:* Continue study.

(7) *Color in White Wine:* Continue study.

*(8) *Color Intensity for Distilled Alcoholic Beverage Products:* Adopt as official first action the spectrophotometric method for color intensity as described by the Associate Referee (*J. Assoc. Off. Anal. Chem.* **65,** 224-226 (1982)); continue study.

(9) *Coumarin in Wine:* Continue study.

(10) *Diethylpyrocarbonate in Beverages:* Continue study.

(11) *Ethanol in Wine by GLC:* Continue study.

(12) *Flavor Compounds in Malt Beverages:* Continue study.

(13) *Glycerol in Wine:* Continue study.

(14) *Hydrogen Cyanide:* Continue study.

(15) *Malic Acid in Wine:* Continue study.

*(16) *Malt Beverages and Brewing Material:* Adopt as official final action the official first action air oven method for moisture in barley, **10.B01;** adopt as official first action the following ASBC methods: *N*-nitrosamines in beer by Celite adsorption (screening method); *N*-nitrosamines in beer by distillation (confirmatory method); barley extract (*J. Assoc. Off. Anal. Chem.* **64,** 1140 (1981)). Continue study.

(17) *Sorbic Acid in Wine:* Continue study.

(18) *Sugar, Reducing:* Continue study.

(19) *Sulfur Dioxide in Wine:* Continue study.

(20) *Tartrates in Wine:* Continue study.

(21) *Volatile Acidity in Wine:* Continue study.

(22) *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; **10.183-10.185,** 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; **13.058-13.059,** alginates; **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:* Adopt the Ad Hoc Committee report on Nitrogen-to-Protein Conversion Factor (*J. Assoc. Off. Anal. Chem.* **65,** 333 (1982)). Delete the official

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The recommendations submitted by Committee D were adopted by the Association. Section numbers refer to *Official Methods of Analysis* (1980) 13th Ed., and "Changes in Methods," *J. Assoc. Off. Anal. Chem.* (1980) **63,** 374-423; and (1981) **64,** 501-540.

first action methods for starch, **14.075-14.080;** continue official first action status of the following methods: **14.049-14.053,** α-amylase; **14.013-14.015,** lactose; **14.117-14.120,** mineral oil; **14.150-14.153,** sterols.

FLAVORS AND NONALCOHOLIC BEVERAGES

(1) *Additives in Vanilla Flavorings:* Continue study.
(2) *Ash and Pungent Principles in Mustard:* Continue study.
(3) *Caffeine and Methyl Xanthenes in Nonalcoholic Beverages:* Continue study.
(4) *Characterization of Natural Spices and Flavors:* Continue study.
(5) *Citral:* Continue study.
(6) *Essential Oils:* Continue study.
(7) *Extractable Color in Capsicum Spices and Oleoresins:* Continue study.
*(8) *Glycyrrhizic Acid and Glycyrrhizic Acid Salts:* Adopt as official first action the HPLC method for glycyrrhizic acid or glycyrrhizic acid salts as described by the Associate Referee; continue study.
(9) *Imitation Maple Flavors, Identification and Characterization:* Continue study.
(10) *Lasiocarpine and Pyrrolizidines in Herbal Beverages:* Continue study.
(11) *Moisture in Dried Spices:* Continue study.
(12) *Monosodium Glutamate in Foods:* Continue study of the official first action method, **20.189-20.191.**
(13) *Organic Solvent Residues in Flavorings:* Continue study.
(14) *Vanillin and Ethyl Vanillin in Foods:* Continue study.
(15) *Vinegar:* Continue study.
(16) *Other Topics:* Continue official first action status of the following methods: **12.017,** dibasic acids; **12.019,** citric acid; **12.020,** total malic acid; **12.021,** laevo malic acid; **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,** and **19.124,** anise and nutmeg extracts; **19.125, 19.126,** and **19.127-19.128,** other extracts and toilet preparations.

FRUITS AND FRUIT PRODUCTS

(1) *Adulteration of Orange Juice by Pulp Wash and Dilution:* Continue study.
(2) *Dimethylpolysiloxane in Fruit Juices:* Discontinue topic.
(3) *Fruit Acids:* Continue study.
(4) *Fruit Juices, Identification and Characterization:* Continue study.
(5) *Isoascorbic Acid (Erythorbic Acid-Antioxidant) in Fruit Purees:* Continue study.
(6) *Isotope Ratio Analysis for Detection of Adulteration of Apple Juice With Corn Sugars* $^{13}C/^{12}C$: This topic has been transferred to the new topic "Stable Carbon Isotope Ratio Analysis," which has been placed under Sugars and Sugar Products.
(7) *Orange Juice Content:* Continue study.

PRESERVATIVES AND ARTIFICIAL SWEETENERS

(1) *Benzoates and Hydroxybenzoates in Food:* Continue study.
(2) *Benzoates, Saccharin, and Caffeine, High Pressure Liquid Chromatography:* Continue study.
(3) *Formaldehyde:* Continue study.
(4) *Meats, Ground, Screening Methods for Chemical Preservatives:* Continue study.
(5) *Organic Preservatives (Thin Layer Chromatography):* Continue study.
(6) *Preservatives (Qualitative Methods):* Continue study.
*(7) *Saccharin and Its Salts:* Adopt as official final action the official first action differential pulse polarographic method, **20.A06-20.A10.**
(8) *o-Toluenesulfonamide in Saccharin:* Discontinue topic.
(9) *Other Topics:* 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,** identifica-

tion of nonnutritive 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.

SUGARS AND SUGAR PRODUCTS

(1) *Chromatographic methods:* Continue study.

(2) *Color, Turbidity, and Reflectance—Visual Appearance:* Continue study.

(3) *Corn Syrup and Corn Sugar:* Continue study.

(4) *Dry Substance:* Continue study.

(5) *Enzymatic Methods:* Continue study.

(6) *Honey:* Continue study.

(7) *Maple Sap and Syrup:* Continue study.

*(8) *Stable Carbon Isotope Ratio Analysis:* Adopt as official first action the mass spectrometric stable isotope procedure for the determination of high fructose corn syrups in orange juices as presented by the Associate Referee; continue study.

(9) *Standardization of Sugar Methods of Analysis:* Continue study.

*(10) *Sugars in Cereal:* Adopt as official first action the high pressure liquid chromatographic method for the determination of sugars in sweetened cereals as presented by the Associate Referee; continue study.

(11) *Sugar in Sugar Cane:* Continue study.

(12) *Sugars, Reducing:* Continue study.

(13) *Weighing, Taring, and Sampling:* Continue study.

VITAMINS AND OTHER NUTRIENTS

(1) *Amino Acids:* Continue study.

*(2) *Automated Nutrient Analysis:* Adopt as official final action the following semi-automated methods: niacin and niacinamide in food products, **43.B05-43.B08,** and riboflavin in foods, **43.B01-43.B04;** continue study.

(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:* 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 stucy.

(12) *Iron Availability:* Discontinue topic.

(13) *Niacinamide (Polarography):* Continue study.

(14) *Pantothenic Acid, Total Activity in Foods:* Continue study.

*(15) *Protein Quality, Evaluation in Foods:* Adopt as official first action the screening in vitro assay for predicting PER as measured by rat bioassay as recommended by the investigators with concurrence of the Associate Referee; 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 for vitamin D in fortified milk and milk powder, **43.B09-43.B15;** adopt as official first action the HPLC method for vitamin D in pet foods and animal feeds as described by the Associate Referee; 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

KENNETH HELRICH (Rutgers University, Cook College, New Brunswick, NJ 08903), *Chairman;* JERRY A. BURKE (Food and Drug Administration, Division of Chemical Technology, Washington, DC 20204); ANTHONY J. MALANOSKI (U.S. Department of Agriculture, Food Safety and Inspection Service, Washington, DC 20205); GERALD R. MYRDAL (Wisconsin Department of Agriculture, Trade and Consumer Protection, Madison, WI 53707); BARTHOLOMEW J. PUMA (Food and Drug Administration, Division of Chemical Technology, Washington, DC 20204), *Secretary;* and STEPHEN W. BUTLER (Food and Drug Administration, Division of Mathematics, Washington, DC 20204), *Statistical Consultant*

CARBAMATE PESTICIDES, FUMIGANTS, AND MISCELLANEOUS

(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:* Obtain collaborators with the specialized equipment required for post-column fluorescent derivative formation and measurement in the Associate Referee's high performance liquid chromatographic method (*J. Assoc. Off. Anal. Chem.* (1980) **63,** 1114–1124), and initiate collaborative study of this method for determining residues of *N*-methyl carbamate insecticides in crops.

(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:* Continue study to evaluate analytical methods for determining residues of fenvalerate in foods.

(6) *Fumigants:* Appoint 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:* 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:* 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) 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 method described by the Associate Referee in 1980, with gas-liquid chromatography of fluoroacetic acid, underivatized, for determining residues of sodium monofluoroacetate in foods.

FUNGICIDES, HERBICIDES, AND PLANT GROWTH REGULATORS

(1) *Anilazine:* Appoint 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:* Appoint Associate Referee; continue study of methods for determining residues of benomyl, methyl 2-benzimidazole carbamate, and thiabendazole in foods.

(3) *Captan and Related Fungicides:* Appoint Associate Referee; continue study of methods for determining captan, captafol, folpet, and their metabolites in foods.

(4) *Carbamate Herbicides:* Appoint Associate Referee; continue study to evaluate methods for determining residues of carbamate herbicides in crops.

(5) *Chlorophenoxy Alkyl Acids:* Conduct ruggedness test of the Associate Referee's improved version of the gas-liquid chromatographic method reported by Cessna (*J. Agric. Food Chem.* (1980) **28,** 1229–1232) for determining residues of (2,4-dichlorophenoxy)acetic acid in green wheat and wheat grain and, if warranted by the outcome, prepare collaborative study protocol for review and comment by the General Referee.

(6) *Chlorophenoxy Alkyl Acids in Water and Waste Water:* Transfer topic to the General Referee for Water; initiate collaborative study of the method outlined by the General Referee (*J. Assoc. Off. Anal. Chem.* (1981) **64,** 394) for determining chlorophenoxy alkyl acids and related esters in water and wastewater by gas-liquid chromatography of the methyl esters derived from the acids and the hydrolyzed esters.

(7) *Chlorothalonil:* Appoint Associate Referee; continue study of gas-liquid chromatographic methods for determining residues of chlorothalonil in crops.

(8) *Dinitro Compounds:* Appoint Associate Referee; continue study of methods for determining dinitro aryl herbicide residues in foods.

(9) *Diquat and Paraquat:* Continue study of the method described by King (*J. Agric. Food Chem.* (1978) **26,** 1460–1463) for determining residues of diquat by gas-liquid chromatography of the amine produced by the reduction of the pyridinium salt with sodium borohydride, to extend the method to residues of paraquat, and to eliminate interferences found in some crop samples.

(10) *Dithiocarbamates, General Residue Methods:* Appoint Associate Referee; continue study to develop methods for distinguishing dialkyldithiocarbamates from ethylenebisdithiocarbamates and for determining the parent fungicides and their metabolites in foods.

(11) *Maleic Hydrazide:* Appoint Associate Referee; continue study of gas-liquid chromatographic or high pressure liquid chromatographic methods for determining maleic hydrazide in foods.

(12) *1-Naphthaleneacetic Acid and 1-Naphthaleneacetamide:* Initiate collaborative study of the Associate Referee's improved version of the method for determining 1-naphthaleneacetic acid and 1-naphthaleneacetamide in apples by high pressure liquid chromatography (*J. Assoc. Off. Anal. Chem.* (1980) **63,** 100–106).

(13) *Organotin Fungicides:* Continue study.

(14) *Pentachlorophenol:* Continue study of the gas-liquid chromatographic method for determining pentachlorophenol, underivatized, in milk and blood, as described by the Associate Referee (*J. Agric. Food Chem.* (1980) **28,** 710–714); conduct intralaboratory trial of an improved version of this method with modifications for the analysis of gelatin, fish, and eggs and, if warranted by the results, initiate interlaboratory trial of the improved method.

(15) *Sodium o-Phenylphenate:* Appoint Associate Referee; continue study to evaluate high pressure liquid chromatographic methods for determining *o*-phenylphenol in foods.

(16) *Substituted Ureas:* Initiate collaborative study of the gas-liquid chromatographic method proposed by the Associate Referee, based on the method of Baunok and Geissbuehler (*Bull. Environ. Contam. Toxicol.* (1968) **3,** 7–17).

(17) *Succinic Acid, 2,2-Dimethylhydrazide:* Appoint Associate Referee; continue study to develop a gas-liquid chromatographic or high pressure liquid chromatographic method for determining succinic acid, 2,2-dimethylhydrazide in foods.

(18) *Thiolcarbamate Herbicides:* Appoint Associate Referee; continue study to evaluate methods for determining residues of

thiolcarbamate herbicides in crops.

(19) *s-Triazines:* Appoint Associate Referee; initiate collaborative study of the gas-liquid chromatographic method for determining atrazine and cyanazine in corn and potatoes, as outlined by the General Referee (*J. Assoc. Off. Anal. Chem.* (1980) **63,** 273).

(20) *Trifluralin:* Appoint Associate Referee; continue study to evaluate methods for determining trifluralin in crops.

METALS AND OTHER ELEMENTS

(1) *Atomic Absorption:* Change title of topic to Atomic Absorption Spectrophotometry (AAS); continue study to consolidate present official methods for determining metals and other elements by atomic absorption spectrophotometry into a general analytical scheme for all food commodities.

*(2) *Cadmium and Lead in Earthenware:* Designate as surplus the official first action rapid screening method for detecting acid-extractable cadmium and lead in earthenware, **25.035-25.037;** submit 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:* Continue study of analytical methods using carbon rod atomization techniques for atomic absorption spectrophotometric determination of trace elements in foods.

(4) *Contamination Control in Trace Elemental Analysis:* Discontinue as separate topic, with each Associate Referee under the direction of the General Referee for Metals and Other Elements to ensure that proper procedures for contamination control are incorporated into methods being considered for collaborative study in their topics.

(5) *Emission Spectrochemical Methods:* Prepare method write-up and protocol for collaborative study of the inductively coupled argon plasma emission spectroscopic method developed by the Associate Referee for multielement analysis of raw agricultural crops for review by the General Referee; monitor progress of the ongoing collaborative study described by the General Referee in 1981 of the method using nitric-perchloric acid digestion of infant formula with multielement determination by inductively coupled plasma emission spectroscopy.

(6) *Fluorine:* Appoint Associate Referee; initiate second collaborative study of the microdiffusion and fluoride-specific electrode method studied by Dabeka and McKenzie (*J. Assoc. Off. Anal. Chem.* (1981) **64,** 1021-1026) for determining fluoride in infant foods, with the method modified for improvement as suggested by the authors of the first study, after approval of the study protocol by the General Referee.

(7) *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 and selenium in foods; continue study of hydride evolution methods for determining tin in foods.

(8) *Mercury (Organic):* Change title of topic to Mercury; continue study of interferences from selenium and tellurium in the method for determining mercury by atomic absorption spectrophotometry or inductively coupled plasma emission spectroscopy using the simplified apparatus described by the Associate Referee (*J. Assoc. Off. Anal. Chem.* (1981) **64,** 1105-1110) for generating elemental mercury vapor from sample digests with stannous chloride reducing solution and, when the interferences are fully defined, design collaborative study of the method for review by the General Referee.

(9) *Methyl Mercury in Fish and Shellfish:* Initiate topic for collaborative study of an improved version of the method by Watts et al. (*J. Assoc. Off. Anal. Chem.* (1976) **59,** 1226-1233) for the gas-liquid chromatographic determination of methyl mercury in fish and shellfish, in-

* 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 E were adopted by the Association. Section numbers refer to *Official Methods of Analysis* (1980) 13th Ed., and "Changes in Methods," *J. Assoc. Off. Anal. Chem.* (1980) **63,** 374-423.

corporating the method modifications developed by J. O'Reilly to eliminate the problems experienced with the diethylene glycol succinate-packed column in the previous collaborative study of this method, as described by the General Referee in 1981.

(10) *Multimetal Residues by Resin Column Separations:* Continue study of an improved version of the resin column chromatographic technique for isolating transition elements and heavy metals from alkali and alkaline earth metals (*J. Agric. Food Chem.* (1975) **23,** 41–45) for use in conjunction with multielement detection systems.

(11) *Organometallics:* Continue study to develop methods for determining organometallic compounds in foods.

*(12) *Polarography:* Adopt as official first action the dry ash, anodic stripping voltammetric method described by the Associate Referee in 1981 for determining cadmium and lead in foods other than fats and oils; continue study.

(13) *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;** complete interlaboratory trial of an improved version of **25.136–25.138** and, if warranted by the results of the trial, initiate collaborative study of the improved method, after submitting the protocol for the study to the General Referee for approval.

(14) *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;** continue study of voltammetric methods applicable to the determination of metals and other elements in foods.

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 after the recent publication of the method in order 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; prepare protocol 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.

(2) *Gas-liquid Chromatography (Alkaline Precolumn):* 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) *Multielement Determination After Closed System Digestion:* Transfer topic to the General Referee for Metals and Other Elements; continue official first action status of the multielement method, **25.A01–25.A05** (*J. Assoc. Off. Anal. Chem.* (1980) **63,** 388–391), for determining arsenic, cadmium, lead, selenium, and zinc in foods; continue study to extend this method to the determination of chromium, copper, and nickel.

(4) *Organophosphorus Pesticide Residues:* Continue study of the official final action carbon column cleanup method for residues of parathion, paraoxon, EPN, carbophenothion and its oxygen analog in apples and green beans, **29.039–29.043,** 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.

(5) *Pesticides in Meat and Meat Products:* Appoint 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.

(6) *Pollutant Phenols in Fish:* Initiate topic to

develop and collaboratively study methods applicable to the analysis of fish for residues of phenols classified as priority pollutants by the U.S. Environmental Protection Agency.

(7) *Whole Blood:* Continue study of the method for extracting Mirex from whole blood (*J. Assoc. Off. Anal. Chem.* (1980) **63,** 965–969) and the application of this method to the extraction of additional organochlorine pesticides; conduct interlaboratory trial of the method and, if warranted by the results of the trial, develop collaborative study protocol for review and approval by the General Referee.

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 to define the terminal residues of chlordane in various foods and to evaluate methods for their determination.

(2) *Chlorinated Dioxins:* Continue study to develop and evaluate analytical methods for determining residues of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) at parts-per-trillion levels in fish, milk, and other foods, and to establish criteria for confirming the identity of TCDD by gas-liquid chromatography/mass spectrometry.

(3) *Chlorobenzilate, Chloropropylate, and Bromopropylate:* Prepare report on the studies 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 Associate Referee; continue study to evaluate methods for the determination of dicofol residues in crops.

(5) *Kepone:* Continue study of analytical methods for determining residues of Kepone in fish and shellfish.

(6) *Low Moisture-High Fat Samples, Extraction Procedure:* Initiate collaborative study of the method combining the sample preparation/extraction procedure reported by the Associate Referee in 1981 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 related low moisture-high fat products.

(7) *Multiresidue Methodology, Miniaturization:* Complete the collaborative study of the Associate Referee's improved version of the miniaturized multiresidue extraction and cleanup procedure for determining residues of organochlorine pesticides and polychlorinated biphenyls in fish (*J. Assoc. Off. Anal. Chem.* (1974) **57,** 567–579) and, if warranted by the evaluation of the results, submit a report on the collaborative study with a recommendation for consideration of the method under the interim first action approval procedure.

(8) *Photochemical Derivatization for Confirmation of Residue Identity:* Continue study of the Associate Referee's method for confirming the identity of heptachlor and heptachlor epoxide by gas-liquid chromatography of their photoderivatives (*J. Assoc. Off. Anal. Chem.* (1977) **60,** 673–678) to determine (a) the identity of the photoderivatives of heptachlor, heptachlor epoxide, and other compounds for which the confirmation procedure has proved to be effective in the absence of sample matrix, and (b) the extent to which coextractives from samples prepared by the multiresidue methods **29.001–29.018** and **29.029–29.034** affect the formation of these derivatives; prepare report for publication of the results obtained with the 56 organochlorine pesticides that have been irradiated in the commercial photochemical reactor.

*(9) *Polychlorinated Biphenyls (PCBs):* Adopt as official final action the official first action methods for determining PCBs in dairy products, poultry fat, and fish, **29.001-29.018,** and paper and paperboard, **29.035-29.038,** including both the total peak area or peak height method and the individual peak area method for quantitation of PCBs by electron capture gas-liquid chromatography, **29.018,** and Table **29:02.**

(10) *Root-Absorbed Residues, Extraction Procedures:* Appoint Associate Referee; continue study to evaluate available information on the extraction of root-absorbed residues and to develop and evaluate effective extraction procedures for root-absorbed residues, for incorporation into the multiresidue method, **29.001-29.018.**

(11) *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**).

(12) *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 electron capture capillary column gas-liquid chromatography and other techniques for the quantitative determination of toxaphene residues in human tissues including milk.

*(13) *Other Topic:* Adopt as official final action the official first action gas-liquid chromatographic method for the determination of hexachlorobenzene and mirex in adipose tissue, **29.A01-29.A04** (*J. Assoc. Off. Anal. Chem.* (1980) **63,** 396).

ORGANOPHOSPHORUS PESTICIDES

(1) *Confirmation Procedures:* Initiate collaborative study of the pentaflurobenzyl 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 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).

(2) *Extraction Procedures:* Appoint Associate Referee; continue study to determine the efficiency of extraction procedures for organophosphorus pesticide residues in crops.

(3) *General Method for Organochlorine and Organophosphorus Pesticides:* Appoint 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.

(4) *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.

(5) *Soils:* Appoint Associate Referee; continue study to develop multiresidue extraction and cleanup methods for determining organophosphorus pesticides and their metabolites in soils.

(6) *Sweep Codistillation:* Continue study to extend the applicability of the present sweep codistillation method for determining organophosphorus pesticide residues in crops, **29.044-29.049,** to the analysis of fatty foods.

(7) *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 evaluate and refine cleanup and enzyme inhibition detection procedures for determining organophosphorus pesticides by optical scanning thin layer chromatography.

(8) *Water:* Discontinue topic, with further work in this area to be carried out by the Associate Referee for Organophosphorus Pesticides in Water under the direction of the General Referee for Water.

RADIOACTIVITY

The position of General Referee for Radioactivity has been vacant since March 1981. The recommendations were drafted by Committee E after consultation with the Associate Referees.

*(1) *Barium-140:* Adopt as official first action the interim first action method for the simultaneous determination of barium-140, cesium-137, and iodine-131 in milk, as described in the official final action method for cesium-137 in milk, **48.025-48.029;** in cooperation with the Associate Referees for Cesium-137 and Iodine-131, revise the report on the 2 collaborative studies supporting the extension of **48.025-48.029** to barium-140 and iodine-131 to correct the statistical and editorial problems delaying publication of the report in the *Journal.*

(2) *Carbon-14:* Appoint Associate Referee; evaluate data from collaborative study conducted by former Associate Referee A.A. Moghissi 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:* Complete the statistical analysis of results from the collaborative study on extending the gamma-ray spectroscopic method for determining cesium-137 in milk, **48.025-48.029,** to the determination of cesium-137 in other foods; revise report in cooperation with the Associate Referees for Barium-140 and Iodine-131 on the collaborative studies conducted to test the extension of the method for cesium-137 in milk, **48.025-48.029,** to the simultaneous determination of barium-140, cesium-137, and iodine-131 in milk (see rec. 1).

(4) *Iodine-131:* In cooperation with the Associate Referees for Barium-140 and Cesium-137, revise the report on the collaborative studies conducted to test the extension of the official final action gamma-ray spectroscopic method for cesium-137 in milk, **48.025-48.029,** to the simultaneous determination of barium-140, cesium-137, and iodine-131 in milk (see rec. 1); continue study of the more sensitive method recommended by the Nuclear Regulatory Commission, as summarized by the former General Referee in *J. Assoc. Off. Anal. Chem.* (1979) **62,** 387-389, for determining iodine-131 in milk.

(5) *Neutron Activation Analysis:* Submit protocol for collaborative study of the method for determining sodium in neutron-irradiated biological materials to the General Referee for review and, if approved, initiate the collaborative study; complete the evaluation 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 of methods for determining additional elements such as aluminum, calcium, copper, magnesium, and manganese in neutron-irradiated samples.

(6) *Plutonium:* Appoint Associate Referee; continue study of the Energy Research and Development method, (1976) *HASL-300; E-Pu-01-01,* for determining plutonium in urine, feces, and water, as summarized by the former General Referee (*J. Assoc. Off. Anal. Chem.* (1977) **60,** 378-379) for application to the determination of plutonium in foods and other biological materials.

(7) *Radium-228:* Prepare manuscript for publication of the method reported by the Associate Referee at the Annual Meeting in 1980 for the determination of radium-228 in foods and water; conduct interlaboratory trial of this method and, if warranted by the results of the trial, initiate collaborative study.

(8) *Strontium-89 and -90:* Initiate collaborative study of the method described by the Associate Referee in *J. Agric. Food Chem.* (1969) **17,** 1337-1339 for the determination of strontium-89 and -90 in foods.

(9) *Tritium:* Appoint Associate Referee; re-evaluate data, and submit revised 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:* Continue study of automated gel permeation chromatography/cesium silicate adsorption chromatography as a cleanup proce-

dure for the gas-liquid chromatographic determination of chemical contaminants in fish.

(2) *Chemical Pollutants in Water and Waste Water:* 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 Associate Referee; continue study to evaluate and collaboratively study methods for determining chlorinated solvents in water and wastewater.

(4) *Organophosphorus Pesticides in Water:* Submit protocol for collaborative study of the method proposed by the U.S. Environmental Protection Agency for determining organophosphorus pesticides in water and wastewater to the General Referee for review and comment, and if approved by the General Referee, initiate the collaborative study.

(5) *Triazine Herbicides in Water:* Appoint Associate Referee; continue study to evaluate and collaboratively test methods for determining triazine herbicides in water and wastewater.

(6) *Other Topics:* 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

MICHAEL WEHR (Oregon Department of Agriculture, 635 Capitol St, NE, Salem, OR 97310), *Chairman;* D. A. SCHIEMANN (Ontario Ministry of Health, Environmental Bacteriology, Box 9000, Terminal A, Toronto, Ontario, Canada M5W 1R5); ARAM BELOIAN (Environmental Protection Agency, Hazard and Evaluation Division, Washington, DC 20460); CHONG PARK (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, Cincinnati, OH 45226); PARIS M. BRICKEY, JR (Food and Drug Administration, Division of Microbiology, Washington, DC 20204), *Secretary;* FOSTER D. MCCLURE (Food and Drug Administration, Division of Mathematics, Washington, DC 20204), *Statistical Consultant*

ANALYTICAL MYCOLOGY OF FOODS AND DRUGS

(1) *Baseline Mold Counts by Blending:* Continue study.

(2) *Chemical Methods for Detecting Mold:* Continue study.

*(3) *Geotrichum Mold in Canned Fruits, Vegetables, and Juices:* Adopt as official first action the method for *Geotrichum* mold in fruit nectars, purees, and pastes; continue study.

(4) *Geotrichum Mold in Frozen Fruits and Vegetables:* Continue study.

(5) *Molds and Yeast in Beverages:* Continue study.

*(6) *Standardization of Plant Tissue Concentrations for Mold Counting:* Adopt as official first action the method for Howard mold count of fruit nectars, purees, and pastes; continue study.

(7) *Tomato Products, Chemical Method:* Continue study.

(8) *Tomato Rot Fragment Count:* Continue study.

DISINFECTANTS

(1) *Antimicrobial Agents Used by Laundries on Fabrics and Materials:* Continue study.

(2) *Sporicidal Tests:* Continue study.

(3) *Tuberculocidal Tests:* Continue study.

(4) *Use-Dilution Test, Variation and Amendments:* Continue study.

(5) *Virucide Tests:* 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 F were adopted by the Association. Section numbers refer to *Official Methods of Analysis* (1980) 13th Edition.

(6) *Textile Antibacterial Preservatives:* Initiate topic.

EXTRANEOUS MATERIALS IN FOODS AND DRUGS

(1) *Asbestos Measurements in Foods, Drugs, and Cosmetics:* Continue study.
(2) *Automated Filth Analysis:* Continue study.
(3) *Botanical Drugs, Adulteration by Foreign Plant Materials:* Continue study.
(4) *Botanicals:* Continue study.
(5) *Brine Extractions, Techniques:* Continue study.
(6) *Cacao Bean Products, Ground:* Discontinue topic.
(7) *Cereals, Breakfast, Ready-to-Eat:* Continue study.
(8) *Chocolate Products:* Continue study.
(9) *Cocoa Powder and Press Cake:* Continue study.
(10) *Dairy Products:* Discontinue topic.
*(11) *Defatting Solvent Economizer, Application to Filth Methods:* Adopt as official first action the use of the solvent economizer apparatus as an alternative defatting procedure for ground turmeric; discontinue topic.
(12) *Fish, Canned:* Continue study.
(13) *Food Supplement Tablets:* Continue study.
(14) *Fruit Products:* Discontinue topic.
*(15) *Grains, Whole, Cracking Flotation Methods:* Adopt as official first action the interim first action method for internal insect infestation in whole grain, *J. Assoc. Off. Anal. Chem.* **64,** 1408–1410 (1981), to replace **44.037.**
(16) *Insect Excreta in Flour:* Continue study.
(17) *Mammalian Excreta Fragments in Milled Food Products:* Continue study.
(18) *Meats, Processed:* Continue study.
(19) *Methods for Urine Detection (formerly Urine TLC):* Appoint Associate Referee.
(20) *Mite Contamination Profiles and Characterization of Damage to Foods:* Appoint Associate Referee.
(21) *Mites in Stored Foods:* Continue study.
(22) *Mushroom Products, Dried:* Continue study.
(23) *Mushrooms, Canned:* Continue study.
(24) *Particulates in Large Volume Parenterals:* Continue study.
(25) *Peanut Butter, Water-Insoluble Inorganic Residues:* Continue study.
*(26) *Rice and Rice Products:* Adopt as official first action the method for filth in rice flour, rice paper, and extruded rice products; discontinue topic.
(27) *Rye Bread:* Continue study.
(28) *Soluble Insect and Other Animal Filth:* Appoint Associate Referee.
(29) *Soups, Canned and Dehydrated:* Continue study.
(30) *Spices:* Appoint Associate Referee.
(31) *Tea:* Discontinue topic.
(32) *Vegetable Products, Dehydrated, Isolation of Extraneous Filth:* Continue study.
(33) *Vertebrate Excreta, Chemical Identification Test (formerly Mammalian Excreta, Chemical Confirmation Test):* Appoint Associate Referee.
(34) *Fecal Sterols:* Establish topic; appoint Associate Referee.
(35) *Mole:* Establish topic.

MICROBIOLOGICAL METHODS

(1) *Automated Methods for Foods and Cosmetics:* Continue study.
(2) *Automated Methods for Fungi:* Continue study.
(3) *Bacillus cereus, Isolation and Enumeration:* Continue study.
(4) *Bacillus cereus Enterotoxin:* Continue study.
(5) *Bacillus cereus Toxin:* Continue study.
(6) *Campylobacter species:* Continue study.
(7) *Canned Foods:* Continue study.
(8) *Cereal Products:* Continue study.
(9) *Clostridium botulinum and Its Toxin, Detection:* Continue study.
(10) *Clostridium perfringens, Isolation and Enumeration:* Continue study.
(11) *Coliform Bacteriology:* Continue study.
(12) *Cosmetic Microbiology:* Continue study.
(13) *Endotoxins by Limulus Amebocyte Lysate:* Continue study.
*(14) *Enteropathogenic Escherichia coli:* Adopt as official first action the method for detection of *Escherichia coli* invasiveness using monolayer HeLa cells prepared in antibiotic-free minimal essential medium with fetal bovine serum.
(15) *Enteropathogenic Escherichia coli, Direct Fluorescent Antibody Procedures:* Continue study.
(16) *Escherichia coli and Coliform Bacteria:* Continue study.
(17) *Parasitology:* Continue study.

(18) *Pathogenic Yeasts, Molds, and Actinomycetes:* Continue study.

*(19) *Salmonella:* Adopt as official final action the official first action revision, *J. Assoc. Off. Anal. Chem.* **64,** 529 (1981), of **46.054-46.067** for the detection of *Salmonella* to delete the combination of brilliant green, *Salmonella-Shigella* (SS), and bismuth sulfite agars andreplace them by a combination of bismuth sulfite, xylose lysine desoxycholate, and Hektoen enteric agars; adopt as official final action the official first action revision, *J. Assoc. Off. Anal. Chem.* **64,** 529 (1981), of **46.054-46.067** for the detection of *Salmonella* species to extend the applicability of the method to edible casein and milk chocolate; in method **46.054-46.067,** revise **46.058(b)** to clarify directions for the treatment of typical or suspicious triple sugar iron (TSI) agar cultures. This section is subject to some interpretation when a typical TSI reaction is obtained and the lysine iron agar is not typical for *Salmonella.* The following revision directs analysis of the TSI culture as a presumptive positive *Salmonella* culture:

(b) *Presumptive reactions.*—Incubate TSI and LIA slants at 35° for 24 ± 2 h and 48 ± 2 h, resp. Cap tubes loosely to maintain aerobic conditions while incubating slants to prevent excessive H_2S production. *Salmonella* cultures typically have alk. (red) slant and acid (yellow) butt, with or without H_2S (blackening of agar) in TSI agar. In LIA, *Salmonella* cultures typically have alk. (purple) reaction in butt. Consider only a distinct yellow coloration in butt of tube as an acidic (neg.) reaction. Do not eliminate cultures that produce discoloration in butt solely on this basis. Most *Salmonella* cultures produce H_2S in LIA. Retain all presumptive pos. *Salmonella* cultures on TSI (alk. slant and acid butt) agar for biochem. and serological tests whether or not corresponding LIA reaction is pos. (alk. butt) or neg. (acid butt). Do not exclude a TSI culture that appears to be non-*Salmonella* if the reaction in LIA is typical (alk. butt) for *Salmonella.* Treat these cultures as presumptive pos. and submit them to further examination. LIA is useful in detection of *S. arizonae* and atypical *Salmonella* strains that utilize lactose and/or sucrose. Discard only apparent non-*Salmonella* TSI agar cultures (acid slant and acid butt) if corresponding LIA reactions are not typical (acid butt) for *Salmonella.* Test retained presumptive pos. TSI agar cultures as directed in **46.058(c)** to det. if they are *Salmonella* sp., **46.062(e)**(*1*), or *S. arizonae* organisms, **46.062(e)**(*2*).

If TSI slants fail to give typical *Salmonella* reactions, pick addnl suspicious colonies from selective medium plate not giving presumptive pos. culture and inoculate TSI and LIA slants as in **(a).**

(20) *Salmonella, Fluorescent Antibody Technique:* Continue study.

(21) *Shigella:* Discontinue topic.

(22) *Somatic Cell, Automatic Optical Counting Method:* Continue study.

(23) *Somatic Cell, Fossomatic Counting Method:* Continue study.

(24) *Somatic Cell, Millipore-DNA Assay:* Continue study.

(25) *Somatic Cell, Rolling Ball Viscometer Procedure:* Continue study.

(26) *Staphylococcal Toxins:* Continue study.

(27) *Staphylococcus:* Continue study.

(28) *Staphylococcus aureus:* Continue official first action status of the surface plating method for *S. aureus* (**46.075-46.076**).

(29) *Sterility Testing of Medical Devices:* Continue study.

(30) *Testing Biological Sterility Indicators:* Continue study.

(31) *Vibrio cholerae and Detection of Its Toxin:* Continue study.

(32) *Vibrio parahaemolyticus:* Continue study.

(33) *Virology and Animal Oncology:* Continue study.

(34) *Yeast and Mold Counts by Spiral Plate Method:* Continue study.

(35) *Yersinia enterocolitica:* Continue study.

(36) *Helium Leaks, Detection in Canned Foods:* Initiate topic.

(37) *Microbe Identification by Capillary GC:* Initiate topic.

(38) *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**).

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); PATRICIA BULHACK (Food and Drug Administration, Division of Color Technology, Washington, DC 20204); GLENN M. GEORGE (Salsbury Laboratories, Charles City, IA 50616); GORDON G. CARTER (Food and Drug Administration, National Center for Antibiotics Analysis, Washington, DC 20204), *Secretary;* and RUEY K. CHI (Food and Drug Administration, Division of Mathematics, Washington, DC 20204), *Statistical Consultant*

ANTIBIOTICS

*(1) *Affinity Qualitative Determination of Penicillin in Milk:* Adopt as official first action the microbiological penicillin-affinity method for qualitative detection of penicillins in milk at levels ≥0.01 unit penicillin G/mL as described by the Associate Referee; continue study.

*(2) *Bacitracin in Feeds:* Adopt as official first action the quantitative microbiological determination of bacitracin in feed premixes at levels ≥10 g/lb as described by the Associate Referee; continue study.

*(3) *Bacitracin in Feeds and Premixes (Chemical Determination):* Adopt as official first action the quantitative HPLC determination of bacitracin in feed premixes at levels ≥10 g/lb, as described by the Associate Referee; 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 methods **42.237-42.240, 42.135-42.138;** continue study.

(10) *Monensin:* Continue official first action status of methods **42.245-42.249, 42.250-42.255;** continue study.

(11) *Oxytetracycline:* Continue study.

*(12) *Quantitative Determination of β-Lactam Antibiotic Residues in Milk:* Adopt as official first action the *B. stearothermophilus* disc method for quantitative determination of penicillins in milk at levels ≥0.016 unit penicillin G/mL as described by the Associate Referees. This recommendation for adoption is made with the provision that, in the case of any dispute or controversy, the official first action method **16.142** be designated as the conclusive method for the quantitative determination of penicillin in milk.

* (13) *Qualitative Determination of β-Lactam Antibiotic Residues in Milk:* Adopt as official first action the *B. stearothermophilus* disc method for qualitative detection of penicillins in milk at levels ≥0.008 unit penicillin G/mL, described by the Associate Referee. Adopt as official first action the *B. stearothermophilus* color-reaction ampule and multitest methods (Delvotest) for qualitative detection of penicillins in milk at levels ≥0.007 unit penicillin/mL, as described by the Associate Referee. 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.

No reports were submitted by the General Referees in the areas of Forensic Sciences, Microbial Mutagenicity Testing, and Toxicological Tests. The recommendations for continued study were initiated by members of Committee G.

BIOCHEMICAL METHODS

(1) *Aminoglycosides in Animal Tissue:* Continue study.

(2) *17-β-Estradiol and Diethylstilbestrol in Tissues (Immunochemical Methods):* Continue study.

(3) *Hormones in Tissues (Immunospecific Affinity Chromatography)*: Continue study.
(4) *Immunochemical Species Identification of Meat*: Initiate topic.
(5) *Performance Evaluation Methods for Non-RIA Procedures Measuring Human Chorionicgonadatropin*: Continue study.
(6) *Performance Evaluation Protocols for Clinical Chemical and Immunochemical Diagnostic Products*: Continue study.
(7) *Steroid Quantitation (Enzymatic Methods)*: Continue study.
(8) *Sulfa Drugs in Animal Tissues (Immunoassay)*: Continue study.

COLOR ADDITIVES

(1) *Arsenic and Heavy Metals*: Continue study.
(2) *Atomic Absorption*: Continue study.
(3) *Cosmetics*: Continue study.
(4) *Color in Candy and Beverages*: 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*: Adopt as official final action method **34.B01-34.B06**. Adopt as official first action the HPLC method for the determination of intermediates and reaction by-products in FD&C Yellow No. 5 as described by the Associate Referee; continue study.
(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, 34.063-34.068**.
(12) *Intermediates, Uncombined in Other Certifiable Colors*: Discontinue topic.
(13) *Subsidiary Colors in Certifiable Color Additives*: Continue study.
(14) *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) *Cyzine*: Discontinue topic.
(3) *Decoquinate*: Discontinue topic.
(4) *Dichlorophene*: Discontinue topic.
(5) *Diethylstilbestrol*: Continue study.
(6) *Dimetridazole*: Continue study.
(7) *3,5-Dinitrobenzamide*: Continue study.
(8) *Ipronidazole*: Discontinue topic.
(9) *Nitrofurans*: Continue study.
(10) *Racephenicol*: Discontinue topic.
(11) *Screening Methods*: Continue study.
(12) *Steroids*: Continue study.
(13) *Sulfa Drugs*: Continue study.
*(14) *Sulfonamide Drugs*: Adopt as official first action the two interim first action methods for the determination of sulfamethazine in swine tissues.
(15) *1-Tetramisole*: Discontinue topic.
(16) *Zeranol*: Discontinue topic.

DRUGS IN FEEDS

(1) *Amprolium*: Continue study.
*(2) *Arprinocid*: Adopt as official first action the spectrophotometric method for the determination of arprinocid in feed premixes as described the the Association Referee; continue study.
(3) *Arsanilic Acid*: Continue study.
(4) *Carbadox*: Continue study.
(5) *Dibutyltin Dilaurate*: Continue study.
(6) *2-Chloro(2,4,5-Trichlorophenyl)Vinyl Dimethyl Phosphate (Rabon)*: Continue study.
(7) *1,2-Dimethyl-5-nitroimidazole (Dimetridazole)*: Continue 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*: Initiate topic.
(13) *Melengestrol Acetate*: Continue study.
(14) *Microscopy*: Continue study.
(15) *Nifursol*: Continue 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) *Sulfadimethoxine-Ormetoprin Mixtures:* Continue study.
(20) *Sulfa Drug Residues:* 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

Initiate General Refereeship.

Report of the Executive Director

DAVID B. MACLEAN
AOAC, 1111 N 19th Street, 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.* **64**, 446–450 (1981)).

The first meeting of the Board of Directors was held January 15, 1981 in Arlington, VA. H. L. Reynolds, President, presided. Other members present were J. P. Minyard, W. R. Bontoyan, C. W. Gehrke, W. P. McKinley, J. B. Kottemann, and B. Larsen. Others present were: D. B. MacLean, C. Cassidy, G. Schwartzman, and K. Fominaya.

The Board of Directors took the following actions at this meeting:

(1) Reaffirmed telephone ballots authorizing the commitment of funds for AOAC to organize and administrate a Technical Advisory Group for ISO/TC 134, Fertilizers.

(2) Approved the revised Long-Range Planning Committee's (LRPC) activities for 1981.

(3) Approved the purchase of word processing equipment.

(4) Requested the LRPC to revise the proposed 5-year plan in accordance with the revised terms of reference, activities list, and comments sent by Ms. Reynolds.

(5) Recommended that the approved terms of reference with current members' names for each Committee be published in *The Referee* with a request for comment.

(6) Directed that the terms of reference for the Committee on Laboratory Quality Assurance be modified.

(7) Approved the terms of reference for the Committee on Safety, subject to annual review.

(8) Approved the terms of reference for the Committee on Methods for Air Sampling and Analysis, subject to annual review.

(9) Directed that the terms of reference for the Official Methods Board be reviewed and revised to be consistent with the October 1980 Bylaw Amendments.

(10) Approved the terms of reference for the Joint Mycotoxin Committee.

(11) Approved the Finance Committee's recommendation to retain the present accounting firm for fiscal year 1981.

(12) Directed the Committee on the Constitution to study the Office of the Treasurer.

(13) Approved the concept of a promotional brochure, which, along with the revised Handbook and Style Manual, was to be presented to the Editorial Board and the Board of Directors for comment before publication.

(14) Increased the price of the *Journal* to $62.00 for 1982.

(15) Directed Dr. MacLean to ask the Committee on Collaborative Studies to provide terms of reference after the Committee's reorganization was completed.

(16) Reaffirmed the request for terms of reference for the Committee on State and Provincial Participation.

(17) Endorsed the idea of a membership directory, with a request that Dr. MacLean work out the details for production.

(18) Approved the terms of reference for the Committee on International Cooperation, subject to annual review.

(19) Approved the terms of reference for the Committee on the Constitution.

(20) Directed that the Committee on Automated Methods and the Committee on Gas and Liquid Chromatography for Pesticide Formulations be combined to deal with the development of specifications for instrumentation and performance. The Committee was to establish its own organizational structure and would receive a charge from the Board of Directors after the Board's May 15th meeting.

(21) Approved the terms of reference for the Long-Range Planning Committee, as modified, subject to annual review.

(22) Approved the terms of reference for the Ways and Means Committee.

(23) Approved the registration fees for the 1981 Annual Meeting.

(24) Reviewed and approved the slide series script. Accepted the estimate for photographer's services as presented.

(25) Accepted the recommended policy on Private Sustaining Membership as amended.

(26) Directed the Staff to review the nominating procedure for Fellows and to make a recommendation for improving and modifying the current procedure.

(27) Requested a preliminary study of the advantages and disadvantages of AOAC entering into a Laboratory Accreditation Program, to be presented to the Board at the May 15th meeting. Suggested a committee be formed to investigate

the feasibility of a laboratory accreditation program. Directed Dr. MacLean to prepare and implement a proposal to evaluate the Utah State Laboratory as requested by the Utah State Department of Agriculture.

(28) Directed Dr. MacLean to prepare and submit a bid to publish and sell the *Manual of Methods for Air Sampling and Analysis.*

The second meeting of the Board of Directors was held May 14, 1981 in Ottawa, Ontario. H. L. Reynolds, President, presided. Other members present were: J. P. Minyard, W. R. Bontoyan, C. W. Gehrke, J. B. Kottemann, and B. Larsen. Others present were: D. B. MacLean and D. Riccard.

The Board of Directors took the following actions at that meeting:

(1) Directed that the terms of reference for the Committee on State and Provincial Participation be rewritten to follow the standard format. Commended the Committee for their accomplishments.

(2) Directed that a chairman for the Committee on Performance of Instrumental Methods and Data Handling be appointed; that a charge to the Committee be established; and that the Committee formulate a timetable and organizational plan. Directed the Committee to plan a symposium for the 1983 Annual Meeting.

(3) Approved all funding commitments made as of May 14, 1981 to speakers at the 1981 Symposia. Directed all future funding be budgeted and limited to travel and subsistence for invited foreign speakers deemed to be essential to the success of the symposium.

(4) Authorized the appointment of the Ad Hoc Committee on Symposia.

(5) Directed Dr. Minyard to prepare a draft of the terms of reference for the AOAC Meeting Program Committee and to submit the draft to the Board at its October 1981 meeting.

(6) Directed the Staff to identify a management consulting firm to review AOAC organizational structure and Bylaws, and to present a proposal to the Board at the September 1981 meeting. Directed Dr. MacLean to ask the Committee on the Constitution to clarify who votes on the adoption of methods and to describe in detail how the voters are proven eligible.

(7) Adopted a point system for the 1982 Fellows selection.

(8) Approved travel funds for M. Wehr to represent AOAC in the Philippines and possibly Taiwan. Approved travel funds for M. Tuinstra to attend the 1981 AOAC Annual Meeting.

(9) Directed B. Larsen to convey to the LRPC the Board's concerns regarding communications.

(10) Directed the Ad Hoc Committee on Laboratory Certification to prepare a detailed analysis of pros and cons of an AOAC-sponsored laboratory accreditation program.

(11) Directed the LRPC to develop guidelines for regional sections with input from staff and persons now active in organizing regional sections.

(12) Directed Dr. MacLean to draft a statement that FDA agree to waive the application of the deduction alternative to income from the Annual Meeting when FDA agrees to supply support for Annual Meeting Symposia.

The third meeting of the Board of Directors was held September 10, 1981 in Arlington, VA. H. L. Reynolds, President, presided. Members present were: J. P. Minyard, B. Larsen, W. R. Bontoyan, J. B. Kottemann, C. W. Gehrke, and W. P. McKinley. Others present were: D. B. MacLean, R. Blakely, G. Schwartzman, and K. Fominaya.

The Board of Directors took the following actions:

(1) Approved the proposed 1981-1982 budget.

(2) Authorized the Executive Director to approve travel extensions inside or outside North America, subject to certain conditions.

(3) Approved financial support for D. A. Biggs to attend ASTM E-29 meetings as an AOAC representative.

(4) Approved a cost of living increase for the AOAC staff.

(5) Modified the AOAC Personnel Policy to extend the Executive Director's authority to postpone a salary step increase from 45 days to the date of the next Board of Directors' meeting.

(6) Directed Dr. MacLean to write a letter of commendation and thanks to Dr. Morton Beroza for his exceptional service to AOAC.

(7) Directed Dr. MacLean to verify information on an individual's service to AOAC, but not to judge performance, when requested by a government agency or private industry to comment on a volunteer's participation in AOAC.

(8) Approved the nomination of William Horwitz as an Honorary Member of AOAC at the Opening Session of the 95th Annual Meeting.

(9) Approved the following recommendations concerning AOAC international activities:

a. Pay 1981 ANSI fees.

b. Request the Committee on International Cooperation to review AOAC participation,

document the value of AOAC involvement in each activity, make recommendations on changes in AOAC involvement in specific activities, and provide a proposed budget for future international activities at the October 1981 meeting.

c. Continue efforts to solicit Private Sustaining Members from the fertilizer industry for support of ISO/TC 134, Fertilizers, and consider a similar effort for support of ISO/TC 34, Agricultural Food Products.

d. Fund international activities as presently budgeted, including Fiscal Year 1982, for ISO/TC 34 and ISO/TC 134, pending review by the Committee on International Cooperation.

(10) Approved the terms of reference for the Committee on State and Provincial Participation.

(11) Directed that the Committee on Meeting Arrangements be disbanded.

(12) Deferred decision on reactivating the Interagency Committee until receipt of a clarification from the LRPC concerning the proposed activities of this Committee.

(13) Changed the name of the Ad Hoc Committee on Symposia to the Special Program Committee.

(14) Directed that the Board of Directors annually review the status and performance of all committees.

(15) Directed the Staff to review the terms of reference for each committee to ensure that they include terms of service and reappointment potential.

(16) Endorsed improving the management of existing requirements to ensure submittal of written reports of committee activities.

(17) Appointed an Ad Hoc Committee on Membership.

(18) Directed the Committee on the Constitution to review the voting procedure and make recommendations on possible changes.

(19) Appointed B. Larsen as Secretary pro tem to handle Association business until a Secretary is appointed, providing the AOAC attorney confirms the legality of such an appointment.

(20) Approved the concept of open meetings for the Committees on Official Methods (A–G) with the condition that anyone wishing to participate or to make a presentation notify the AOAC office 2 weeks before the committee meeting.

(21) Approved the purchase of a mini-computer for the AOAC office.

The fourth meeting of the Board of Directors was held October 18, 1981 in Washington, DC. H. L. Reynolds, President, presided. Members present were: W. P. McKinley, J. P. Minyard, W. R. Bontoyan, C. W. Gehrke, J. B. Kottemann, and B. Larsen. Others present were: D. B. MacLean, R. Blakely, G. Schwartzman, and K. Fominaya.

The Board took the following actions at that meeting:

(1) Acknowledged receipt of the audited financial report and decided to await the report of the Finance Committee before taking any actions.

(2) Endorsed all but one recommendation made by the LRPC for improving methods output; i.e., the Board believes that contract analysis is not the most efficient way to improve and streamline protocols for interlaboratory collaborative studies.

(3) Commended the LRPC for a splendid report on methods output.

(4) Directed the Committee on Collaborative Studies to consider the idea of using a truncated collaborative study which uses fewer laboratories and more samples than presently used to arrive at candidate methods, and to report to the Board of Directors within 6 months.

(5) Instructed the Staff to pursue a marketing study for animal feed drug reference standards and pesticide reference standards.

(6) Directed the Staff to engage the Federation of the American Societies for Experimental Biology (FASEB) to do a limited evaluation of AOAC with emphasis on involvement in the biological areas and international activities.

(7) Endorsed the Editorial Board's recommendations to establish a Search Committee to find a replacement for Helen Reynolds as Editor of the *Journal*.

(8) Postponed consideration of the centennial issue of *Official Methods of Analysis* until more data are gathered on advantages and disadvantages of publishing the book 3 to 6 months early.

(9) Directed the Executive Director to announce the need for an assistant or administrator to work with the Centennial Committee.

(10) Established the policy that every Board of Directors member serve a maximum of 6 years.

(11) Appointed the Treasurer to act as Secretary of the AOAC when necessary.

(12) Requested the President to announce the AOAC Name Change Contest to revise the name of the Association to incorporate *all* scientists and still maintain the AOAC acronym. The prize will be an all-expense-paid trip to the 1984 Spring Training Workshop in Philadelphia.

Important events affecting AOAC during 1981

included:

(1) Leonard Stoloff, Division of Chemistry and Physics, Bureau of Foods, Food and Drug Administration, Washington, DC, was named 1981 winner of the Harvey W. Wiley Award.

(2) The following scientists were named 1981 Fellows of the AOAC: James P. Minyard, State Chemist, Mississippi State, MS; Louis L. Gershman, FDA, Boston, MA; Kenneth Helrich, Rutgers University-Cook College, New Brunswick, NJ; Arthur R. Johnson, FDA, Washington, DC; Valva C. Medkiff, University of Kentucky, Lexington, KY; Robert D. Stubblefield, USDA, Peoria,IL; and Charles C. Clark, DEA, Miami, FL.

(3) Rita A. Manning, a student at the University of Texas, San Antonio, received the 1981-82 Scholarship Award, the sixteenth given by AOAC.

(4) *Optimizing Chemical Laboratory Performance Through the Application of Quality Assurance Principles,* and *Test Protocols for the Environmental Fate and Movement of Toxicants,* the proceedings of symposia held at the 94th Annual Meeting, were published.

(5) Sources of financial support during 1981 were:

Government:

Agricultural Research Service
Alberta Agriculture
Bureau of Alcohol, Tobacco and Firearms
Consumer Product Safety Commission
Environmental Protection Agency, Office of Pesticide Programs
Fish and Wildlife Service
Food and Drug Administration
Food Safety and Inspection Service
Health and Welfare Canada, Health Protection Branch
National Marine Fisheries Service
Alabama Department of Agriculture and Industries
Arizona Office of the State Chemist
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 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 and Consumer Affairs
Oklahoma State Department of Agriculture
Oregon Department of Agriculture
Pennsylvania Department of Agriculture
South Carolina Department of Agriculture
South Dakota State Chemical Laboratories
Tennessee Department of Agriculture
Utah State Department of Agriculture
Vermont Agricultural Experiment Station
Virginia Division of Consolidated Laboratory Services
Wisconsin Department of Agriculture, Trade, and Consumer Protection
Wyoming Department of Agriculture

Industry:

Agrico Chemical Co.
Alcon Laboratories, Inc.
Allergan Pharmaceuticals, Inc.
American Cyanamid Co.
Andersons, The
Bacardi Corp.
Cargill, Inc.
Ciba-Geigy Corp.
Coca Cola Co.
CPC North America-CPC International, Inc.
Duphar B.V.
DuPont Co.
E & J Gallo Winery
Eastman Chemical Products, Inc.
Eli Lilly and Co.
Endo Laboratories
FBC Limited
FMC Corp.
GB Fermentation Industries, Inc.
Fertilizer Institute, The
General Foods Corp.
General Mills, Inc.

Heinz U.S.A.
Hershey Foods Corp.
Hoechst-Roussel Pharmaceuticals, Inc.
Hoffmann-La Roche, Inc.
ICI Americas, Inc.
International Minerals and Chemicals (IMC) Corp.
Kraft, Inc.
Kroger Co., The
Lehn & Fink Products Co.
Mead Johnson & Company
Monsanto Agricultural Products Co.
O. M. Scott & Sons Co.
Ortho Pharmaceutical Corp.
Pennwalt Corp.
Pfizer, Inc.
Pillsbury Co., The
Procter & Gamble Co., The
Raltech Scientific Services
Rhone-Poulenc Chemical Co.
Joseph E. Seagram & Sons, Inc.
Shaklee Corp.
Smith Kline Corp.
Sunkist Growers, Inc.
Swift & Co.
Technicon Industrial Systems
Upjohn Co., The
Velsicol Chemical Corp.

(6) The Technical Advisory Group (TAG) to the American National Standards Institute (ANSI) for the International Organization for Standardization (ISO) Technical Committee (TC) 34 on Agricultural Food Products is actively reviewing, commenting, and voting on methods which have been submitted for consideration as International Standards.

(7) The AOAC was represented at various international meetings as follows:

Robert W. Weik, 2nd Session of the Codex Committee on Cereals and Cereal Products, Washington, DC, April 27, 1981;

Robert W. Weik, 14th Session of the Codex Alimentarius Commission, Geneva, Switzerland, June 29–July 10, 1981;

Warren Bontoyan, CIPAC Symposium, Gembloux, Belgium, June, 1981;

William Horwitz, 12th Session of the Codex Committee on Methods of Analysis and Sampling, Budapest, Hungary, May 11–15, 1981;

William Horwitz, IUPAC International Conference on Harmonisation of Collaborative Studies, Helsinki, Finland, August 20–21, 1981; and

William Horwitz, Nordic Committee for Food Analysis, Oslo, Norway, August 20–21, 1981.

(8) The 6th Annual Spring Training Workshop and Exhibition was held in Ottawa, Ontario, May 12–14, 1981, the first such meeting to be held outside the borders of the United States. Approximately 600 persons attended, including 400 Canadian and 200 American representatives. Technical sessions included laboratory quality assurance, chromatography, food additives, nitrosamines, mycotoxins, metals, extraneous materials, microbiology, pesticides, vitamins, animal drugs, human drugs, drug standards, and forensic science.

(9) The 95th Annual Meeting was held October 19–22, 1981 at the Marriott Twin Bridges Hotel, Washington, DC. Approximately 1100 persons attended. At the General Session, the 11th Award for the best Associate Referee Report of the Year was presented to Raymond J. Gajan, Senior, Research Chemist, Division of Chemical Technology, FDA, for his report, "Collaborative Study: Determination of Lead and Cadmium in Foods by Anodic Stripping Voltammetry." Special awards were presented to Donald Grant, Murray Sutton, and Darrell Wood for organizing the 1981 Spring Training Workshop in Ottawa. William Horwitz was named an Honorary Member of AOAC. Helen L. Reynolds presented her Presidential Address, "Growing Pains," and Leonard Stoloff presented his Wiley Award Address, "The Total Picture."

The Annual Meeting featured five symposia: AOAC Methods Development—Challenge of the Next Decade; Analytical Methodology for Lead in Foods; Computers in the Laboratory; Infant Formula Regulation and Infant Food Problems; and Problems and Solutions in Trichothecene Methodology. Helen Reynolds presided over the banquet on Monday, October 19, and Richard Ronk served as Toastmaster. Leonard Stoloff was presented the 25th Harvey W. Wiley Award, consisting of $750 and a plaque. Arthur H. Hayes, Jr, Commissioner of FDA, gave the keynote address. Entertainment for the evening was provided by Steve Hudson. Banquet attendance was 284. The 2nd Annual Collaborative Studies Luncheon was held on Tuesday, October 20, 1981.

(10) On October 22, James P. Minyard, Jr, took office as President and Warren Bontoyan as President-Elect. Bernhard Larsen was re-elected as Treasurer. D. Earle Coffin was elected as a new Board member. James Kottemann and Charles Gehrke continued as Board members. Helen Reynolds, as 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, Science Program, Washington, DC 20250

Other members: T. G. Alexander, Jr, P. G. Harrill

The Treasurer and the Finance Committee have confirmed in Fiscal Year 1981 (*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 pleasure to be able to report that, again for Fiscal Year 1981, the year-end Financial Statement had been completed and professionally audited before the Association's Annual Meeting, October 18–22, 1981, and reviewed by the Finance Committee before the Business Meeting that closed that Annual Meeting. This prompt closing of the books for Fiscal Year 1981 is attributable in large measure to the internal accounting practices put into place by the Association's Comptroller, Richard Blakely. When put into service, a new computer will complement these new fiscal practices. The Finance Committee believes that the Association's bookkeeping and accounting practices are now fully attuned to the rapid evolution of the Association.

STATEMENT OF FINANCIAL CONDITION – SEPTEMBER 30, 1981

Assets

Current Assets:		
Cash, Bank of Virginia		$156,933.06
Cash, Bank of Virginia, payroll		1,225.94
Cash, office fund		500.00
Accounts receivable, books and publications		36,954.83
Accounts receivable, contracts and grants		72,091.54
Accrued interest receivable		17,468.26
Inventory, books and publications		180,995.85
Prepaid expenses		4,125.19
Advances		2,642.50
Total Current Assets		$472,937.17
Investments:		
Securities	$89,783.01	
Certificates of deposit	831,586.98	
Savings	1,838.35	923,208.34
Fixed Assets:		
Office furniture, fixtures and equipment	$56,011.68	
Less: Accumulated depreciation	22,823.56	33,188.12
Deferred Costs:		
Spring Workshop, 1981	$3,835.14	
Spring Workshop, 1982	3,513.28	
Spring Workshop, 1983	145.65	
Annual Meeting, 1981	24,969.87	
Methods of Analysis, 14th Edition	16,283.69	
Statistical Manual	500.00	
Handbook Revision	3,158.01	
Mid-West Regional Meeting	34.00	52,439.64
Total Assets		$1,481,773.27

Liabilities and Fund Balance

Current Liabilities:		
Accounts payable		$38,425.31
Accrued and withheld payroll taxes		1,153.81
Total Current Liabilities		$39,579.12
Deferred Income:		
Journal subscriptions	$118,076.83	
Annual Meeting, 1981	29,197.50	
Prepaid rental income	526.02	
State support, 1981-82	14,775.00	
Total Deferred Income		162,575.35
Reserve for Publications		200,000.00
Restricted Reserve for the 14th Edition		308,884.00
Restricted Fund—Harvey Wiley		43,816.75
Fund Balance:		
Balance, October 1, 1980	$850,959.56	
Add: Symposium, Quality Assurance, Fall, 1980	4,428.98	
Adjusted Balance, October 1, 1980	$855,388.54	
Less: Restricted Reserve for the 14th Edition	308,884.00	
	$546,504.54	
Add: Excess Income over expenses for the twelve months ended September 30, 1981	$180,413.51	
Balance, September 30, 1981		726,918.05
Total Liabilities and Fund Balance		$1,481,773.27

The Finance Committee has had the task of tracking and advising on the Association's investing and safeguarding of its funds. The Committee believes that the Association's staff has done this conscientiously and with results as satisfactory as could be expected in the present business climate.

The Finance Committee recommended to the Board of Directors that the Association's present accounting firm be retained for Fiscal Year 1983.

Accepted.

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. H. Pomerantz, H. L. Reynolds, and C. H. VanMiddelem

The Editorial Board convened twice this past year: a full-day session at the AOAC offices, February 17, 1981, and a half-day meeting at the AOAC Annual Meeting, Marriott Twin Bridges Hotel, October 18, 1981.

Sales of the 13th edition of the *Official Methods of Analysis* continue to exceed those of the 12th edition for the same time interval. Through September, 1981, 11,225 copies of the 13th edition have been sold. This is 712 more copies (7% increase) than the number of 12th editions distributed for a like period. Foreign sales have

made up 43% of the sales for the current edition.

The publication of the *Quality Assurance Symposium* held at the 94th Annual Meeting has been successful. One thousand copies were originally printed. Inventory at the end of September was 259. Reprinting has been authorized by the Board.

The *Statistical Manual* continues to be in high demand and reprinting has been authorized.

Policy for the publication of symposia, previously adopted by the Editorial Board, was editorially revised. The revised policy is referred to the Symposia and Special Programs Committee.

The 1982 *Journal* budget was reviewed and adopted. Projected estimates into 1983 dictate another increase in subscription rates. The Editorial Board therefore proposed that 1983 *Journal* subscriptions be set at $75.00.

The Board moved to remove restrictions on advertising in the *Journal*. This, in effect, opens the *Journal* to advertisers of products and commodities regulated by governmental agencies. However, recognizing the inherent pitfalls of this action, the Board provided that a 3-member subcommittee establish guidelines and serve as an advisory body to the Managing Editor.

For planning purposes, the Board needs to know the potential for selling space for advertising within the *Journal*. At the direction of the Board, the Managing Editor has obtained an estimate of cost for determining that potential. This estimate is $3,000–$5,000. The Editorial Board is requesting that a consultant be hired for this sum to obtain the needed information.

The subcommittee concerned with the Wernimont statistical manuscript reported to the Board and recommended acceptance of the manuscript but with rewriting. The Board accepted this recommendation and has been seeking an individual to perform this task. Reviews of the manuscript praise the content but note numerous technical errors.

Terms of reference for the Editorial Board, requested by the Board of Directors, has been adopted by the Editorial Board, and referred to the Directors.

Nomenclature of terms used in papers submitted for publication on the subjects of GC (Gas Chromatography) and LC (Liquid Chromatography) must henceforth conform to ANSI/ASTM standards E682 and E355. This requirement has been adopted by the Board and referred to the Official Methods Board.

Consideration is to be given to tentative proposals from FDA and CIPAC on publishing arrangements for manuals. Efforts of this nature must always consider the work load already imposed on the small editorial staff.

The subcommittee on permanent numbering of official methods submitted its final report and proposal. The Board adopted the recommendation. This action dictates that each method appearing in the 14th edition of *Official Methods of Analysis* be identified with a unique number. This number will be of seven digits; the first three represent the year of adoption, the next three reflect the numerical sequence of adoption, and the seventh digit will be a check derived mathematically from the first six. The number will be assigned only to that given method, and any technical change in that method will require a new numerical assignment in the same manner. The older number will not reappear except to identify the method as originally written.

The Editorial Board recognizes the added effort required of the staff to implement this numbering procedure, especially as it applies to established methods. This additional load requirement is referred to the Board of Directors who must weigh it against the benefits of every AOAC method being uniquely identified by an unvarying number: user convenience of not having to keep track of changing numbers for unchanging methods; reduced error of citation, especially in official regulations; and consistency for legal reference in courts of law.

To coincide with the centennial celebration of AOAC, the Board proposes that a centennial issue of the 14th edition be available in October 1984. This date disrupts the normal 5 year cycle of publication. This proposal is referred to the Board of Directors.

A survey was made of users of *Official Methods of Analysis*. The responses obtained from 229 users have been tabulated and will be useful as we plan future editions. A readership survey is presently being conducted for the *Journal*.

The *Handbook of the AOAC* and the *Style Manual of the AOAC* have been updated and combined by the staff. The revision is now ready for review.

The most important and critical subject faced by the Board is that imposed by Helen Reynolds' announcement that she wishes to be relieved of the editorship of the *AOAC Journal*. Under her leadership, and with the assistance of Norma Yess in recent years, the *AOAC Journal* has gained world renown as being equal to, if not better than, journals of other technical associations and

societies. The Board expresses its deep appreciation to Helen Reynolds for her dedication in this endeavor.

The Board has directed that a search committee immediately begin the process whereby this anticipated vacancy may best be filled. Accordingly, a committee composed of the following has been named: David MacLean, Kenneth Hill, Irwin Pomerantz, Alan Hanks, and Charles Jelinek. Helen Reynolds will serve on the committee in an ex-officio role.

Accepted.

Report on the Twenty-Fifth Annual Meeting of the Collaborative International Pesticides Analytical Council (CIPAC)

WARREN R. BONTOYAN, *AOAC Representative*, and JAMES E. LAUNER,[1] *AOAC Correspondent*
Environmental Protection Agency, Office of Pesticide Programs, Beltsville, MD 20705

The 25th Annual CIPAC Meeting was held at the Belgium Agriculture Research Center, Gembloux, Belgium, in June 1981 and was hosted by J. Henriet and Centre de Recherches Agronomiques et Station de Phytopharmacie, Ministere de L'Agriculture, Gembloux. Among the papers presented at the one-day symposium were 2 on physical properties of pesticides, 2 on analysis by high pressure liquid chromatography for temephos and carbamic herbicides, and 3 by various assay procedures: methazol by infrared, ethion by gas-liquid chromatography, and herbicides in multicomponent formulations by thin layer chromatography-spectrodensitometry.

The informal meeting of FAO (Food and Agriculture Organization) and GIFAP (International Group of National Association of Agrochemicals), which represents the major producers of pesticides in Europe and North America, preceded the CIPAC Symposium. Information on the discussions and decisions of the FAO-GIFAP Informal Meeting can be obtained from L. Brader, Food and Agriculture Organization of the United Nations, Via della Terme di Caracalla 00100, Rome, Italy.

The official CIPAC organization is:

Officers:

Chairman—J. Henriet (Belgium)
Secretary—A. Martijn (Netherlands)
Treasurer—M. J. P. Harrington (United Kingdom)
Assistant Secretary—J. F. Lovett (United Kingdom)

Committee of Management:

J. Henriet—Belgium
A. Martijn—Netherlands
M. J. P. Harrington—United Kingdom
W. R. Bontoyan—USA
H. P. Bosshardt—Switzerland
H. H. Povlsen—Denmark
W. Weinman—Federal Republic of Germany
F. Sanchez Rasero—Spain

Members, Country:

United States W. R. Bontoyan
United Kingdom J. Lovett
Denmark and Scandinavia H. H. Povlsen
West Germany W. Weinman
Czechoslovakia V. Batora
Belgium J. Henriet
Netherlands A. Martijn
Switzerland H. Bosshardt
Australia T. J. Beckman
France F. Declercq
Spain F. Sanchez Rasero
Portugal A. S. Fernandes
Honorary member R. de B. Ashworth

Corresponding Members or Observers, Country:

Poland S. Fulde; A. Kotarski
Canada P. Barrette
Sweden M. Akerblom
Yugoslavia A. Sovljanski
Malaysia S. H. Tan
Greece P. Balayannis
Spain P. Hitos
Bangladesh M. R. Amin
Sri Lanka R. C. Boon
South Africa H. Carstens

[1] State Department of Agriculture, Laboratory Services, Salem, OR 93710

Agency or Organizations:	*Correspondent:*
EPA	W. R. Bontoyan
USDA	J. R. Plimmer
World Health Organization (WHO)	G. Quelennec
Food and Agriculture Organization (FAO)	Vacant
European Common Market	G. Hudson
AOAC	W. R. Bontoyan
Ministry of Agriculture, Fisheries and Food (UK)	Vacant
Groupement International des Associations Nationales de Fabricants de Pesticides	R. Goulden
Laboratory of the Government Chemist (UK)	D. Abbott
Agricultural College of Norway	H. Friestad
Texas A & M University	A. Hanks
U. S. Public Health Service	J. Miles
Oregon Department of Agriculture	J. Launer
Director of Laboratory Analysis, Liege, Belgium	A. Benoit

The summaries of the decisions taken at the 25th meeting of the Technical Committee of CIPAC in Gembloux, Belgium, June 22–23, 1981 are:

Code Number	*Chemical*	*Status of the Method*
1	2,4-D	The method for the determination of extractable acids on 2,4-D technical, CIPAC 2935/R, app. D, was adopted as full CIPAC method.
2	MCPA	The method for the determination of extractable acids in MCPA technical, CIPAC 2939/R, app. D, was adopted as full CIPAC method.
6	2,4,5-T	The method for the determination of extractable acids in 2,4,5-T technical, CIPAC 2942/R, app. D, was adopted as full CIPAC method.
11	dichlorvos	The GLC method for the determination of dichlorvos technical, CIPAC 2947/R, app. V, was adopted as provisional WHO-CIPAC method.
19	DNOC	The changes, CIPAC 2928/R, app. A, to the existing full CIPAC method, CIPAC/2854/R, app. A, for DNOC salt nonaqueous solutions were accepted.
45	mevinphos	The HPLC method for the determination of mevinphos in mevinphos technical and formulations, CIPAC 2969/R, was adopted as provisional CIPAC method.
46	dinoseb	The changes, CIPAC 2929/R, app. A, to the existing full CIPAC method, CIPAC 2841/R, app. A, for dinoseb salt nonaqueous solutions were accepted. The spectrophotometric method for dinoseb petroleum oil formulations, CIPAC 2930/R, app. A, was adopted as full CIPAC method.

Code Number	*Chemical*	*Status of the Method*
50	MCPB	The method for the determination of extractable acids in MCPB technical, CIPAC 2940, app. D, was adopted as full CIPAC method.
51	mecoprop	The method for the determination of extractable acids in mecoprop technical, CIPAC 2941/R, app. C, was accepted as full CIPAC method.
59	dimethoate	The TLC and GLC methods for dimethoate technical and emulsifiable concentrates, CIPAC 2926/R, app. H, were adopted as provisional CIPAC methods.
61	maneb + 103. fentin	The CS_2 evolution method for the determination of maneb in maneb-fentin mixtures, CIPAC 2932/R, app. A, was adopted as full CIPAC method.
71	mercury	The methods for determining impurities in technical and formulated mercurials, CIPAC 2863/R, app. Y to AD, were adopted as full CIPAC methods. The colorimetric method for the determination of mercury on treated seeds, CIPAC 2865/R, app. D, was adopted as full CIPAC method.
83	2,4-DB	The method for the determination of extractable acids in 2,4-DB technical, CIPAC 2936/R app. C, was adopted as full CIPAC method.
84	dichlorprop	The method for the determination of extractable acids in dichlorprop technical, CIPAC 2937/R, app. D, was adopted as full CIPAC method.
118	fenoprop	The method for the determination of extractable acids in fenoprop technical, CIPAC 2938/R, app. B, was adopted as full CIPAC method.
152	disulfoton	The GLC method, **6.A27-6.A31**, for disulfoton formulations was adopted as provisional AOAC-CIPAC method.
170	nitrofen	The GLC method for nitrofen technical and formulations, CIPAC 2727/M, was adopted as full CIPAC method.
177	propineb	The CS_2 evolution method for propineb and propineb dispersible powders, CIPAC 2931/R, app. A, was adopted as full CIPAC method.
231	pirimicarb	The GLC method for pirimicarb technical and formulations, CIPAC 2984/(M), was adopted as provisional CIPAC method.
339	diflubenzuron	The HPLC method for diflubenzuron technical concentrates and formulations, CIPAC 2908, was adopted as full CIPAC method.
340	temephos	The HPLC method for temephos technical and temephos emulsifiable concentrates and water dispersible powders, CIPAC 2982a/M, was adopted as full WHO-CIPAC method.
345	MGK 264	The provisional method, **6.A15-6.A17**, was adopted as full AOAC-CIPAC method.

Code Number	Chemical	Status of the Method
353	triazophos	The HPLC method for triazophos technical concentrates and emulsifiable concentrates, CIPAC 2961/(M), was adopted as provisional CIPAC method.
360	triforine	The HPLC method for triforine emulsifiable concentrates, CIPAC 2964/(M), was adopted as provisional CIPAC method.
RE 135	mercury(II)	The method for the standardization and determination of mercury(II) chloride, CIPAC 2863/R, app. AE, was accepted.
MT-	water solubility	The provisional method for the determination of the water solubility of compounds with low solubility, CIPAC 2963/M, was adopted as full CIPAC method.

Accepted.

Report of the Committee on Collaborative Studies

WILLIAM HORWITZ, *Chairman*
Food and Drug Administration, Bureau of Foods, Washington, DC 20204

Other members: R. Albert, G. Carter, P. R. Caudill, T. Dols, H. Egan, M. Ihnat, A. J. Malanoski, K. McCully, F. W. Quackenbush, W. Stellar, E. Stoddard, E. S. Windham, J. Winter

The Committee on Collaborative Studies was established during the year for the following purposes: (1) To provide guidelines for designing, conducting, and interpreting collaborative studies of methods of analysis for commodities of interest to the Association; for reviewing the results of these studies by the Committee on Official Methods; and for approving the methods of analysis associated with these studies; (2) To recommend, assist in, review, or approve, in coordination with the Editorial Board, preparation of publications containing information on requirements for conducting collaborative studies and for reviewing the resulting data; (3) To recommend studies to develop policies for the statistical selection, and the physical removal and handling of samples.

Eight subcommittees under a main committee have been established to develop the desired material. The main committee and subcommittees and the status of their work at the time of the 1981 Annual Meeting are as follows:

Main Committee.—William Horwitz, FDA, Washington, DC, *Chairman;* John Winter, EPA, Cincinnati, OH; Harold Egan, Laboratory of the Government Chemist, London, England.

Definitions.—Thomas Dols, FDA, Rockville, MD, *Chairman.* Draft of definition of "method of analysis" prepared.

Design and Conduct of Collaborative Studies.—William Stellar, American Cyanamid, Princeton, NJ, *Chairman;* Michael Margosis, FDA, Washington, DC; Leonard Stoloff, FDA, Washington, DC. Draft of a short outline of the collaborative study, procedure to validate the performance of a collaborative study, and the outline of a tabular guide prepared.

Guidelines for Statistical Analysis.—Milan Ihnat, Agriculture Canada, Ottawa, Canada.

Guidelines for Performance of Methods.—Anthony J. Malanoski, USDA, Washington, DC.

Guidelines for Systems Control of Collaborative Studies.—Keith McCully, Health and Welfare Canada, Ottawa, Canada, *Chairman;* Stephen Sherken, FDA, Rockville, MD.

Sampling and Sample Preparation.—Paul R. Caudill, Mississippi State Chemical Laboratory, MS, *Chairman;* Edwin Glocker, Glenelg, MD; H. S. Ragheb, Purdue University, W. Lafayette, IN.

Reporting Forms and Data Base.—Richard Albert, FDA, Washington, DC, *Chairman.* Draft data base prepared.

Applications to Biological Tests.—Gordon Carter,

FDA, Washington, DC, *Chairman;* John O'Rangers, FDA, Washington, DC; Stanley Katz, Rutgers University, New Brunswick, NJ.

All subcommittees have an outline of their draft documents and those subcommittees that had not yet prepared a draft intended to submit a first draft for circulation by mid-November 1981.

The Committee has held 2 meetings in which the outline of the work was reviewed. A paper on "The Role of the AOAC in Harmonization of Collaborative Analytical Studies" was prepared by the Committee Chairman for presentation at the conference on Harmonisation of Collaborative Studies, Helsinki, Finland, August 20–21, 1981, organized by the International Union for Pure and Applied Chemistry (IUPAC).

As drafts are prepared and reviewed by the Committee, their availability will be announced in *The Referee.* The target date for the final report is the 1984 Centennial Meeting.

Accepted.

Report of the Committee on the Constitution

D. EARLE COFFIN, *Chairman*
Health and Welfare Canada, Tunney's Pasture, Ottawa, Ontario, Canada K1A 0L2

Other members: R. Frank, F. Johnson, J. B. Kottemann, M. Rhodes, E. D. Schall

During 1980-81, the Committee on the Constitution has considered the need for bylaw changes in relation to voting rights of members, succession within the Board of Directors, and effect of proposed OMB regulations on the process of adoption of methods.

The Committee concluded that bylaw changes were not needed in relation to succession within the Board of Directors or to satisfy the proposed OMB regulations.

Bylaws on membership and voting are difficult to interpret and lead to confusion about voting rights within the AOAC. In the next year, this Committee plans to develop a detailed description and interpretation of these rules and indicate potential problems for consideration by the Association.

Accepted.

Report of the European Representative

MARGREET TUINSTRA-LAUWAARS
Langhoven 12, 6721 SR Bennekom, The Netherlands

The work of the European Representative consists of several activities: attending meetings and, in relation to the new developments in AOAC, establishing personal contacts with institutes and their experts in Europe.

The European Representative attended the following meetings:

(1) International Dairy Federation (IDF) Permanent Committee of Commission E—Analytical Standards; Laboratory Techniques, November 10, 1980, at IDF Headquarters, Brussels, Belgium. The Committee, jointly with the International Organization for Standardization (ISO) and AOAC, reviewed topics under consideration in Commission E. The chairman was R. Demeter, Belgium.

(2) IDF/ISO/AOAC Group E 46—Determination of Vitamin A content of dried milk at IDF Headquarters, November 28, 1980, Brussels, Belgium; chairman, Ch. J. J. Olling, The Netherlands.

(3) IDF Permanent Committee of Commission E, January 21, 1981, Leeuwarden, The Netherlands. Joint IDF/ISO/AOAC topics were reviewed; chairman, R. Demeter, Belgium.

(4) IDF/ISO/AOAC Group E 8—Nitrate, ni-

trite, phosphorus, and chloride in cheese and other dairy products, March 9–10, 1981, Bern, Switzerland: chairman, L. J. Poortvliet, The Netherlands.

(5) ISO/Technical Committee (TC) 34/Sub-Committee (SC) 5—Milk and Milk Products, Chemical Week, June 1–5, 1981, Prague, Czechoslovakia. The following Joint ISO/IDF/AOAC Groups of Experts met (also attended by observers from various countries and interested organizations):

Group E 38—Sampling techniques; chairman, K. Steen, Denmark.

Group E 17—Characterization of dried milk according to heat treatment and usage; chairman, J. C. D. White, United Kingdom.

Group E 31—Fat Determination (Gravimetric methods); chairman, J. Eisses, The Netherlands.

Group E 26—Numerical selection of samples; chairman, D. C. Bettes, United Kingdom.

Group E 47—Antibiotics; chairman, W. Heeschen, Federal Republic of Germany.

Group E 11—Analysis of casein; rapporteur, P. de Hoog, The Netherlands.

IDF Permanent Committee of Commission E met on June 2, 1981, at Prague; chairman L. J. Poortvliet. The ISO/TC 34/SC 5 plenary session, chaired by L. J. Poortvliet, followed the above sessions.

(6) Collaborative International Pesticides Analytical Council (CIPAC), Technical Committee, June 22, 1981, Gembloux, Belgium; chairman, J. Henriet, Belgium. This, the first attendance by the AOAC European Representative at a CIPAC Meeting, offered an opportunity to meet AOAC people and experts from other interested organizations and countries.

(7) IDF/ISO/AOAC group E 42—Edible ices, July 13, 1981, IDF Headquarters, Brussels, Belgium; chairman, R. W. Weik, USA.

(8) IDF/ISO/AOAC Group E 5—Water content of milk and milk products, July 14, 1981, IDF Headquarters, Brussels, Belgium; chairman, R. W. Weik, USA

(9) IDF/ISO/AOAC Group E 8—Nitrate, nitrate, etc. in dairy products, September 15, 1981, Hillerød, Denmark; chairman, L. J. Poortvliet, The Netherlands.

In recent years, the practice of conducting collaborative studies for testing methods is becoming accepted by the IDF/ISO/AOAC Groups of Experts. Moreover, in the past year both IDF and ISO/TC 34/SC 5 have accepted the collaborative study as part of the working procedure for elaborating standards for methods of analysis. The procedure of IDF/ISO/AOAC cooperation will be revised accordingly.

In the past year standards have been published for: milk and milk products, yeasts and molds; milk and milk products, guide to sampling techniques; dried milk, determination of titratable acidity (routine method); dried milk, determination of titratable acidity (reference method); liquid milk, enumeration of microorganisms (colony count at 30°C); dried milk, guideline for the detection of neutralizers; liquid milk, psychrotrophs by colony count at 6.5°C; and milk and milk products, detection of *Salmonella*.

Besides representation of AOAC at the above meetings, increasing attention has been given to making personal contacts in Europe for the purpose of expanding European membership in AOAC.

To inform people about AOAC and its aims, the European Representative gave various talks at interested laboratories, made personal contacts during meetings, and visited institutes. These contacts are expected to increase in the near future and should help AOAC to increase the number of members, sustaining members, and Associate Referees in Europe.

W. Horwitz visited 3 laboratories in The Netherlands in August and September 1981: the State Institute for Quality Control of Agricultural Products, Wageningen; the National Institute of Public Health, Bilthoven; and Duphar Laboratories, Weesp. These visits were coordinated by the European Representative.

Accepted.

Report of the Committee on International Cooperation

BARRY SMITH, *Chairman*
Health and Welfare Canada, Tunney's Pasture, Ottawa, Ontario, Canada E1A 0L2

Other members: L. Appleqvist, W. R. Bontoyan, B. Borsje, H. Egan, E. R. Elkins, I. Hoffman, W. Horwitz, B. Larsen, E. H. Losiewicz, J. P. Minyard, Jr, D. L. Park, R. C. Rund, D. C. Smith, J. Vidaurreta, R. W. Weik

The Committee, at its recent session, was pleased to welcome several members and delegates from outside North America: Roger Wood of the UK Ministry of Agriculture, Fisheries, and Food; Harold Egan of the UK Laboratory of the Government Chemist; C. Zanini of the Brazilian Ministry of Health; Ben Borsje of Duphar, The Netherlands; and Margreet Tuinstra, European representative for the AOAC.

The Committee discussed the cost, and benefits of participation in certain international organizations. In light of the complexity of this issue, a working group was formed to study the matter in detail and report to the Board of Directors early in 1982.

The Committee reviewed its terms of reference and noted that, in most cases, objectives for the year 1980/81 had been met. The subject of term of membership of the Committee chairman and members will be considered by the Committee at its next session.

Several of the international delegates stressed the need for translation of AOAC official methods into other languages, particularly Spanish. This action would further underline the international status of AOAC. A Committee member volunteered to investigate the feasibility of translation through the United Nations system.

Reports from representatives indicated a continuing high level of cooperation with the Analytical Division of the Chemical Society, the International Union of Pure and Applied Chemistry, Collaborative International Pesticides Analysis Council, Codex Committee on Methods of Analysis and Sampling, and the Joint International Dairy Federation/International Organization for Standardization/AOAC Committee.

The Committee was pleased to receive a detailed report from Roger Wood concerning method development and collaborative studies within the European Economic Community.

Accepted.

Report on the Annual Meeting of the International Union of Pure and Applied Chemistry (IUPAC) Commission on Oils, Fats, and Derivatives

DAVID FIRESTONE, *Representative*
Food and Drug Administration, Division of Chemical Technology, Washington, DC 20204

Approximately 40 Commission members and observers attended the annual meeting held as part of the 31st IUPAC General Assembly, August 26–28, 1981, Leuven, Belgium. Among the observers were M. Pike, representing the Federation of Oils, Seeds, and Fat Associations, Ltd, and E. Hopkin of the International Dairy Federation. Another observer, V. Rubajlo, expects to be appointed this year as the National Representative to the Committee from Russia. Rodica Stoica has been appointed National Representative from Romania.

C. Paguot (France) concluded 4 years as chairman of the Commission. He will be succeeded by D. Firestone who was elected for a two-year term (1981–1983) along with M. Naudet (France) as vice-chairman. A. Hautfenne (Belgium) will continue as secretary (1981–1983), becoming vice-chairman during the period 1983–1985. The Commission, which began its

activities involving standardization of analytical methods for oil and fat products in 1930 as the International Commission for the Study of Fat Products, is the source of uniform methods frequently adopted by other national or international organizations including the International Organization for Standardization (ISO) and the Codex Alimentarius Commission.

The Commission reviewed 25 projects including methodology for glycerines and alkaline soaps, erucic acid, solid content of fats by nuclear magnetic resonance (NMR) techniques, polycyclic aromatic hydrocarbons, industrial lecithin products, total sterols, erythrodiol in grapeseed and olive oils, mineral oil residues, tocopherols, chlorinated pesticides in wool wax, linoleic acid content of margarines, emulsifiers, heated fats, plastic polymers and plastic monomers in fats, triglyceride composition by gas chromatography (GC), thiobarbituric acid value, and solvent residues in oils and oilseed cakes. Project work was completed on the following methods which were adopted after successful collaborative study: tocopherols in oils, fats, and margarines by thin layer chromatographic (TLC) fractionation and colorimetric or GC determination; *cis, cis* linoleic acid in oils and margarines by the lipoxidase enzymatic method combined with GC; oxidation products in heated fats by the Guhr and Waibel chromatographic procedure; polyethylene type polymers in fats by gravimetric and infrared spectrophotometric procedures; erucic acid by TLC-GC; and solid content of fats by NMR techniques. Part 3 of the first supplement of the commission's *Standard Methods for the Analysis of Oils, Fats and Derivatives* was published recently. Part 3 includes methods for determining *cis, cis*-methylene interrupted polyunsaturated fatty acids (enzymatic method) and polyethylene type polymers (gravimetric and infrared spectrophotometric procedures). A list of current projects and projects completed within the past 2 years is shown in the accompanying table. The Commission is striving continuously to improve operating procedures and shorten completion times of its projects.

Current and recently completed projects of IUPAC Commission on Oils, Fats, and Derivatives are:

Project	*Study Period*	*Completion, Years*	*Project Leader*
Total Oxidized Fat by TLC/ Densitometry	1972–1981	9	M. Naudet
Tocopherols in Oils and Margarines	1972–1981	9	P. Hendrikse
Chlorinated Pesticides in Fats and Oils	1972–1979	7	E. L. Delvaux
cis, cis Polyunsaturated Fatty Acids	1974–1981	7	O. Levin
Changes in Fats during Deep Fat Frying	1974–1981	7	H. Wessels
Polyethylene Polymers in Oils and Fats	1975–1980	5	J. Vander Weel
Determination of Erucic Acid	1978–1981	3	H. Wessels
Publication of 6th Edition of Methods	1979–1982	3	A. Hautfenne
Methods of Analysis For Glycerines	1979–1982	3	A. Møller
Methods of Analysis for Alkaline Soaps	1979–1982	3	H. Bruschweiler
Solids Content of Fats by NMR	1979–1981	2	M. Naudet
Emulsifiers in Oils and Fats	1979–		H. Bruschweiler
Polycyclic Aromatic Hydrocarbons	1979–		D. Firestone
Erythrodiol in Grapeseed and Olive Oil	1979–		J. Gracian Tous
Total Sterols	1979–		M. Naudet
Pesticides in Woolwax	1979–		G. Zwerenz
Mineral Oil Residues	1979–		H. Vos; J. P. Wolff
Analysis of Commercial Lecithin	1979–		R. Ohlson
Phospholipids in Commercial Lecithin	1980–		O. Levin; J. Beare Rogers

Project	Study Period	Completion, Years	Project Leader
Thiobarbituric Acid Value	1981-		J. Pokorny
Glyceride Composition by GLC	1981-		W. D. Pocklington
Solvent Residues in Oils	1981-		W. D. Pocklington
Solvent Residues in Cakes	1981-		J. P. Wolff

Accepted.

Report of Intersociety Committee (ISC) on the Manual of Methods for 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 25, 1981, in Philadelphia, PA. The secretary reported a considerable drop in sales of the second edition of the manual because of lack of promotion by the American Public Health Association (APHA). As of June 1, 1981, 3300 copies had been sold, and 2600 remained in inventory. Several Committee members stated that most professionals in the field were unaware of the existence of the book. This problem will be discussed with the new APHA Director of Publications, Adrienne Ash.

Two proposals for publication of the third edition were discussed. AOAC made a proposal similar to the current APHA arrangement, under which AOAC would advance publication costs. Both ISC and AOAC would equally share net book sales proceeds, but ISC would use its initial money to reimburse AOAC for publication costs. Another proposal, made by Macmillan, would provide ISC a 15% royalty of net sales receipts for the first 3000 copies and 18% thereafter. This would provide funds to ISC sooner, but, if sales exceeded 6000 copies, the total received would be less. The decision will be made by a mail ballot to members after additional information on costs is obtained and comparisons of estimated income under various assumptions of book sales are prepared. Macmillan claims it has a very effective sales organization.

The editor presented a report listing reviews of new developments in air sampling and analysis. He will prepare guidelines for submission of new manuscript. Reports were presented for the 10 substance subcommittees. They are reviewing and updating the methods in the second edition of the manual and preparing new ones. If a method is to be deleted as obsolete, they will provide reasons for such a recommendation. Several subcommittees in need of new members have been inactive. Candidates were suggested. The chairman reported that new representatives to ISC have been designated by the American Society of Mechanical Engineers and the American Public Works Association.

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, W. R. Bontoyan, F. M. Garfield, J. Martini, S. Sherken, P. Smith, and J. Winters

The initial report of the Committee, presented at last year's annual business meeting, outlining the terms of reference, short- and long-term objectives, and goals was accepted by the Board of Directors with minor changes. The Committee meeting of Monday, October 19, 1981, in Washington, DC, concentrated on the preparation of a 5-year plan.

The purposes and responsibilities of the Committee are to advise and make recommendations to the Board of Directors for the adoption and application of quality assurance principles for the improvement of analytical laboratory operations. To achieve this, the following plan was developed:

(1) To assist the AOAC in the preparation and publication of a quality assurance manual for analytical laboratories, the Committee will, by January 1982, provide advice and guidance as to content and format of a quality assurance manual and will review and comment, on a timely basis, on material prepared for the manual.

(2) By January 1982, the Committee will develop a plan for identifying and obtaining existing or planned government and industry manuals and programs.

(3) By October 1982, the Committee will develop a plan for encouraging analytical laboratories to improve laboratory operations through the adoption and application of quality assurance principles.

(4) To promote AOAC nationally and internationally as a leader in the field of quality assurance for analytical laboratories, the Committee will determine, by October 1982, which organizations, associations, etc., are active in quality assurance activities and, by October 1983, advocate that AOAC join and/or encourage the organization of conferences or symposia on quality assurance for laboratories.

(5) By October 1982, the Committee will develop a plan, to be approved by the Board of Directors, for encouraging the use of quality control principles in AOAC approved methodology and by General Referees, Associate Referees, and others presenting methods or reports at AOAC meetings.

(6) The Committee will assist the AOAC staff, as requested, in reviewing and commenting on regulations, proposed by federal or state agencies, which involve quality control/assurance or good laboratory practices.

Accepted.

Report of the Long-Range Planning Committee

H. MICHAEL WEHR, *Chairman*
Oregon Department of Agriculture, Salem, Oregon 97310

Other members: C. Andres, F. Baur, G. H. Boone, W. Y. Cobb, W. Furman, F. M. Garfield, K. A. Helrich, I. Hoffman, P. D. Jung, D. Larsen, A. Munson, H. L. Reynolds, J. Rodricks

The primary responsibility of the Long-Range Planning Committee is to provide recommendations to the Board of Directors on ways to improve the functioning of the Association.

During the past year the Committee met three times, March 5–6, 1981, July 23–24, 1981, and October 18, 1981. The Committee directed its efforts to those areas with significant long-term

impact on the functions and operations of AOAC. Project areas covered by the Committee included methods output, AOAC committee structure, liaison activities, membership, regional sections, and an AOAC foundation or fund. Ad hoc committees were established in the areas of methods output, committee structure review, liaison activities, and membership.

The results of Committee discussion and recommendations to the Board on these project areas are summarized below.

Methods Output

The Committee report to the Board suggested reasons for the decline in methods output observed over the past several years and made recommendations for improving the output of approved methods. Recommendations included:

(1) Improved communication and promotion of AOAC with government agencies, industry, and other groups.

(2) Improved identification of methods needs and sources of methods available for collaborative study.

(3) Consideration of the use of a candidate method approach for those methods which have received ruggedness testing for sensitivity, precision, and accuracy within a single laboratory, but which have not undergone collaborative study. The Committee recommended that a task force be established to develop the concept for review by the Board of Directors.

(4) Expediting implementation of the collaborative study process by (a) improving management of the collaborative study system, (b) implementing, on at least an annual basis, a meeting of the Methods Board to discuss administration and operation of the methods validation process, and (c) improving and streamlining protocols for interlaboratory collaborative study including consideration of contract analysis for portions of the collaborative study process.

Committee Structure Review

A report was submitted to the Board of Directors outlining recommendations relating to the Committee structure as follows:

(1) Place the Joint Committee on Statistics and the Committee on Collaborative Studies under the Methods Board. This would bring all committees directly involved with methods development and validation under a single board.

(2) Discontinue the Committee on Meeting Arrangements. Many functions of this committee have been absorbed by staff or can be delegated to a new Programs Committee.

(3) Reactivate the Interagency Committee responsible for improving communications between the Association and Federal agencies.

(4) Establish a Program Committee to assist in symposia planning, technical paper review, short course development, and related items.

(5) Establish a Membership Committee to develop and implement membership activities and to help form and guide regional sections.

(6) Implement procedures for establishment of new Committees, specifically requiring all new Committees to be formed initially on an ad hoc basis for a period of one year, with conversion to full regular committee status on Board approval.

(7) Implement procedures for dissolution of Committees, including provisions for mandatory periodic review of Committee activities by the Board.

(8) Provide for uniformity of terms of service for Committee members and rotation of Committee member replacement to provide for Committee continuity.

Liaison Activities

A proposal was prepared for the Board of Directors review recommending that AOAC liaison activities with other professional associations be reviewed and strengthened. The objective of the project would be to obtain active, concerned, knowledgable communication representatives from individual technical societies, trade associations, and other appropriate organizations. The project would involve assessing current liaison activities, strengthening where needed, and establishing new liaisons as appropriate.

Functions of Liaisons would be to:

(1) Inform their respective organizations and members of the AOAC, its goals, objectives, and purposes;

(2) Provide information on methods for collaborative study and recommend individuals to serve as referees and collaborators; and

(3) Serve as a source of information on key contacts within companies for possible financial or technical assistance to the Association.

Membership

Continuing topics of individual dues structure, complimentary membership, and membership growth and retention as related to the long-term effect on the Association were discussed. The Committee considered recommending publication in *The Referee* of an article and membership

response survey dealing with dues structure and related membership items. The Committee recommended that if membership activities are important and/or play a substantial financial role in the support of AOAC, a Membership Committee be established to handle membership development and related activities. Further, the Board should have the primary responsibility of reviewing dues structure or assigning its review to a Committee of the Association.

In the coming year the Committee will study membership activities including a review of classes, rights, and privileges of membership.

Other Items

(1) *Regional Sections.*—At the request of the Board of Directors, the Committee began development of model bylaws for regional sections. The Committee considered areas to be incorporated into model bylaws, leaving specific drafting of the model bylaws to the Committee on the Constitution. The Committee also considered concerns relating to possible conflict of regional section meeting structure with that of the Spring Training Workshop. The Committee concluded that, at present, development of specific guidelines relating to regional section meeting programs was unnecessary; however, regional section programs should be reviewed by staff, and conflicts resolved.

(2) *AOAC Foundation Funds.*—The Committee considered the relative benefits of establishing a foundation versus the use of a special fund for attracting funds for a specific purpose(s). It was reported that raising of funds can be achieved equally well by both means, but administratively and legally the fund approach is simpler. The Committee recommended, therefore, that the fund approach be considered as the method of choice in specialized fund raising efforts.

In the coming year the Committee will also be discussing with Federal, State, and industry representatives the future needs from AOAC with regard to methods and other services.

Accepted.

Report of the Joint Mycotoxin Committee

PETER SCOTT, *Chairman*
Food Research Division, Health Protection Branch, Ottawa, Ontario, Canada K1A 0L2

Other members: A. Pohland, J. Wessel (AOAC), T. Romer, O. Shotwell, L. Stoloff (AACC), L. Goldblatt, R. Stubblefield, A. Waltking (AOCS), A. Campbell (IUPAC)

The following are selected notes from the last meeting, held Oct. 22, 1981, in Washington, DC.

The International Scene

Egypt: A mycotoxin symposium and workshop was held September 6–16, 1981 in Cairo. This convention was cosponsored by the U.S. Food and Drug Administration and the National Research Center of Egypt. A 3-day symposium was followed by a 7-day workshop. A total of 225 scientists from 42 countries attended the symposium. The workshop, planned for 60, had 102 participants who were given hands-on training in analytical methodology ranging in complexity from minicolumn and TLC to HPLC and immunoassay. Khayria Naguib from the National Research Center was Director of this project.

Canada: The problem of deoxynivalenol (vomitoxin) contamination of the 1980 wheat crop of Ontario and Quebec was discussed last year. This year, an average of 0.18 ppm was found in Ontario soft winter wheat and up to 5 ppm in Quebec hard wheat. It was recommended that an upper limit of 0.3 ppm vomitoxin be allowed in Ontario soft wheat to be used in nonstaple foods and that 1981 Quebec hard wheat not be used in the production of foods for human consumption.

The United States Scene

A symposium on trichothecene methodology was held at the AOAC 95th Annual Meeting. Trichothecenes, such as T-2 toxin, diacetoxyscirpenol, and deoxynivalenol (vomitoxin) are the top priority mycotoxins being studied by the

AOAC at this time. Known signs of toxicity include dermal lesions, lack of appetite, and vomiting. As a result of the symposium, it was recommended that efforts to obtain pure reference standards and adequate analytical methodology be increased.

Future Mycotoxin Meetings

April 13–15, 1982, AOAC Spring Workshop, New Orleans, LA

September 1–3, 1982, IUPAC Symposium on Mycotoxins and Phytotoxins, Vienna, Austria

Accepted.

Report of the Ad Hoc Committee on Nitrosamines

THOMAS FAZIO, *Chairman*
Food and Drug Administration, Division of Chemistry and Physics, Washington, DC 20204

Other members: C. Cross, H. M. Davis, T. Davis, W. Fiddler, J. B. Fox, E. L. Greenfield, D. C. Havery, J. Hotchkiss, A. J. Malanoski, I. O'Neill, B. D. Page, R. A. Scanlan, N. P. Sen, F. Suhre, G. Thomas

The Committee met twice in the past year, May 13, 1981, in Ontario, Canada, and October 21, 1981, in Washington, DC.

Nitrate, nitrite methodology.—The present AOAC method was reported to be inadequate for determining nitrite in some foods, especially samples containing high levels of ascorbate or other reductants. At the May meeting, W. Fiddler suggested that a comparative study of various methods (Thermal Energy Analysis (TEA), polarographic, Griess colorimetric) be carried out for the determination of nitrite in meat products. The chemiluminescent method (using TEA), developed by the Eastern Regional Research Laboratory (ERRL), Philadelphia, was reported to have high sensitivity, good reproducibility and to be superior to that reported by C. L. Walters of England. The possibility of using a less expensive commercial NO_x analyzer instead of TEA is being investigated. As of the October meeting, 4 methods were under evaluation or development: charcoal column, chemiluminescent detection (CLD), alkaline AOAC, high pressure liquid chromatographic (HPLC)-ion exchange. A collaborative study was recommended in spite of problems of instability of nitrite in foods. By spring 1982, J. B. Fox and F. Suhre will make a recommendation to the Committee as to which method(s) should be collaboratively tested. J. B. Fox will serve as Associate Referee for nitrite and nitrate as food additives.

The AOAC method for nitrate was also reported to be inadequate and obsolete. T. Fazio's laboratory had tried Sen's method for determining nitrate and nitrite in baby food, cured meats, etc., and it worked satisfactorily. Problems had developed in a comparative study of the specific ion electrode method for nitrate. F. Suhre and W. Fiddler recommended that the Committee consider a comparative or collaborative study of the cadmium reduction method which they believe to be more applicable.

American Society for Brewing Chemists (ASBC) collaborative study on determining N-nitrosodimethylamine (NDMA) in beer and malt.—T. Fazio and G. Thomas reported in May that the Celite column method (survey) and the barium hydroxide distillation method (reference) were highly satisfactory for determining NDMA in beer. ASBC recommended adoption of these 2 methods. Two other methods studied, the direct extraction and the Preptube method, were both unsatisfactory. No methods for malts were satisfactory. As of the October meeting, ASBC had adopted the distillation method and the Celite column method for NDMA in beer. The Committee agreed to recommend that the Celite column method be granted interim first action status by AOAC. A. J. Malanoski will study the ASBC data in detail and report to the committee on its suitability for use by AOAC before official action is taken. The method of Sen et al. was also proposed for interim first action. R. A. Scanlan outlined the current ASBC study on NDMA in dried malt: samples of malt have been distrib-

uted to collaborators; the mineral oil vacuum distillation, Celite column, and wort extraction methods are to be tested; and results will be reported to the ASBC by May 1982. The Committee will evaluate the data generated by ASBC and make recommendations based on such data, providing it meets AOAC standards.

Proposed AOAC collaborative study of the Celite method for the determination of NDMA in nonfat dry milk powder (NFDM) and ground malt.—T. Fazio proposed that the Celite column method for NFDM developed by FDA be collaboratively studied. The need for a study was prompted by FDA's pending Regulatory Compliance Program for this commodity in 1982. This method, once verified, could be adapted to similar dried products. Only verification studies would be needed to expand the method to other matrices. The Committee adopted this proposal and T. Fazio agreed to head a collaborative study and to report the outcome within the year. Five laboratories, International Agency for Research on Cancer (IARC), Canadian Health Protection Branch, USDA-ERRL, Ontario Research Foundation, and R. A. Scanlan, have agreed to participate. USDA, Beltsville, tentatively agreed to participate.

Dry-cured bacon.—A Food Safety and Inspection Service (FSIS) comparative study of 4 methods for dry-cured fried bacon has been completed but the data have not yet been fully evaluated. The 4 methods were vacuum oil distillation, dry column, distillation (Sen), and multi-detection GC-MS. A report will be made at a future meeting.

Determination of volatile nitrosamines in bacon drippings.—N. P. Sen reported a rapid liquid-liquid extraction sample preparation method for GLC-TEA determination of volatile nitrosamines in cooked-out bacon fat. AMI and Oscar-Meyer are also active in this area.

Cosmetics.—H. M. Davis discussed the FDA method for determining NDELA in cosmetics, a method based on liquid-liquid extraction on a Celite column and final determination by HPLC-TEA with recoveries of 80–103%. Polarographic and TEA methods give comparable results. Thus far, no collaborative study of the method has been carried out. S. Hecht indicated that certain cosmetic products might also contain traces of other nitrosamines (e.g., N-nitrosodiisopropylamine, N-nitrosomethylsterylamine, N-nitrosomethyldecylamine).

Total nitroso methods.—The Committee discussed an "improved" method based on Walter's earlier method and developed by Dr. Bavin. They agreed that the method had problems, was not widely used, and no current action was needed, but that the latest revisions in the method should be distributed to the Committee.

Precursor amines.—I. O'Neil, representing IARC, emphasized the need to measure levels of nitrosamine precursors (secondary and tertiary amines, nitrates, and nitrites) and inhibitors (ascorbate, alpha-tocopherol) of nitrosamine formation in foods, an area of study that has been neglected relative to nitrosamines. Most members agreed, but noted that the cost of these many analyses for each food item would be prohibitive.

Only R. A. Scanlan reported working on these precursors. However, he and J. Hotchkiss believe this area may gain importance as in vivo nitrosation is better understood.

Accepted.

Report of the Committee on Performance of Instrumental Methods and Data Handling

JACK R. PLIMMER, *Chairman*
U.S. Department of Agriculture, Science and Education Administration, Beltsville Agricultural Research Center, Beltsville, MD 20705

Other members: S. E. Chappell, R. H. Collier, R. Greenhalgh, P. Kane, B. A. Leonhardt, M. Margosis, L. L. Wall, R. W. Wayne, P. Whittier

The Committee was established in 1981 at the request of the Board of Directors to address quality assurance of instrumental methods and is responsible for the general area of instrumental methods and data handling. The first meeting was held at the AOAC Annual Meeting,

October 1981, to define Committee objectives, approaches, and priorities.

The goal of the Committee is to prepare guidelines for analytical methods in which the apparatus to be used is described in generic terms and in terms of performance. The performance of the analytical method must be assured, but instrumentation need only be described within the context of the method. Avoidance of brand names in description of analytical methods should provide greater flexibility for selection of conditions by the operator who conducts the analysis.

Routinely used instrumentation is usually adequate if performance criteria for the method are established and checked. The Committee will not attempt to write instrument specifications, but rather to describe the instrument that is suitable for the method in terms of its performance. For the analyst to be able to ensure the performance of the method, the selection of appropriate standards will be critical, and the analytical method may require a full description of suitable standards.

Analytical methods that require instrumental techniques, automated techniques, and/or data handling will be described in terms of performance of the methods. Quality assurance will be a major concern. Problems of performance tests, standards needed in tests, and instrument specification protocols will receive Committee attention, as will terminology for use in describing methods. The Subcommittees Instrumental Specifications, Gas and Liquid Chromatographic Specifications are involved in the achievement of the overall goal.

Examples of methods written in generic style are now in preparation and will be submitted for Committee review in 1982. It will be necessary to review AOAC methods in the context of the Committee charge. This process will require the cooperation of Referees, and the Committee will seek to establish liaison with other organizations.

The Committee was also charged with the preparation of a possible chapter on instrumentation for the 14th edition of *Official Methods of Analysis* and instructions to Referees on performance descriptions of instrumentation.

Accepted.

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, M. Cunningham, R. Hall, J. C. Kissinger, F. Lundgren, L. Mell, R. J. Noel, T. Tomczak, G. D. Wyer

The Committee reviewed its short-term goals, deleted the use of the proposed questionnaire (developed during the 1980 meeting) concerning the use of and the need for a chapter on safety. Instead, the Committee proposed to use *The Referee*, the *Journal* and the supplements to *Official Methods of Analysis* for dissemination of safety information.

The Committee requested that the next user survey contain questions pertaining to safety, prepared by the Committee on Safety.

The Committee proposed that the precautionary statements following the title of the method be expanded to include precautionary words, such as explosive, toxic, and carcinogen.

The Committee reviewed the new methods for inclusion of safety cautionary statements.

Accepted.

Report of the Committee on State and Provincial Participation

HOWARD P. MOORE, *Chairman*
Ohio Department of Agriculture, Division of Plant Industry, Feed and Fertilizer Section, Reynoldsburg, OH 43068

Other members: H. Bradford, J. Counts, P. Ferrara, B. Hines, T. Jensen, S. Katz, D. Lewis, D. McDaniel, J. Martini, H. Morris, S. Padmore, P. Rexroad, M. Rhodes, M. Schreiber, V. Thorpe, G. Tichelaar, L. Torma

Twelve members of the present committee and nine other interested persons met October 21, 1981, to discuss implementation of the responsibilities of the Committee as delineated in the terms of reference approved by the AOAC Board of Directors.

The role of this Committee is to develop a network of liaison persons from each state and province to serve as contacts between the AOAC central office and the individual units. Each individual will be contacting laboratories in his or her area to encourage greater participation in the methods development process, particularly as Associate Referees and collaborators. Liaison people may also be able to give guidance to AOAC staff on sources of financial support for AOAC and on follow up to initial contacts.

As the regional organization concept develops, liaison people will form the nucleus of regional representation. Those willing to serve in this capacity should contact the AOAC office. In the future, a representative from each region will organize a steering committee to guide participation.

Regional groups will help satisfy the need felt by states and provinces for a system to bring their laboratories in closer contact with the Association, thereby promoting the sense of belonging needed to justify financial support of AOAC.

This Committee reiterates the need for a directory of laboratories, listing each laboratory's area of expertise, organized by chapters as in the book of methods. Such a directory will improve communications among the listed laboratories and will assist analytical chemists in locating help in method development and problem solving. A draft format to be used in collecting data for this directory was furnished by the AOAC office, who agreed to prepare the questionnaire and to mail it to all laboratories who might have an interest in the work of the AOAC. The Committee also asked that the new brochure, "AOAC Today," be included in the mailing.

Accepted.

Report of the Committee on Statistics

JAMES S. WINBUSH, *Chairman*
Food and Drug Administration, Division of Mathematics, Washington, DC 20204

Other members: C. Annello, P. R. Caudill, D. E. Coffin, E. Glocker, R. C. Rund, M. Siewierski

Members of the Statistics Committee are willing to work with the members of the Committee on Collaborative Studies and to serve on the subcommittees. When sufficient information is available about the individual subcommittees for Statistics Committee members to decide on the subcommittee best fitted to their talents, they will make their preferences known.

Goals of the Statistics Committee were discussed. The Committee intends to initiate a column in *The Referee* on statistical aspects of collaborative studies.

The Committee agreed to provide a session at the AOAC Annual Meeting at least every other year, where a panel of statisticians and chemists could informally discuss statistical issues, such as innovations in the design and analysis of col-

laborative studies, the solution of some existing problem, or pertinent issues encountered as statistical consultants to the subcommittees.

The Committee will study statistical problems associated with the mini-collaborative study, studies involving automated methods, and minor changes in approved methods.

The Committee wants to emphasize, through education, the importance and necessity of ruggedness tests and to stress the contribution of these tests to the evaluation of collaborative study results and to the success of the studies.

The Committee will study and make a recommendation as to which outlier procedures are acceptable for use in the evaluation of collaborative studies.

Accepted.

Report of the Ways and Means Committee

STANLEY E. KATZ, *Chairman*
Rutgers University-Cook College, Department of Biochemistry and Microbiology, New Brunswick, NJ 08903

Other members: R. Blinn, J. Bourke, C. Gehrke, J. Goleb, M. Malina, L. Perlman, W. Phillips, M. Ready

The Ways and Means Committee was charged as follows: to recommend to the Board overall fund-raising strategies for the AOAC; to recommend fund-raising techniques and specific sources to the staff; and, in cooperation with the staff, to prepare two 4-year projections of money needs.

The committee met October 19th to explore approaches by which the charges could be met.

Committee opinions on questions discussed are as follows:

(1) *Broadening the Financial Base of the AOAC by Expansion of Private Sustaining Membership Rolls.*—Expansion of the rolls should be predicated on full membership being available to industrial scientists. The question of full membership is fundamental to a concerted effort in this area. Industry would be unlikely to contribute substantial funding to an organization in which its scientists do not have full status. Analytical methodology is useful to industry as well as to regulatory agencies. There is no reason to assume that industrial scientists will not contribute to developing analytical methodology which can be used as a basis for guidelines for ensuring public health and safety. Keeping analytical methodology at the forefront of science is politically beneficial and is the primary mission of the AOAC.

(2) *Program of Standards.*—Because of the trend toward laboratory certification, AOAC should work to become a leader in marketing primary and matrix standards. AOAC should work with organizations that have a long and distinguished history of providing standard materials.

(3) *Workshops and Training Courses.*—AOAC should provide courses and/or training in the following areas: (a) legal: to include training as expert witnesses and in the rules of evidence, (b) instrumental: theoretical and practical aspects of the newest analytical methodology, and (c) statistical: the need, theory, and application of statistical techniques.

(4) *Consultation Services.*—AOAC should act as a source and/or clearing house for consultants and experts for industry and regulatory organizations. The reservoir of talent would include retired experts. Providing this service would place AOAC in a central role in industrial and regulatory functions.

(5) *Publications and Membership.*—Membership fees could be linked with a larger and more varied *Referee*. *The Referee* could be issued more frequently, cover a wider scope, increase circulation, and possibly carry advertising. The *Journal* would be optional with membership. Expanded circulation might promote increased advertising

(6) *Investments.*—The Finance Committee should study the investments of the AOAC with the aim of improving the return on all investments.

(7) *Sale of Mementos.*—T-shirts are known to be a source of revenue. Their sale could provide modest funding of the national meeting.

The above points were discussed in a preliminary fashion and need further study before consideration by the AOAC staff.

Accepted.

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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

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*

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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)

Microbiological Contamination: Arvey C. Sanders (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., Box 54, 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): W. W. Dada (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): Gerald G. Dull (U.S. Department of Agriculture, Agricultural Research Service, Box 5677, Athens, GA 30604)

Meat and Meat Products (ISO/TC 34/SC 6): Bernhard Larsen (U.S. Department of Agriculture, Washington, DC 20250)

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)

Pesticides Analysis Committee of the Ministry of Agriculture in the United Kingdom:

Dithiocarbamates Panel: Warren R. Bontoyan (Environmental Protection Agency, Office of Pesticide Programs, Beltsville, MD 20705)

Emulsifiability Panel: Keith G. Seymour (Dow Chemical Co., Agricultural Research Department, Midland, MI 48640)

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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*

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FEEDS

Referee: Clyde E. Jones, State Department of Agriculture, 2331 W 31st Ave, Denver, CO 80211

Amino Acid Analysis in Mixed Feeds

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
Rodney J. Noel, 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
James Lange, Raltech Scientific Services, PO Box 7545, Madison, WI 53707

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, Zacatecas 80, Mexico 7 DF. Mexico

Boron
James R. Melton, Texas A&M University, Agricultural Analytical Services, College Station, TX 77843

Calcium and Magnesium
Donald N. Willett, State Department of Agriculture, Hill Farms Laboratory, 4702 University Ave, Madison, WI 53705

Copper
Theo N. Maris, State Department of Agriculture, Mayo Building, Tallahassee, FL 32304

Elemental Analysis of Liming Materials
——

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, Experiment 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, 1525 Sherman St, Denver, CO 80203

Sulfur
Virginia A. Thorpe, Michigan Department of Agriculture, Laboratory Division, 1615 S Harrison Rd, East Lansing, MI 48823

Water-Soluble Methylene Ureas
Allan Davidson, DM 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: ——

Ammonia as a Product Ingredient
Alonza G. Nero, Consumer Product Safety Commission, 230 S Dearborn St, Chicago IL 60604

Benzene in Consumer Products
Wayne G. Wamer, Consumer Product Safety Commission, 200 C St, SW, Washington, DC 20204

Carbolic Acid (Phenolic) Compounds
John C. Williams, Consumer Product Safety Commission, 6 World Trade Center, New York, NY 10048

Chlorinated Hydrocarbons
Patricia Davidson, Consumer Product Safety Commission, San Francisco Area Office Laboratory, 50 United Nations Plaza, San Francisco, CA 94102

Diethylene Glycol and Ethylene Glycol
——

Flammable Substances in Pressurized Containers
Lawrence Feldman, Consumer Product Safety Commission, 6 World Trade Center, New York, NY 10048

Flash Point of Solids and Semisolids

Formaldehyde
Ping Y. Peng, Consumer Product Safety Commission, 7440 S Pulaski Rd, Chicago, IL 60629

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
John Izzi, Consumer Product Safety Commission, 6 World Trade Center, New York, NY 10048

Selenium

Toxic Metals in Paints
Warren K. Porter, Jr, Consumer Product Safety Commission, 200 C St, SW, Washington, DC 20204

Turpentine
Thomas J. Reiss, Consumer Product Safety Commission, 90 Church St, New York, NY 10007

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 428, Woodbine, GA 31569

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

3,5-Dimethyl-4-(methylthio)phenyl Methylcarbamate (Methiocarb®)
C. J. Cohen, Mobay Chemical Corp., Agricultural Chemicals Division, Box 4913, Kansas City, MO 64120

***O*-Isopropoxyphenyl Methylcarbamate (Propoxur®)**
C. J. Cohen

Methomyl
James E. Conaway, Jr, E. I. du Pont de Nemours & Co., Analytical Study Group, 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
Lilia Rivera, State Department of Food & Agriculture, Pesticide Formulations Laboratory, 3292 Meadow View Rd, Sacramento, CA 95832

Captan
A. Aner Carlstrom, Chevron Chemical Co., 940 Hensley St, Richmond, CA 94804

Carboxin

Chlorothalonil
Brian H. Korsch, Diamond Shamrock Co., PO Box 348, Painesville, OH 44079

Copper Naphthenate

Dinocap

Dithiocarbamate Fungicides

Folpet

Oxycarboxin

Pentachloronitrobenzene
Alan R. Hanks, Texas A&M University, Agriculture Analytical Services, College Station, TX 77843

***o*-Phenylphenol**

Triphenyltin

PESTICIDE FORMULATIONS: GENERAL METHODS

Referee: Warren R. Bontoyan, Environmental Protection Agency, Office of Pesticide Programs, Beltsville, MD 20705

Atomic Absorption Spectroscopy
Paul D. Jung, Environmental Protection Agency, Chemical Laboratory, Beltsville, MD 20705

Contaminants in Pesticide Formulations
Warren R. Bontoyan

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., Box 344, Niagara Falls, NY 14302

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., 5000 Richmond St, Philadelphia, PA 19137

Diflubenzuron
A. Van Rossum, Duphar BV, Graneland, 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
Anne L. Ochs, Department of Agriculture, Division of Laboratory Services, St Paul, MN 55107

Tetradifon
Albertus Martijn, Plantenzietenkundige Dients, Postbus 9102, 6700 HC Wageningen, The Netherlands

Toxaphene
William H. Clark, Hercules, Inc., Analytical Division, Research Center, Wilmington, DE 19899

Trichlorfon (Dylox®)
Michael Sabbann, Department of Agriculture, Division of Laboratory Services, St Paul, MN 55107

PESTICIDE FORMULATIONS: HERBICIDES I

Referee: Warren R. Bontoyan, Environmental Protection Agency, Office of Pesticide Programs, Beltsville, MD 20705

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

Diphacinone
Violet M. Stephens, State Department of Agriculture, Division of Laboratory Services, 510 State Office Bldg, St. Paul, MN 55155

Pentachlorophenol
Elmer H. Hayes, Environmental Protection Agency, Chemistry Laboratory, Beltsville, MD 20705

Picloram
Timothy S. Stevens, Dow Chemical Co., Analytical Labs, Midland, MI 48640

Plant Growth Regulators
Richard K. Gard, Office of Indiana State Chemist, Purdue University, West Lafayette, IN 47907

2,3,6-Trichlorobenzoic Acid
Arthur H. Hofberg, Ciba-Geigy Corp., Analytical Chemistry Division, Greensboro, NC 27409

PESTICIDE FORMULATIONS: HERBICIDES II

Referee: Laszlo Torma, State Department of Agriculture, Montana State University, Bozeman, MT 59715

Alanap
George Fuller, 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
Gregory S. Grimes, Purdue University, Department of Biochemistry, West Lafayette, IN 47907

Fluometuron
Arthur H. Hofberg

Linuron
Glenn A. Sherwood

Metalochlor
Arthur H. Hofberg

Methazole
John Forrette, Velsicol Chemical Corp., 341 E Ohio St, Chicago, IL 60611

Monuron

Oryzalin

Paraquat, HPLC Analysis
Lynn Hageman, Montana Dept. of Agriculture, Montana State University, Bozeman, MT 59717

Penoxalin
Gregroy S. Grimes

Profluralin

Siduron
Glenn A. Sherwood

Thiocarbamate Herbicides
William Y. Ja

Trifluralin (Treflan®) and Benefin (Balan®)
Gregory S. Grimes

PESTICIDE FORMULATIONS: HERBICIDES III

Referee: Thomas L. Jensen, State Department of Agriculture, 3703 S 14th St, Lincoln, NE 68502

Alachlor 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

Glyphosate (Isopropylamine Salt *N*-(Phosphoromethyl) Glycine)
Arnold J. Burns, Monsanto Agricultural Products Co., PO Box 174, Luling, LA 70070

Metribuzin
Roger Bishop, Iowa Department of Agriculture, State Chemical Laboratory, Des Moines, IA 50319

Monosodium Methane Arsenate

Propanil
Delmas Pennington, Rohm and Haas, PO Box 591, Knoxville, TN 37901

Terbuthylazine

Triazine Herbicides
Arthur H. Hofberg, Ciba-Geigy Corp., Analytical Chemistry Division, 410 Swing Rd, Greensboro, NC 27409

PESTICIDE FORMULATIONS: INORGANIC PESTICIDES

Referee: Warren R. Bontoyan, 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

Dipropyl Isocinchomeronate (MGK 326®)
Dave Carlson, McLaughlin Gormley King Co., 8810 Tenth Ave, Minneapolis, MN 55427

Fumigants
Lorraine Kroposki, Dow Chemical Co., Analytical Laboratories, Pittsburg, CA 94565

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 ORGANOPHOSPHATE 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. Estein, 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. Estein

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: THIOPHOSPHATE INSECTICIDES

Referee: Edwin R. Jackson, Mississippi State Chemical Laboratory, Box CR, Mississippi State, MS 39762

Acephate
———

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
———

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 D vision, Box 400, Princeton, NJ 08540

Dioxathion
William H. Clark, Hercules, Inc., Analytical Division, Wilmington, DE 19899

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
Chan Caldwell, Mobil Chemical Co., Analytical Chemistry Department, Box 240, Edison, NJ 08817

***O*-Ethyl *O*-(4-Methylthio) Phenyl *S*-Propyl Phosphorothioate (Sulprofes)**
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
Herman Moya, Chemical & Hop Laboratory, 2017 First St S, Yakima, WA 98903

Malathion
Richard S. Wayne

Methidathion
———

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: ———

Ashing Methods
J. Benton Jones, Jr, University of Georgia, Horticulture Department, Athens, GA 30602

Atomic Absorption Methods
Robert A. Isaac, University of Georgia, College of Agriculture, Athens, GA 30601

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, Emporia Kansas State College, Department of Chemistry, Emporia, KS 66801

Fluoride
Jay S. Jacobson, Boyce Thompson Institute, 1086 N Broadway, Yonkers, NY 10701

Nitrogen, Nonprotein
———

Plasma and Emission Spectroscopy
Robert A. Isaac

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, University of Kentucky, Department of Agronomy, Lexington, KY 40506

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, Lorrillard 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); Joseph V. Thom (State Department of Health, Laboratory Services Program, 2151 Berkeley Way, Berkeley, CA 94704); Thomas Layloff (Food and Drug Administration, 1114 Market St, St. Louis, MO 63101); James B. Kottemann (Food and Drug Administration, Division of Drug Chemistry, Washington, DC 20204), *Secretary;* Chang S. Lao (Food and Drug Administration, Bureau of Medical Devices, 8757 Georgia Ave, Silver Spring, MD 20853), *Statistical Consultant*

DRUGS, ACIDIC AND NEUTRAL NITROGENOUS ORGANICS

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

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

Disulfiram
Edward J. Wojtowicz, Food and Drug Administration, 599 Delaware Ave, Buffalo, NY 14202

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

Sulfonamides (Thin Layer Chromatography)
Charlotte A. Brunner, Food and Drug Administration, Division of Drug Chemistry, Washington, DC 20204

DRUGS, ALKALOIDS

Referee: Edward Smith, Food and Drug Administration, Division of Drug Chemistry, Washington, DC 20204

Atropine in Morphine, Atropine Tablets, and Injections
Ira J. Ho comb, Parke Davis and Co., GPO Box 118, Detroit, MI 48232

Belladonna Alkaloids

Colchicine in Tablets
Eugene A Breault, Food and Drug Administration, 50 Fulton St, San Francisco, CA 94102

Curare Alkaloids
John R. Hohmann, Food and Drug Administration, Division of Drug Biology, Washington, DC 20204

Ephedrine
Charles C. Clark, Drug Enforcement Administration, 5205 NW 84th Ave, Miami, FL 33166

Ergot Alkaloids
Thomas C. Knott, Food and Drug Administration, 900 Madison Ave, Baltimore, MD 21201

Neostigmine
Rita E. Kling, Food and Drug Administration, 2nd and Chestnut Sts, Philadelphia, PA 19106

Physostigmine and Its Salts
Norlin W. Tymes, Food and Drug Administration, 900 Madison Ave, Baltimore, MD 21201

Pilocarpine

Rauwolfia Alkaloids
Susan Barkan, Food and Drug Administration, Division of Drug Chemistry, Washington, DC 20204

Rauwolfia serpentina
William M. Smith, Food and Drug Administration, 900 Madison Ave, Baltimore, MD 21201

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)
Jack Fasanello, Drug Enforcement Administration, Northeast Regional Laboratory, 555 W 57th St, New York, NY 10019

Heroin
Harold F. Hanel, Drug Enforcement Administration, 5205 NW 84th Ave, Miami, FL 33166

Lysergic Acid Diethylamide (LSD)

Marihuana and Synthetic Tetrahydrocannabinol (THC)
Ivette Vallejo, Drug Enforcement Administration, 5205 NW 84th Ave, Miami, FL 33166

Methadone
Eugene McConigle, Ortho Laboratories, Raritan, NJ 08869

Methamphetamine
Gene Tracey, U.S. Customs Laboratory, 103 S Gay St, Baltimore, MD 21202

Methaqualone Hydrochloride
Harold F. Hanel

Methylphenidate Phenidine Hydrochloride
Stanley Schrieber, Drug Enforcement Administration, 5205 NW 84th Ave, Miami, FL 33166

Optical Crystallographic Properties of Drugs
Robert S. Ferrera, Drug Enforcement Administration, 7704 Old Springhouse Rd, McLean, VA 22101

Phencyclidine (PCP)
Charles C. Clark

DRUGS, MISCELLANEOUS

Referee: Ted M. Hopes, Food and Drug Administration, 850 Third Ave, Brooklyn, NY 11232

Benzoyl Peroxide
John L. Mietz, Food and Drug Administration, 2nd and Chestnut Sts, Philadelphia, PA 19106

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

Identification of Drugs (Mass Spectrometry)
Robert R. Barron, Food and Drug Administration, Division of Drug Chemistry, Washington, DC 20204

Medicinal Gases

——

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 3rd Ave, Brooklyn, NY 11232

Metals in Drug Bulk Powders
Walter Holak

Microcrystalline Tests

——

Nitroglycerin Tablets (Colorimetry)
Yvonne H. Juhl, Food and Drug Administration, National Center for Drug Analysis, 1114 Market St, St. Louis, MO 63101

Potassium Guaiacol Sulfonate
Joseph B. Proctor, Food and Drug Administration, Division of Drug Chemistry, Washington, DC 20204

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
Mae E. Biesemeyer, Food and Drug Administration, Division of Drug Chemistry, Washington, DC 20204

Thyroid by Differential Pulse Polarography
Walter Holak

DRUGS, OTHER NITROGENOUS BASES

Referee: Thomas G. Alexander, Food and Drug Administration, National Center for Antibiotic Analysis, Washington, DC 20204

Aminacrine
Elaine A. Bunch, Food and Drug Administration, 909 First Ave, Seattle, WA 98174

Antihistamines, Adrenergic Combinations by HPLC
William J. Bachman, Food and Drug Administration, 60 Eighth St NE, Atlanta, GA 30309

Chlorpromazine
Donald J. Smith, Food and Drug Administration, Division of Drug Chemistry, 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

Homatropine Methyl Bromide in Tablets
Duane Hughes, Food and Drug Administration, 1009 Cherry St, Kansas City, MO, 64106

Mephentermine
Elaine J. Friedman, Food and Drug Administration, 50 Fulton St, San Francisco, CA 94102

Phenothiazines in Drugs
Edward G. Lovering, Health Protection Branch, Drug Research Lab., Tunney's Pasture, Ottawa, Ontario, Canada K1A 062

Quaternary Ammonium Compounds

——

Thioridazine
Kurt Steinbrecher, Food and Drug Administration, 909 First Ave, Seattle, WA 98174

Tropane Aminoxides

——

DRUGS, STEROIDS AND TERPINOIDS

Referee: ——

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

Benzothiazine Derivatives
F. Raymond Fazzari, Food and Drug Administration, Division of Drug Chemistry, Washington, DC 20204

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

Progestational Components of Oral Contraceptives

———

Steroid Acetates
Halver C. Van Dame, Merck Sharpe & Dohme Research Laboratories, West Point, PA 19486

Steroid Phosphates
Richard M. Venable, Food and Drug Administration, Divis on 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

COMMITTEE C

D. Earle Coffin (Health and Welfare Canada, Nutritional Services, Tunney's Pasture, Ottawa, Ontario, Canada K1A 0L2), *Chairman*; Thomas R. Romer (Ralston Purina Co, 835 S Eighth St, St. Louis, MO 63188); Alfred D. Campbell (Food and Drug Administration, Office of Science, Washington, DC 20204); 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); Donald N. Willett (State Department of Agriculture, Trade & Consumer Protection, Hill Farms Laboratory, 4702 University Ave, Madison, WI 53705); 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 Adm nistration, 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

Chlorogenic Acid in Coffee
James A. Yeransian, General Foods Corp., White Plains, NY 10625

Crude Fiber in Tea

———

Moisture in Coffee and Tea
William P. Clinton, General Foods Corp., White Plains, NY 10625

Solvent Residues in Decaffeinated Coffee
B. Denis Page, Health and Welfare Canada, Health Protection Branch, Ottawa, Ontario, Canada K1A 0L2

Solvent Residues in Decaffeinated Tea

———

Theophylline in Tea

———

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

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 (Karl Fischer Method)
Gary H. Richardson, Utah State University, De-

partment 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.

Shellfish
Theodore L. Chambers, Food and Drug Administration, Division of Food Technology, Washington, DC 20204

TLC Determination of Amines in Fishery Products
Thomas L. 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

EGGS AND EGG PRODUCTS

Referee: Wallace S. Brammell, Food and Drug Administration, Division of Color Technology, Washington, DC 20204

Color
Marvin E. Winston, Winston Laboratories, 23–25 Mount Vernon St, Ridgefield Park, NJ 07660

Fat
Lawrence E. Taber, Seymour Foods, 101 N Kansas Ave, Topeka, KS 66601

Phosphorus
Wallace S. Brammell

Sterols (Gas Chromatography)
Alan J. Sheppard, Food and Drug Administration, Division of Nutrition, Washington, DC 20204

Total Solids
Herbert C. Sorensen, Columbia Laboratories, Inc., PO Box 40, Corbett, OR 97019

ENZYMES

Referee: Lester Hankin, Connecticut Agricultural Experiment Station, PO Box 1106, 123 Huntington St, New Haven, CT 06504

Amylase Activity in Cereal and Cereal Products
John R. Vercellotti, Labs, Inc., E 25th & Jefferson Ave, Covington, LA 70433

Catalase in Frozen Vegetables
David L. Burton, Food and Drug Administration, Division of Food Technology, Washington, DC 20204

Papain

Peroxidase in Frozen Vegetables
David L. Burton

Proteolytic Enzymes in Treated Meats

Rennet
V. W. Christensen, Miles Laboratories, Inc., PO Box 592, Madison, WI 53701

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, PO Box 61, 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

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

FOOD ADDITIVES

Referee: Thomas Fazio, Food and Drug Administration, Division of Chemistry and Physics, Washington, DC 20204

Anticaking Agents
——

Antioxidants
Dennis Page, Food Research Division, Bureau of Chemical Safety, Tunney's Pasture, Ottawa, Ontario, Canada K1A OL2

Brominated Oils
James F. Lawrence, Food Research Division, Bureau of Chemical Safety, 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
Nisua 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
——

GELATIN, DESSERT PREPARATIONS, AND MIXES

Referee: ——

Gel Strength
Katherine G. Sloman, General Foods Corp., White Plains, NY 10602

MEAT AND MEAT PRODUCTS

Referee: Richard L. Ellis, U.S. Department of Agriculture, Scientific Services, Food Safety and Inspection Service, Washington, DC 20250

Ashing Methods

Automated Methods
Jon L. Schermerhorn, Department of Agriculture and Markets, New York State Food Laboratory, Albany, NY 12235

Bone Content
Paul Corrao, U.S. Department of Agriculture, Food Safety and Inspection Service, Beltsville, MD 20705

Chlorinated Hydrocarbons in Poultry
James Ault, ABC Labs, Box 1097, Columbia, MO 65205

Fat in Meat Products
John 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

Fluoride in Deboned Meat and Poultry
Thomas S. Dolan, U.S. Department of Agriculture, Scientific Services, Food Safety and Inspection Service, Washington, DC 20250

Identification of Meats, Serological Tests
Richard P. Mageau, U.S. Department of Agriculture, Scientific Services, Food Safety and Inspection Service, Beltsville, MD 20705

Moisture, Automated Karl Fischer Titrator Method
Robert Bredimus, Howard Johnsons, 9720 Springfield Blvd, Queens Village, NY 11429

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

Proximate Composition Relationships
Anthony J. Malanoski, U.S. Department of Agriculture, Food Safety and Inspection Service, Washington, DC 20250

Sodium and Potassium in Meat Products

Specific Ion Electrode Applications

Sugars and Sugar Alcohol

Temperature, Minimum Processing
Julio D. Pettinati

MICROCHEMICAL METHODS

Referee: Al Steyermark, Rutgers University, Newark College of Arts and Sciences, Department of Chemistry, Newark, NJ 07102

HPLC of Vegetable Material
Laverne Scroggins, U.S. Department of Agriculture, Eastern Regional Research Center, Philadelphia, PA 19118

MYCOTOXINS

Referee: Leonard Stoloff, Food and Drug Administration, Division of Chemistry and Physics, Washington, DC 20204

Aflatoxin M
Robert D. Stubblefield, U.S. Department of Agriculture, Northern Regional Research Center, Peoria, IL 61604

Aflatoxin Methods
Alfred D. Campbell, 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
Colette P. Levi, General Foods Corp., White Plains, NY 10602

Grains
Odette L. Shotwell, U.S. Department of Agriculture, Northern Regional Research Center, Peoria, IL 61604

Mixed Feeds
Thomas R. Romer, Ralston Purina Co., 835 S Eighth St, St. Louis, MO 63188

Ochratoxins
Stanley Nesheim, Food and Drug Administration,

Division of Chemistry and Physics, Washington, DC 20204

Patulin
Peter M. Scott, Health and Welfare Canada, Health Protection Branch, Tunney's Pasture, Ottawa, Ontario, Canada, K1A 0L2

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
Glen Bennett, U.S. Department of Agriculture, Science and Education Administration, Northern Regional Research Center, Peoria, IL 61604

NUTS AND NUT PRODUCTS

Referee: Glenn Fuller, U.S. Department of Agriculture, Western Regional Research Center, Berkeley, CA 94710

Antioxidants

Composition (Ash, Fat, Fiber, Protein, Water)

Moisture and Water Activity

Oils, Hydrogenated, in Peanut Butter

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., Box 54, 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

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

Paralytic Shellfish Poisoning (Immunoassay Method)
Edward P. Ragelis

Shellfish Poisons
William L. Childress, Food and Drug Administration, 585 Commercial St, Boston, MA 02109

Tetradotoxins

COMMITTEE D

John C. Kissinger (U.S. Department of Agriculture, Eastern Regional Research Center, 600 E Mermaid Lane, Philadelphia, PA 11918), *Chairman*; Robert A. Martin (Hershey Food Corp., Hershey Technical Center, 1025 Reese Ave, Hershey, PA 17033); Elmer George, Jr (Department of Agriculture and Markets, State Food Laboratory, 1220 Washington Ave, Albany, NY 12235); Harry G. Lento (Campbell Soup Co., Campbell Place, Camden, NJ 08151); Donald B. Parrish (Kansas State University, Department of Biochemistry, Manhattan, KS 66506); Laura Zaika (U.S. Department of Agriculture, Eastern Regional Research Center, 600 E Mermaid Lane, Philadelphia, PA 11918); Benjamin Krinitz (Food and Drug Administration, 850 Third Ave, Brooklyn, NY 11232), *Secretary*; Dennis Ruggles (Food and Drug Administration, Division of Mathematics, Washington, DC 20204), *Statistical Consultant*

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

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
Gordon J. Pilone, The Christian Brothers, Mont La Salle Vineyards, PO Box 420, Napa, CA 94558

Color in White Wine
Robert Dowrie, Almaden Vineyards, 1530 Blossom Hill Rd, San Jose, CA 95118

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

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, Reducing
Brother Maluvius, The Christian Brothers, Mont LaSalle Vineyards, Box 420, Napa, CA 94558

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

Volatile Acidity in Wine
Gordon J Pilone

CACAO PRODUCTS

Referee: Jay C. Musser, 214 Merrita Ave, Mount Joy, PA 17552

Caffeine and Theobromine
Wesley A. Kreiser, Hershey Foods Corp., Hershey, PA 17033

Carbohydrates in Chocolate Products
William H. 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 A. Kreiser

CEREAL FOODS

Referee: Doris Baker, U.S. Department of Agriculture, Nutrition Institute, Beltsville, MD 20705

Iron

Phytates
Barbara F. Harland, Food and Drug Administration, Division of Nutrition, Washington, DC 20204

Starch in Raw and Cooked Cereals
Robin M. Saunders, U.S. Department of Agriculture, Western Regional Research Center, 800 Buchanan St, Albany, CA 94710

FLAVORS

Referee: ———

Additives in Vanilla Flavorings
Sidney Kahan, Fitelson Laboratories, 350 W 31st St, New York, NY 10001

Characterization of Natural Foods

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

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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, Food and Drug Administration, 50 Fulton St, San Francisco, CA 94102

Citral

Lasiocarpine and Pyrrolizidines in Herbal Beverages

Monosodium Glutamate in Foods

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: ——
Ash and Pungent Principles in Mustard

Characterization of Natural Spices

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
J. D. Henry, Allied Foods Inc., 93326 Martech Station, 1450 Hills, Pl, NW, Atlanta, GA 30318

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

Honey
Jonathan W. White, Jr, 217 Hillside Dr, Navasota, TX 77868

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 11918

Standardization of Sugar Methods of Analysis
Whitney J. Newton II, Holly Sugar Co., PO Box 1052, Colorado Springs, CO 80901

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

Sugars, Reducing

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, Southern Regional Research Center, Oil, Seed and Foods Laboratory, New Orleans LA 70179

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
Harry Lento, Campbell Soups, Campbell Place, Camden, NJ 08151

Folic Acid
Patric G. Bryan, 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, Raltech, Box 7545, Madison, WI 53707

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, Meade Johnson Co., 2404 Pennsylvania Ave, Evansville, IN 47721

COMMITTEE E

Jerry A. Burke, (Food and Drug Administration, Division of Chemical Technology, Washington, DC 20204), *Chairman*; Anthony J. Malanoski (U.S. Department of Agriculture, Food Safety and Inspection Service, Washington, DC 20250); Kenneth Helrich (Rutgers University–Cook College, Department of Entomology–Economic Zoology, New Brunswick, NJ 08903); William A. Steller (American Cyanamid Co., PO Box 400, Princeton, NJ 08540); Gerald R. Myrdal (Wisconsin Department of Agriculture, Bureau of Chemistry, 4702 University Ave, Madison, WI 53705); Wendell F. Phillips (Campbell Soup Co., Campbell Pl, Camden, NJ 08151); Henry F. Enos (Environmental Protection Agency, Pesticide Research Laboratory, Gulf Breeze, FL 32561); Bartholomew Puma (Food and Drug Administration, Division of Chemical Technology, Washington, DC 20204), *Secretary*;

CARBAMATE PESTICIDES, FUMIGANTS, AND MISCELLANEOUS

Referee: Robert W. Storherr, Environmental Protection Agency, 1921 Jefferson Davis Hwy, Arlington, VA 20460

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 and Metabolites
Sujit Witkontin, FMC Corp., 100 Niagara St, Middleport, NY 14105

Ethylene Oxide and Its Chlorohydrin
A. R. Stemp, Kraft Co., 801 Waukegan Rd, Glenview, IL 60025

Fenvalerate
Gobind P. Makhijani, Environmental Protection Agency, Benefits and Field Studies Division, Beltsville, MD 20705

Fumigants

Inorganic Bromides in Grains
King T. Zee, Environmental Protection Agency, Benefits and Field Studies Division, Beltsville, MD 20705

Permethrin
Gobind P. Makhijani

Phosphine
T. Dumas, Research Institute, University Sub Post Office, London, Ontario, Canada N6A 3K0

Resmethrin
Calvin Corbey, Environmental Protection Agency, Benefits and Field Studies Division, Beltsville, MD 20705

Sodium Monofluoroacetate
Henry M. Stahr, Iowa State University, College of Veterinary Medicine, Ames, IA 50010

FUNGICIDES, HERBICIDES, AND PLANT GROWTH REGULATORS

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

Carbamate Herbicides

Chlorophenoxy Alkyl Acids
Allan E. Smith, Agriculture Canada, Research Branch, Box 440, Regina, Saskatchewan, Canada S4P 3A2

Chlorothalonil

Dinitro Compounds

Diquat and Paraquat
Harry A. McLeod, 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
William P. Cochrane, Agriculture Canada, Plant Products Division, Ottawa, Ontario, Canada K1A 0L5

Organotin in Fungicides
Richard D. Cannizzaro, Thompson-Hayward Chemical Co., 5200 Speaker Rd, Kansas City, KS 66110

Pentachlorophenol
Arnold P. Borsetti, Food and Drug Administration, Division of Chemical Technology, Washington, DC 20204

Sodium *o*-Phenylphenate

Substituted Urea
Robert E. Leitch, E. I. Du Pont de Nemours & Co. Inc., Industrials and Biochemicals Department, Wilmington, DE 19898

Succinic Acid, 2,2-Dimethylhydrazide

Thiocarbamate Herbicides

***s*-Triazines**
J. F. Lawrence, Health and Welfare Canada, Health Protection Branch, Tunney's Pasture, Ottawa, Ontario, Canada K1A 0L2

Trifluralin

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 0C6

Cadmium and Lead in Earthenware
Benjamin Krinitz, Food and Drug Administration, 850 Third Ave, Brooklyn, NY 11232

Carbon Rod Atomization Techniques

Emission Spectrochemical Methods
Fred L. Fricke, Food and Drug Administration, 1141 Central Pkwy, Cincinnati, OH 45202

Fluorine
Robert W. Dabeka, Health and Welfare Canada, Bureau of Chemical Safety, Tunney's Pasture, Ottawa, Ontario, Canada K1A 0L2

Hydride Generating Techniques
Stephen G. Capar, Food and Drug Administration, Division of Chemical Technology, Washington, DC 20204

Mercury, Organic
Ronald Suddendorf, Food and Drug Administration, Division of Nutrition, Washington, DC 20204

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

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, Food and Drug Administration, 850 Third Ave, Brooklyn, NY 11232

Organometallics
Ronald Suddendorf

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

ORGANOCHLORINE 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

Chlorobenzilate, Chloropropylate, and Bromopropylate
Roy S. Brosdal, Food and Drug Administration, 240 Hennepin Ave, Minneapolis, MN 55401

Dicofol
———

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

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

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

ORGANOPHOSPHORUS PESTICIDES

Referee: Keith A. McCully, Health and Welfare Canada, Field Operations Directorate, Ottawa, Ontario, Canada K1A 1B7

Confirmation Procedures
A. S. Y. Chau, Inland Waters Directorate, Water Quality Branch, 867 Lakeshore Rd, PO Box 5050, Burlington, Ontario, Canada L7R 4A6

Extraction Procedures
———

General Method for Organochlorine and Organophosphorus Pesticides
———

High Fat Samples
Ronald Scharfe, Agriculture Canada, Pesticide Laboratory, Ottawa, Ontario, Canada K1A OC5

Soils

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

Radium-228
Edmond J. Baratta

Strontium-89 and Strontium-90
Edmond J. Baratta

Tritium

WATER

Referee: Alfred S. Y. Chau, Canada Centre for Inland Waters, PO Box 5050, Burlington, Ontario, Canada L7R 4A6

Chemical Pollutants in Aquatic Biota
David L. Stalling, Department of the Interior, Bureau of Sport Fisheries and Wildlife, Columbia, MO 65201

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
S. Michael McCown, Water Air Research, Box 1121, Gainesville, FL 32602

Organophosphorus Pesticides in Water
Patricia Smith, Woodson-Tenent Laboratories, Box 2135, Memphis, TN 38101

Triazine Herbicides in Water

COMMITTEE F

Michael Wehr (Oregon Department of Agriculture, 635 Capitol St, NE, Salem, OR 97310), *Chairman*; Donald Mastrorocco (Hershey Foods Corp., 19 E Chocolate Ave, Hershey, PA 17033); Chong Park (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); Paris M. Brickey, Jr (Food and Drug Administration, Division of Microbiology, Washington, DC 20204), *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

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, 599 Delaware Ave, Buffalo, NY 44202

Molds and Yeasts in Beverages
Stanley M. Cichowicz

Standardization of Plant Tissue Concentrations for Mold Counting
Stanley M. Cichowicz

Tomato Products, Chemical Method for Detecting Mold
Ruth Bandler

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

Textile Antibacterial Preservatives
Luther B. Arnold

Tuberculocidal Tests
Joseph Ascenzi, Arbrook Inc., Arlington, TX 76010

Use-Dilution Test, Variation and Amendments
George Walter, S. C. Johnson and Son, 1525 Howe St, Racine, WI 53403

Virucide Tests
Charles R. McDuff, Economics Laboratory, Inc., 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

Automated Filth Analysis
Jack L. Boese and Harriett R. Gerber, Food and Drug Administration, Division of Microbiology, Washington, DC 20204

Botanical Drugs, Adulteration by Foreign Plant Materials
Frank D. Amelio, Bio Botanica, 2 Willow Park Center, Farmingdale, NY 11735

Botanicals
Arnold E. Schulze, Food and Drug Administration, Division of Microbiology, Washington, DC 20204
Larry E. Glaze, Food and Drug Administration, Division of Scientific Investigations, Rockville, MD 20857
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

Cereals, Breakfast, Ready-to-Eat
Russell G. Dent, Food and Drug Administration, Division of Microbiology, Washington, DC 20204

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

Fish, Canned
Russell G. Dent

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

Mammalian Excreta Fragments in Milled Food Products
Michael Loges, Food and Drug Administration, 599 Delaware Ave, Buffalo, NY 14202

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 McClymont, Health and Welfare Canada, Bureau of Microbiological Hazards, Tunney's Pasture, Ottawa, Ontario, Canada K1A 0L2

Mites in Stored Foods
Jack L. Boese

Mole, Filth in

Mushroom Products, Canned
Jack L. Boese
Alan R. Olsen, Food and Drug Administration, 1521 W Pico Blvd, Los Angeles, CA 90015

Particulates in Large-Volume Parenterals
Gordon Oxborrow, Food and Drug Administration, Minneapolis Center for Microbiological Investigations, 240 Hennepin Ave. Minneapolis, MN 55401

Peanut Butter, Water-Insoluble Inorganic Residues
Roy J. Speeg, Procter and Gamble Co., Winton Hill Technical Center, Cincinnati, OH 45224

Rye Bread
Richard R. Haynos, Food and Drug Administration, 850 Third Ave, Brooklyn, NY 11232

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

MICROBIOLOGICAL METHODS

Referee: Arvey C. Sanders, 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

Automated Methods for Fungi
Marilyn Zipkes, Food and Drug Administration, 1141 Central Pkwy, Cincinnati, OH 45202

***Bacillus cereus*, Isolation and Enumeration**
Stanley M. Harmon, Food and Drug Administration, Division of Microbiology, Washington, DC 20204

***Bacillus cereus* Enterotoxin**
Stanley M. Harmon
Gayle Lancette, Food and Drug Administration, Minneapolis Center for Microbiological Investigations, 240 Hennepin Ave, Minneapolis, MN 55401

***Bacillus cereus* Toxin**
Reginald W. Bennett, Food and Drug Administration, Division of Microbiology, Washington, DC 20204

Campylobacter Species

Canned Foods
Cleve B. Denny, National Food Processors Association, 1133 20th St, NW. Washington, DC 20036

Cereal Products
Nino F. Insalata, General Foods Corp., White Plains, NY 10602

***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
Ana Maria Placencia, Food and Drug Administration, Minneapolis Center for Microbiological Investigations, 240 Hennepin Ave, Minneapolis, MN 55401

Coliform Bacteriology

Cosmetic Microbiology
John P. Lucas, Food and Drug Administration, Division of Microbiology, Washington, DC 20204

Endotoxins By Limulus Amebocyte Lysate
Christine Twohy, Food and Drug Administration, 240 Hennepin Ave, Minneapolis, MN 55401

Enteropathogenic *Escherichia coli*
Ira J. Mehlman, Food and Drug Administration, Division of Microbiology, Washington, DC 20204

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 Coliform Bacteria**
Daniel A. Hunt, Food and Drug Administration, Division of Food Technology, Washington, DC 20204
L. Anne Otto, Food and Drug Administration, 1521 W Pico Blvd. Los Angeles, CA 90015

Helium Leaks, Canned Foods
George J. Jackson, Food and Drug Administration, Division of Microbiology, Washington, DC 20204; Richard A. Rude, Food and Drug Administration, Minneapolis Center for Microbiological Investigations, 240 Hennepin Ave, Minneapolis, MN 55401

Microbe Identification by Capillary GC
Michael McDowell, State Public Health Laboratories, 1409 Smith Tower, Seattle, WA 98104

Parasitology
George J. Jackson
Richard A. Rude

Pathogenic Yeasts, Molds, and Actinomycetes
Philip B. Mislivec, 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

Somatic Cell, Millipore-DNA Assay

Somatic Cell, Rolling Ball Viscometer Procedure
Gary H. Richardson, Utah State University, Department of Nutrition and Food Science, Logan, UT 84322

Staphylococcus

Staphylococcus aureus

***Staphylococcus* Toxin**
Reginald W. Bennett

Sterility Testing of Medical Devices
Daniel A. Quagliaro, Food and Drug Administration, 850 Third Ave, Brooklyn, NY 11232

Testing Biological Sterility Indicators
Gordon Oxborrow, Food and Drug Administration, 240 Hennepin Ave, Minneapolis, MN 55401

***Vibrio cholerae* and Detection of Its Toxins**

Vibrio parahaemolyticus
John Liston, University of Washington, College of Fisheries, Institute for Food Science and Technology, Seattle, WA 98195
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 and Mold Counts

Yersinia enterocolitica
Ira J. Mehlman

COMMITTEE G

Richard L. Brunelle (Bureau of Alcohol, Tobacco and Firearms, National Laboratory Center, 1401 Research Blvd, Rockville, MD 20850), *Chairman*; Patricia Bulhack (Food and Drug Administration, Division of Color Technology, Washington, DC 20204); Valva C. Midkiff (University of Kentucky, Division of Regulatory Services, Kentucky Agricultural Experiment Station, Lexington, KY 40506); Alexander MacDonald (Hoffmann-La Roche Co., 340 Kingsland St, Nutley, NJ 07110); Glenn M. George (Salsbury Laboratories, Research Division, 2000 Rockford Rd, Charles City, IA 50616); Harold Thompson (National Center for Toxicological Research, Jefferson, AR 72079); Gordon G. Carter (Food and Drug Administration, National Center for Antibiotic Analysis, Washington, DC 20204), *Secretary*; Ruey Chi (Food and Drug Administration, Division of Mathematics, Washington, DC 20204), *Statistical Consultant*

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

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, Ralston Purina Co., Checkerboard Sq, St. Louis, MO 63188

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, 500 Peshtigo Ct, Chicago, IL 60690
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, Rutgers University, Department of Biochemistry and Microbiology, New Brunswick, NJ 08903

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

Immunochemical Species Identification of Meat
Alonza R. Hayden

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

---

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 Adams, 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 Administra-

tion, 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

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
J. Westheimer, Hoffmann-La Roche Inc., Animal Health Research, 340 Kingsland St, Nutley, NJ 07110

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
Mercy Araujo, Hoffman-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
William T. Van Antwerp, Agri Science Laboratories, 2122 S Granville, Los Angeles, CA 90025

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
Mercy Araujo

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; Joseph McLaughlin, Consumer Product Safety Commission, Division of Biological Sciences, Washington, DC 20204

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

Cholinesterase
Richard Pfeiffer, 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

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

Selenium in Animal Tissue
James E. Roof, State Veterinary Diagnostic Laboratory, PO Box 1430, Harrisburg, PA 17105

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 from the date of publication of this report, March 15, 1982. 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 **.C01.** Subsequent sections are numbered **.C02, .C03, .C04,** et seq. The **C** signifies that the method was adopted in 1981. Methods adopted in 1979 and 1980 are designated by the chapter number plus **.A01, .B01,** 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.*

DEFINITIONS OF TERMS AND EXPLANATORY NOTES

The following should be added under *"Standard Operations,"* p. xvii:

(a) Insert as a new paragraph (25):

(25) *Recovery (R) of analyte from fortified sample by a method of analysis.*—Fraction of an analyte added to a sample (fortified sample) prior to analysis, which is measured (recovered) by the method. When the *same* analytical method is used to analyze both the unfortified and fortified samples, calculate %*R* as follows:

$$\%R = [(C_F - C_U)/C_A] \times 100$$

where C_F = concn of analyte measured in fortified sample;
C_U = concn of analyte measured in unfortified sample;
C_A = concn of analyte *added* in fortified sample.

(Note: C_A is a calcd value, not a value measured by the method being used.)

Concn of added analyte should be no less than concn of analyte in unfortified sample. Sum of concn of added analyte plus analyte present before fortification should be in same range as analyte concn sought in actual samples. Addn of analyte must not cause measuring instrument to exceed linear dynamic range of std curve. Both fortified and unfortified samples must be treated identically during analysis to minimize experimental bias.

(b) Renumber current paragraph (25) to be new (26), (26) to be new (27), etc., through (29) to be new (30).

1. AGRICULTURAL LIMING MATERIALS

No additions, deletions, or changes.

2. FERTILIZERS

The following azomethine H microcolorimetric method for the determination of acid- and water-soluble boron in fertilizers, *J. Assoc. Off. Anal. Chem.* **65,** 234 (1982), was adopted official first action:

Acid- and Water-Soluble Boron
Official First Action

2.C01 ***Apparatus and Reagents***

(a) *Spectrophotometer.*—Beckman Model 24/25, or equiv.

(b) *Precision pipet.*—100 μL Sherwood Lancer (Sherwood Medical Industries, Inc., St. Louis, MO 63103), or equiv.

(c) *Dispenser pipet.*—Automatic (Repipet, Labindustries, Berkeley, CA 94710), or equiv., 5 mL capacity.

(d) *Boron std solns.*—(*1*) *Stock soln.*—100 μg/mL. Dissolve 0.5716 g boric acid in H_2O and dil. to 1 L with H_2O. Mix well and transfer to plastic bottle. (*2*) *Working solns.*—0, 5, 10, 15, 20, 25, 30, and 45 μg/mL. Pipet 0, 5, 10, 15, 20, 25, 30, and 45 mL stock soln into sep. 100 mL vol. flasks, dil. to vol. with 1% HCl, mix well, and transfer to plastic bottles. Solns are stable.

(e) *Azomethine H color reagent.*—Dissolve 0.9 g azomethine H (Pierce Chemical Co., Rockford, IL 61105) and 2.0 g ascorbic acid in 100 mL H_2O. Store in refrigerator and discard after 14 days.

(f) *Buffer-masking soln.*—Dissolve 140 g ammonium acetate, 10 g potassium acetate, 4 g nitrilotriacetic acid, disodium salt 99+% (Aldrich Chemical Co., Inc., Milwaukee, WI 53233), 10 g (ethylenedinitrilo)tetraacetic acid, and 350 mL 10% acetic acid (v/v) in H_2O and dil. to 1 L with H_2O. Soln is stable.

(g) *Color developing reagent.*—Place 35 mL azomethine H color reagent and 75 mL buffer-masking soln into 250 mL vol. flask and dil. to vol. with H_2O. Prep. fresh daily.

2.C02 ***Preparation of Sample Solutions***

(a) *Acid-soluble boron.*—Weigh 2.00 g sample into 100 mL vol. flask, add 30 mL H_2O and 10 mL HCl, stopper, and shake 15 min. Dil. to vol. with H_2O, mix well, and filter immediately into plastic bottle. Dil. as necessary, so final soln for color measurement falls within std curve.

(b) *Water-soluble boron.*—Weigh 2.00 g sample into 250 mL beaker, add 50 mL H_2O, and boil ca 10 min. Filter hot thru Whatman No. 40 paper, or equiv., into 100 mL vol. flask. Wash ppt 6 times with hot, boiled H_2O until vol. in flask is ca 95 mL. Cool, add 1.0 mL HCl, dil. to vol. with H_2O, and mix. Transfer to plastic bottle immediately; dil. as necessary so final soln for color measurement falls within std curve.

2.C03 ***Determination***

Pipet 100 μL aliquots of 0, 5, 10, 15, 20, 25, 30, and 45 μg/mL std and 100 μL aliquots of sample solns into sep. 10 mL erlenmeyers. Add 5.0 mL color developing reagent by automatic pipet dispenser (5 mL pipet is suitable but slower) and let stand 1 h at room temp. Transfer to 1 cm cell and read A at 420 nm against H_2O. Correct for reagent blank (0 mg/mL std). Construct std curve by plotting A against μg/mL stds and read concns (μg/mL) of sample solns from std curve.

2.C04 ***Calculation***

Boron, % = (μg/mL from std curve) × diln factor × (100/g sample) × 10^{-6}

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) Copper naphthenate, **6.065-6.066**

(b) Captan, AOAC-CIPAC gas-liquid chromatographic method, **6.215-6.219**

(c) Methyl parathion or ethyl parathion in microencapsulated formulations, **6.409-6.414** (as modified, "Changes in Methods," *J. Assoc. Off. Anal. Chem.* **63,** 380 (1980))

(d) Triphenyltin compounds, **6.436-6.439**

(e) Captan, AOAC-CIPAC high pressure liquid chromatographic method, **6.A09-6.A14**

(f) Tetradifon technical, **6.B09-6.B14**

(g) Chlorpyrifos, **6.B15-6.B19**

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(2) The following methods were declared surplus:

(a) Chlordane, colorimetric method, **6.223-6.227**

(b) AG chlordane, α- and γ-isomers in technical products, **6.232-6.235**

(c) AG chlordane in granular formulations, **6.236-6.240**

(d) Heptachlor in AG chlordane, **6.241-6.244**

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(3) The official first action method for sampling of pressurized containers, **6.002,** was deleted.

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(4) The following interim official first action infrared spectrophotometric method for the determination of methazole, *J. Assoc. Off. Anal. Chem.* **64,** 1185 (1981), was adopted official first action:

Methazole(2-(3,4-Dichlorophenyl)-4-methyl-1,2,4-oxadiazolidine-3,5-dione)

Infrared Spectrophotometric Method

Official First Action

(Applicable to wettable powder contg methazole as only active ingredient)

6.C01 ***Apparatus and Reagents***

(a) *Infrared spectrophotometer.*—Capable of measuring A from 700 to 900 cm^{-1}, with matched 0.5 mm NaCl or KBr cells.

(b) *Methazole std soln.*—Weigh, to nearest mg, 0.48-0.52 g ref. std methazole (available from Velsicol Chemical Corp., 341 E Ohio St, Chicago, IL 60611) into 4 oz polyethylene screw-cap bottle, pipet in 50.0 mL acetone, and mech. shake 15 min to dissolve.

(c) *Acetone.*—Anal. reagent grade (Mallinckrodt, or equiv.).

6.C02 ***Determination***

Weigh, to nearest mg, 0.63-0.67 g sample into 4 oz polyethylene screw-cap bottle, pipet in 50.0

mL acetone, and mech. shake 1 h. Centrfg. 30 min to obtain clear supernate.

Fill both cells of spectrophtr with acetone, and place in instrument. Optimize gain; set 100% adjust to give 95–98% T at 755 cm^{-1}. Set slit in program or manual mode for optimum sensitivity and resolution. Fill sample cell with std soln, and scan region from 860 to 700 cm^{-1} (A'). Using same conditions, fill same cell with sample soln and scan twice (A). Measure A and A' at 755 cm^{-1}, using min. at 845 cm^{-1} as baseline.

6.C03 ***Calculation***

$$\% \text{ Methazole} = (W' \times A \times P \times 100)/(W \times A')$$

where W and W' = g sample and std, resp.; and P = % purity of std.

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(5) The following interim official first action gas-liquid chromatographic method for the determination of pirimicarb, *J. Assoc. Off. Anal. Chem.* **64,** 1315 (1981), was adopted official first action:

Pirimicarb (2-(Dimethylamino)benzenediazo Sodium Sulfonate)

Gas-Liquid Chromatographic Method

Official First Action

6.C04 ***Principle***

Pirimicarb is detd by gas-liq. chromatgy, using nonadecane as internal std and flame ionization detection. Peak areas are compared with that of std of known purity.

6.C05 ***Apparatus***

(**a**) *Gas chromatograph.*—With heated, glass-lined, injection port and flame ionization detector. Conditions given are for Hewlett-Packard Model 5710A. Other instruments may require changing operating parameters to obtain good resolution and response. Temps (°)—column 210, injection port 240, detector 250; gas flow rates (mL/min)—N carrier gas 40, H 60, air 240; attenuation 32 × 10; sample size 1.0 μL; retention times (min)—pirimicarb 6.8, internal std 8.9. Adjust parameters to assure complete sepn of peaks, and peak hts ca 60–80% full scale on chart at quoted retention times.

(**b**) *Column.*—1.8 m (6 ft) × 0.25 in. (od) × 2 mm (id) glass column packed with 10% silicone SE-30 on 100–120 mesh Chromosorb W(HP) (Applied Science Laboratories, Inc.). Silanize with 30 μL Silyl 8 (Pierce Chemical Co., PO Box 117, Rockford, IL 61105) and heat to 300° for 16 h before use.

6.C06 ***Reagents***

(**a**) *Nonadecane internal std soln.*—Accurately weigh ca 1 g nonadecane (Aldrich Chemical Co., Cat. No. N2890-6) and dissolve in 100 mL $CHCl_3$. Store in tightly capped bottle to avoid evapn. Check internal std soln for interfering components by injecting 1 μL into chromatograph.

(**b**) *Pirimicarb std soln.*—Accurately weigh ca 150 mg pirimicarb std of known purity (ICI Americas Inc., PO Box 208, Goldsboro, NC 27530) into vial. Pipet 10.0 mL internal std soln into vial, cap, and shake to dissolve pirimicarb. Store tightly capped to avoid evapn.

6.C07 ***Determination***

(**a**) *Powder and technical material samples.*—Accurately weigh amt sample contg ca 150 mg pirimicarb into vial. Pipet 10.0 mL internal std soln into vial, cap, and shake to dissolve pirimicarb. Keep tightly capped to avoid evapn. Allow insoluble inerts to settle before use.

(**b**) *Granular formulations.*—Grind sample in mortar and pestle or mech. mill. Accurately weigh amt sample contg ca 150 mg pirimicarb into vial. Add 5.0 mL MeOH and mix to release pirimicarb. Add 10.0 mL internal std soln, cap, and shake to dissolve pirimicarb. Store tightly stoppered to avoid evapn. Allow insoluble inerts to settle before use.

Inject 2 or more aliquots of std soln 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 = area pirimicarb peak/area internal std peak

$$\text{Pirimicarb, \%} = (R/R') \times (W'/W) \times P$$

where R and R' = av. response factor for sample and std solns, resp.; W and W' = mg sample and std, resp.; and P = purity (%) of std.

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(6) The following gas-liquid chromatographic method for the determination of pentachloronitrobenzene, *J. Assoc. Off. Anal. Chem.* **59,** 708 (1976); **65,** 110 (1982), was adopted official first action:

Pentachloronitrobenzene (PCNB) – Gas-Liquid Chromatographic Method

Official First Action

6.C08 ***Principle***

Sample is dissolved in $CHCl_3$, *o*-terphenyl is added as internal std, and PCNB is detd by GLC with flame ionization detection.

6.C09 ***Apparatus and Reagents***

(**a**) *Gas chromatograph with recorder.*—With flame ionization detector and 1.8 × 4 mm (id) glass column packed with 5% SE-30 on 80–100 mesh Chromosorb W (dimethylchlorosilane-treated) (Analabs, Inc.). Condition newly packed column 24 h at 285° with low N flow. Operating conditions: temps (°)—inlet 200, column 175–180, detector 250; carrier gas flow to elute PCNB at ca 4.5 min; adjust H and air as recommended for detector by manufacturer; sensitivity to give peak hts 60–80% full scale.

(**b**) *PCNB std soln.*—2.0 mg/mL $CHCl_3$. Accurately weigh ca 0.2 g PCNB (Olin Corp., Agriculture Products Dept., PO Box 991, Little Rock, AR 72203) into 100 mL vol. flask and dil. to vol. with $CHCl_3$.

(**c**) *Internal std soln.*—0.8 mg/mL $CHCl_3$. Accurately weigh ca 0.2 g *o*-terphenyl (Eastman Kodak Co.) into 250 mL vol. flask and dil. to vol. with $CHCl_3$. Discard after 3 days.

(**d**) *Mixed std soln.*—1.0 mg PCNB + 0.4 mg *o*-terphenyl/mL. Pipet 25 mL each of PCNB and internal std solns into vial and mix.

6.C10 ***Preparation of Sample***

(**a**) *Solid formulations.*—Grind 100 g well mixed sample to pass 1 mm sieve. Accurately weigh portion of well mixed, ground sample contg ca 0.2 g PCNB into 250 mL g-s erlenmeyer and add 100 mL $CHCl_3$; stopper and shake 2 h on rotary shaker. Let insol. matter settle.

(**b**) *Wettable powders.*—Accurately weigh portion of well mixed sample contg ca 0.2 g PCNB into 250 mL g-s erlenmeyer and proceed as for solids.

(**c**) *Liquids.*—Accurately weigh portion of well mixed sample contg ca 0.2 g PCNB into 100 mL vol. flask and dil. to vol. with $CHCl_3$.

(**d**) *Soln for analysis.*—Pipet 10 mL sample ext above and 10 mL internal std soln into vial, cap, and mix.

6.C11 ***Determination and Calculation***

Inject 4 μL aliquots of mixed std soln until variation in response ratio (area or peak ht) for PCNB (first peak) to *o*-terphenyl (second peak) is ca 1%. Inject mixed std, inject sample twice, and repeat injection of mixed std. Retention times must be the same for sample and std. Calc. av. ratios of PCNB to *o*-terphenyl for the 2 mixed std and sample injections, and calc. % PCNB.

$$\% \text{ PCNB} = (R/R') \times (W'/W) \times P$$

where R and R' = av. response ratios for sample and mixed std, resp.; W' = g PCNB/100 mL std soln; W = g sample extd; and P = purity (%) of PCNB std.

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(7) The following gas-liquid chromatographic method for the determination of diazinon in microencapsulated formulations, *J. Assoc. Off. Anal. Chem.* **65,** 115 (1982), was adopted official first action:

Diazinon (*O,O*-Diethyl *O*-(2-Isopropyl-6-methyl-4-pyrimidinyl) Phosphorothioate) in Microencapsulated Formulations

Gas-Liquid Chromatographic Method

Official First Action

6.C12 ***Principle***

Sample is ground in tissue grinder and extd with CH_3CN, dibutyl phthalate is added as internal std, and diazinon is detd by GLC with flame ionization detection.

6.C13 ***Apparatus***

(**a**) *Gas-liquid chromatograph.*—Equipped with flame ionization detector (Perkin-Elmer 900, or equiv.).

(**b**) *GLC column.*—6 ft × 1/4 in. od, 2 mm id, glass, packed with 3% OV-17 on 80–100 mesh Supelcoport. Operating conditions: injection port 200°; column 190° (isothermal); detector 250°; He flow 35 mL/min; H flow optimum for instrument detector; chart speed 0.2 in./min; sample size: 1 μL.

(**c**) *Tissue grinder.*—40 mL capacity (Corning Glass Works, No. 441969, Corning, NY 14830), or equiv.

6.C14 ***Reagents***

(**a**) *Internal std soln.*—Accurately weigh ca 2.0 g dibutyl phthalate into 100 mL vol. flask. Dissolve in and dil. to vol. with CH_3CN.

(**b**) *Diazinon std soln.*—Accurately weigh ca 0.2 g diazinon (W') into 50 mL vol. flask. Pipet in

10.0 mL internal std soln, dil. to vol. with CH_3CN, and mix well.

6.C15 ***Determination***

Mix sample thoroly. With medicine dropper or disposable pipet, transfer ca 2 g sample to Al weighing dish and weigh accurately (*W*). Transfer to tissue grinder, add 30 mL CH_3CN, and grind 3 min. When sample is thoroly ground, quant. transfer to 100 mL vol. flask, wash grinder with CH_3CN, and add washings to vol. flask. Pipet in 20.0 mL internal std soln and dil. to vol. with CH_3CN. Using 10 μL syringe, make duplicate 1 μL injections of sample and std solns.

Measure peak hts of first peak, diazinon, in sample (*PH*) and std soln (*PH'*) and second peak, dibutyl phthalate, in sample (*IS*) and std soln (*IS'*).

$$\text{Diazinon, wt\%} = (PH/PH') \times (IS'/IS) \times (W'/W) \times P$$

where *W* and *W'* = g sample and diazinon std resp.; *P* = % purity of diazinon std.

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(8) The following CIPAC-AOAC liquid chromatographic method for the determination of temephos was adopted official first action:

Temephos (*O,O'*-(Thiodi-4,1-phenylene)-bis(*O,O*-dimethyl Phosphorothioate)

CIPAC-AOAC Liquid Chromatographic Method Official First Action

(Method is suitable for tech. temephos and formulations with temephos as only active ingredient.)

6.C16 ***Principle***

Sample is dissolved in ethyl acetate, *p*-nitrophenyl *p*-nitrobenzoate is added as internal std, and, after diln with *n*-hexane, sample is injected into liq. chromatgc column. LC response ratio of insecticide to internal std is compared with response ratio of std to give content in sample.

6.C17 ***Apparatus and Reagents***

(**a**) *Liquid chromatograph.*—Able to generate >2000 psi and measure *A* at 254 nm.

(**b**) *Chromatographic column.*—Stainless steel, 300 × 3.9 mm id packed with 10 μm silica gel (μ-Porasil, Waters Associates, Inc., is suitable).

(**c**) *Ethyl acetate.*—Burdick & Jackson Laboratories, Inc. Dry over molecular sieve, 5Å, 8–12 mesh beads (Davison Specialty Chemical Co., PO Box 2117, Baltimore, MD 21203). Filter thru 0.45 μm Millipore filter (Millipore Corp., Bedford, MA 01730).

(**d**) *n-Hexane.*—Non-spectro, distd in glass (Burdick & Jackson Laboratories, Inc.). Dry over molecular sieve, 5Å, 8–12 mesh, and filter thru 0.45 μm Millipore filter.

(**e**) *p-Nitrophenyl p-nitrobenzoate internal std.*—1.5 g/250 mL ethyl acetate. React *p*-nitrophenyl *p*-nitrobenzoate (Aldrich Chemical Co.) with excess *p*-nitrophenyl Na salt (Eastman Kodak Co.) in CH_3CN. Alternatively, prep. 1.1% (w/v) dimethyl 4-nitrophthalate in ethyl acetate.

(**f**) *Reference std soln.*—Accurately weigh ca 50, 60, and 70 mg temephos, anal. reagent (American Cyanamid Co.) into sep. 50 mL vol. flasks. Add by pipet 5 mL internal std soln and 25 mL dry ethyl acetate to each flask. Shake flasks to ensure dissolution of std, and dil. to vol. with *n*-hexane. Designate solns as A, B, and C. Use soln B as working std soln for liq. chromatgy; use solns A and C to check linearity of liq. chromatograph (see *Linearity Check*) and to guard against weighing error in prepn of std soln. Supply of soln B can be replenished from time to time without prepg new supplies of solns A and C, provided linearity requirement described under *Linearity Check* can be met.

(**g**) *LC mobile phase.*—Add 100 mL dry ethyl acetate to 1 L vol. flask and dil. to vol. with dry *n*-hexane.

(**h**) *LC operating conditions.*—Column temp. ambient; flow rate 1.0 mL/min (ca 450 psi); retention times: internal std ca 9.6 min, temephos ca 11.5 min. Pump 50 mL anhyd. MeOH thru column followed by 100 mL dry ethyl acetate. Pump LC mobile phase thru column until system is equilibrated (flat baseline). Inject 5 μL aliquots of std soln B until const. response is obtained. If necessary, adjust instrument or injection vol. (usually 3–6 μL) to give 50–60% FSD for internal std peak. Use same injection vol. and instrument settings for all samples and stds.

6.C18 ***Linearity Check***

Inject triplicate aliquots of appropriate vol. (as detd above) of std solns A, B, and C into liq. chromatograph, det. response ratio for each injection, and av. resulting ratios for each soln. Divide av. response ratio for each soln by corresponding content (in mg) and compare resulting response factors. These factors should agree within 2%.

Liq. chromatograph should be checked for linearity at least once a week, and same check should be carried out whenever new std solns are prepd and whenever column, new or used, is installed in instrument.

6.C19 *Sample Preparation*

(a) *Technical and emulsifiable concentrates.*—Accurately weigh amt sample contg ca 60 mg temephos directly into tared 50 mL vol. flask. For temephos tech., warm and thoroly mix before sampling. Add by pipet exactly 5 mL internal std soln and 25 mL dry ethyl acetate. Shake flask to ensure dissolution and dil. to vol. with *n*-hexane.

(b) *Water-dispersible powders and sand granules.*—Accurately weigh amt sample contg ca 60 mg temephos directly into 2 oz bottles fitted with plastic screw cap. Add by pipet 5 mL internal std soln and 25 mL dry ethyl acetate and shake 1 min. Add 20 mL *n*-hexane, mix thoroly, and let particles settle. Filter portion of soln and hold for LC analysis. (In some cases, centrifugation may be sufficient to remove particles before LC analysis.)

6.C20 *Analysis of Sample Solutions*

Inject duplicate aliquots of std soln B. Calc. response ratios by dividing area (or ht) of temephos peak by that of internal std peak. Response ratios should agree within 2%. Average duplicate response ratios obtained with std solns.

Inject duplicate aliquots of each sample soln. Average duplicate response ratios for each sample soln. Note: After first injection of any sample, let instrument run ≥30 min after emergence of temephos peak to det. late-eluting peaks due to impurities. Subsequent injections should be timed so that late-eluting peaks from sample injections do not interfere with internal std or temephos peaks of subsequent samples.

Inject duplicate aliquots of std soln B. Average response ratios of stds immediately before and after sample solns, which should agree within 2%. Use this av. to calc. temephos content of sample solns.

6.C21 *Calculations*

For each injection, response ratio (R) = (area temephos peak/area internal std peak).

$$\text{Temephos, wt\%} = (R \times W' \times P)/(R' \times W)$$

where R' and R = av. response ratio for std soln B and sample soln, resp.; W' and W = wt (mg) of temephos std taken (for std soln B) and sample, resp.; and P = purity of temephos std (%).

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(9) The following gas-liquid chromatographic method for the determination of piperonyl butoxide and pyrethrins, *J. Assoc. Off. Anal. Chem.* **65**, 249 (1982), was adopted official first action:

Pyrethrins and Piperonyl Butoxide Gas-Liquid Chromatographic Method Official First Action

6.C22 *Principle*

Sample is dild with acetone contg dicyclohexyl phthalate internal std and detd by GLC with flame ionization detection. Method is applicable to tech. piperonyl butoxide [80% butylcarbityl 6-propylpiperonyl ether and 20% related compds] and most formulations contg pyrethrins and piperonyl butoxide except shampoo products. Occasionally, an oil diluent will interfere with GLC detn. Method may not be applicable to samples contg <0.1% pyrethrins. Variation in active constituents of pyrethrin ext may cause minor deviations from expected results.

6.C23 *Apparatus and Reagents*

(a) *Gas chromatograph.*—Equipped with flame ionization detector and 122 cm × 4 mm id glass column packed with 5% OV-101 or 5% OV-1 (Analabs, Inc.) on 80–100 mesh Chromosorb W(HP). Operating conditions: column 210°, injection port 250°, detector 250°; gas flows (mL/min)—N carrier gas flow 50, air 350–400, and H 40–50; sensitivity 10^{-10} AUFS. Adjust attenuation to maintain 50–75% FSD for 1.0–1.5 μg piperonyl butoxide. Before use, condition column 2–3 h at 275° with N flow 50 mL/min. If necessary, vary column temp. or gas flow to attain retention times of ca 13–15 min for internal std. Theoretical plates/ft must be >400, based on dicyclohexyl phthalate peak.

Calc. theoretical plates/ft (N) as follows: $N = 16 \times (L^2/M^2 \times F)$, where L = retention of GLC peak (mm); M = peak baseline (mm) produced by drawing tangents to points of inflection of peak; and F = length of column (ft).

(b) *Internal std soln.*—8.0 mg Dicyclohexyl phthalate (Chem Serv, Inc., West Chester, PA 19380)/mL acetone.

(c) *Std soln.*—(*1*) Std soln A.—0.5 mg piperonyl butoxide/mL. Accurately weigh ca 0.25 g piperonyl butoxide (available from McLaughlin Gormley King Co., 8810 Tenth Ave N, Minneapolis, MN 55427) into 50 mL vol. flask and dil. to vol. with acetone. Pipet 10 mL this soln into 100 mL vol. flask, add 5 mL internal std soln by

pipet, and dil. to vol. with acetone. Use this soln for detn of tech. piperonyl butoxide. (2) Std soln B.—Accurately weigh ca 0.25 g piperonyl butoxide into 50 mL vol. flask. Add weighed amt of pyrethrins such that ratio of active ingredients closely resembles that which is expected in sample. Dil. to vol. with acetone. Pipet 10 mL of this soln into 100 mL vol. flask, add 5 mL internal std soln by pipet, and dil. to vol. with acetone. Use this soln for detn of pyrethrins and piperonyl butoxide in formulations.

6.C24 *Preparation of Sample*

(a) *Technical piperonyl butoxide.*—Accurately weigh ca 0.25 g sample into 50 mL vol. flask and dil. to vol. with acetone. Pipet 10 mL this soln into 100 mL vol. flask, add 5 mL internal std soln by pipet, and dil. to vol. with acetone.

(b) *Pyrethrins piperonyl butoxide formulations.*—(1) *Liqs.*—Accurately weigh sample contg ca 0.05 g piperonyl butoxide into 100 mL vol. flask, add 5 mL internal std soln by pipet, and dil. to vol. with acetone. (2) *Aerosol formulations.*—Caution: Open aerosol behind safety shield and in hood. Weigh aerosol can to nearest 0.1 g (*G*). Puncture as *small a hole as possible* in top of can with sharp punch and hammer to allow propellant to release very slowly. (Best results can be obtained by allowing punctured can to stand overnight.) After hiss of escaping propellant is no longer evident, cut open top of can with hand can opener. Leave ca 1 cm attached to can and bend top open. Carefully warm can in beaker of warm tap H_2O several minutes to ensure complete removal of propellant. Transfer aerosol nonvolatiles to vol. flask with aid of acetone. Rinse can thoroly, adding rinses to vol. flask. If aerosol is 8 oz, use 2 L (*V*) vol. flask. This vol. is necessary to ensure complete miscibility of oil phase of aerosol contents. Dil. to vol. with acetone and mix thoroly. Dry empty can and weigh (*T*). Transfer aliquot (*A*) (must be ≤90 mL), equiv. to 50 mg piperonyl butoxide, to 100 mL vol. flask, add 5 mL internal std soln by pipet, and dil. to vol. with acetone.

$$\text{Wt sample} = (G - T) \times (A/V)$$

6.C25 *Gas Chromatography*

Inject 2–3 μL aliquots of std soln until internal std ratios vary ≤2% for successive injections. Det. baseline by drawing straight line to min. on either side of peak of interest. For pyrethrins, use combined ht of cinerin I and pyrethrin I peaks for internal std ratio. Repeat injection procedure with sample soln, followed by injection of std soln. If std peak ratios differ ≥ ±2.0%, repeat series of injections. Injection vol. should not vary > ±10%. Calc. peak ht ratios (sample peak ht/internal std peak ht) of std injections before and after sample injections and average std ratio preceding and following sample injections. Calc. av. peak ht ratios for sample injections. After elution of piperonyl butoxide, allow ca 7 min for elution of extraneous peaks.

$$\% \text{ Piperonyl butoxide or pyrethrins} = (W_s \times P \times R_x)/(W_x \times R_s)$$

where W_s = g std in final diln; W_x = g sample in final diln; P = % purity of std; R_s = ratio of std; and R_x = ratio of sample.

7. ANIMAL FEED

(1) The official final action method for crude fat or ether extract, **7.055–7.060,** was editorially revised to delete the word "entirely" in its two occurrences in the applicability statement. The modified applicability statement reads as follows:

"Use method **7.056** or **7.057** for mixed feeds other than (*1*) baked and/or expanded, (*2*) dried milk products, or (*3*) contg urea."

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(2) The official final action AOCS–AOAC method for crude fiber, **7.061–7.065,** was revised official first action to substitute the use of ceramic fiber for asbestos fiber, *J. Assoc. Off. Anal. Chem.* **65,** 265 (1982). The use of asbestos fiber in method **7.061–7.065** was repealed official first action. The following changes are required:

Revise **7.062**(c) to read:

(c) *Prepared ceramic fiber.*—Place 60 g ceramic fiber (Cat. No. 1740M, Lab Safety Supply Co., PO Box 1368, Janesville, WI 53545) in blender, add 800 mL H_2O, and blend 1 min at low speed.

Det. blank by treating ca 2 g (dry wt) of prepd ceramic fiber with acid and alkali as in detn. Correct crude fiber results for any blank, which should be negligible (ca 2 mg).

Revise **7.065** as follows:

In line 3, replace "Add ca 1 g prepd asbestos . . ." with "Add ca 1.5–2.0 g dry wt of prepd ceramic fiber . . ."

In **7.065**(a), (b), and (c) change "asbestos" to read "ceramic fiber" at each occurrence.

8. BAKING POWDERS AND BAKING CHEMICALS

No additions, deletions, or changes.

9. BEVERAGES: DISTILLED LIQUORS

(1) The following spectrophotometric method for the determination of color intensity of whisky, *J. Assoc. Off. Anal. Chem.* **64,** 541 (1981); **65,** 224 (1982), was adopted official first action:

Color

Official First Action

9.C01 ***Definition***

Whisky color units are defined as $1000 \times A$ at 525 nm of turbidity-free sample measured by spectrophtr or colorimeter with bandwidth of ≤10 mm, using 1 cm cell and H_2O as ref.

9.C02 ***Calibration of Spectrophotometer and Procedure***

Check acccuracy of wavelength scale of spectrophtr or colorimeter with didymium or Ho_2O_3 glass filter or Hg lamp. Use manufacturer's operating procedure to calibrate and operate instrument. Place turbidity-free sample in 1 cm cell and det. A at 525 nm against H_2O as ref. Color intensity units = $1000 \times A$.

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(2) The following method for the determination of alcohol content of beverages by oscillating U-tube density meter, *J. Assoc. Off. Anal. Chem.* **62,** 653 (1979); **64,** 550 (1981); **65,** 218 (1982), was adopted official first action:

Alcohol by Density Meter

Official First Action

9.C03 ***Principle***

Meter dets sp. gr. at 20° by measuring change in frequency of oscillating U-tube filled with sample compared with frequencies of oscillation when filled with 2 stds. Sp. gr. is converted to % alcohol at 15.56°.

9.C04 ***Apparatus and Reagents***

(**a**) *Density meter.*—Mettler/Paar DMA 55D, with adapter No. 5771 which permits continuous flow of sample thru U-tube (Mettler Instrument Corp., Hightstown, NJ 08520).

(**b**) *Water bath.*—Const temp., controlled at 20.00 ± 0.01°.

(**c**) *Syringe.*—10 mL, with Luer fitting, No. 15 needle.

(**d**) *Water.*—Double-distd or treated by ion exchange resin and filtered, freshly boiled (ion exchange cartridge, research model, sold by Illinois Water Treatment Co., 840 Cedar St, Rockford, IL 61105; Gelman Capsule Filter, Cat. No. 12106, 0.2 μm, Gelman Sciences, Inc., 600 S Wagner Rd, Ann Arbor, MI 48105).

(**e**) *Barometer.*

(**f**) *Thermometer.*—18.9 to 25.1°, 0.01° scale divisions (No. 116-C, H & B Instrument Co., American and Bristol St, Philadelphia, PA 19140).

9.C05 ***Standardization of Density Meter***

Rinse U-tube with stream of acetone, and completely dry with air stream. Set switches on density meter in the following positions: power-on, display-T, sampling rate-either 2 or 3. With clean, dry U-tube at 20 ± 0.01°, note and record T for air.

Turn on light and open shutter to view U-tube. Fill U-tube with freshly boiled double-distd (or ion exchange-treated) H_2O by dipping plastic tube connected to inlet (lower) end of U-tube into H_2O std and slowly pulling plunger on syringe, equipped with No. 15 needle, connected by plastic tubing to outlet (upper) of U-tube. View U-tube to ensure that it is full of H_2O and contains no bubbles. Leave end of filler tube submerged in H_2O std and syringe connected while taking reading. Turn off viewing light and close shutter. T value of H_2O on digital display will continue to change until sample temp. reaches equilibrium with const temp. bath (ca 2–3 min). Record T for H_2O.

Calc. app. consts A and B as follows:

$$A = [T^2(H_2O) - T^2(air)]/[\text{sp. gr.}(H_2O) - \text{sp. gr.}(air)]$$

$$B = T^2(H_2O) - [A \times \text{sp. gr.}(H_2O)]$$

Sp. gr. of air = 0.00119 at 20° and 746–752 torr, and 0.00120 at 20° and 753–758 torr. Sp. gr. of H_2O = 1.00000 regardless of barometric pressure.

Enter calcd values of consts A and B into app. memory by rotating appropriate dials. Reset display switch to ρ (sp. gr.), and check reading for sp. gr. of H_2O. Then drain U-tube, dry, and check sp.gr. of air. Numerical displays should be 1.00000 for H_2O, and 0.00119 or 0.00120 for air, depending on barometric pressure. If display values differ >1 in fifth decimal place from correct sp. gr. values, recheck temp. of H_2O bath and T for air and H_2O.

9.C06 ***Specific Gravity Measurement of Samples***

Turn on light and open shutter to view U-tube. Slowly fill U-tube with sample by same method

used to fill with H_2O std, being careful not to introduce bubbles. Turn off viewing light and close shutter. Sp. gr. of sample on digital display will continue to change until sample temp. reaches equilibrium with const. temp. bath (ca 2–3 min). Record sp. gr. of sample, and draw another sample into U-tube. Replicate readings should vary $\leq \pm 0.00001$ sp. gr. unit. Refer to Table **52.003** to convert sp. gr. at 20° to percent alcohol at 15.56°.

9.C07 ***Changing Samples***

Turn on light and open shutter to view U-tube. Lift inlet end of plastic tubing from below surface of old sample and slowly pull syringe plunger to empty tubing and U-tube. Disconnect syringe from plastic tubing and discard syringe contents. Reconnect syringe, submerge tip of tubing below surface of new sample, and slowly draw 8–10 mL sample thru U-tube into syringe to eliminate air bubbles and to rinse system with new sample. Turn off viewing light, close shutter, and take first reading of new sample after temp. equilibrium.

After each 10 samples, or when erratic digital displays are noted, rinse U-tube with acetone and dry with air stream. Let empty U-tube stabilize at $20 \pm 0.01°$.

10. BEVERAGES: MALT BEVERAGES AND BREWING MATERIALS

(1) The official first action ASBC–AOAC method for the determination of moisture (loss on drying) in malting barley, **10.B01,** was adopted official final action.

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(2) The following method for the determination of *N*-nitrosodimethylamine in beer after isolation by Celite adsorption (screening method) or by distillation (confirmation method), *Am. Soc. Brew. Chem. J.* **38,** 111 (1980); **39,** 35, 99 (1981), was adopted official first action as an ASBC–AOAC method:

N-Nitrosodimethylamine (NDMA) in Beer
Gas-Liquid Chromatographic Method
Official First Action
Method I
ASBC-AOAC Method

10.C01 ***Principle***

NDMA is isolated by either adsorption on Celite or distn and is detd by GLC with either electrolytic conductivity or thermal energy analyzer with *N*-nitrosodipropylamine (NDPA) internal std.

10.C02 ***Safety Precautions***

Nitrosamines are considered potent carcinogens. Exercise extreme care in handling nitrosamines or solns of nitrosamines. Avoid skin contact. Use mech. pipetting aids for all pipetting procedures. All samples contg nitrosamines should be properly labeled as "spiked with nitrosamines" or "not for consumption," or with other adequate warning.

10.C03 ***General Precautions***

Thoroly clean all glassware used for nitrosamine analyses with Chromerge, or equiv., and thoroly rinse with H_2O and CH_2Cl_2.

Some nitrosamines degrade when exposed to UV light. Avoid prolonged exposure to fluorescent lights unless lights are covered with yellow translucent shields to filter out UV light. Alternatively, cover sample containers with foil or other suitable material to provide protection from light.

Store stds and CH_2Cl_2 exts in freezer in amber bottles or foil-covered containers

10.C04 ***Reagents***

(**a**) *Celite 545.*—Not acid-washed (Fisher Scientific Co. No. C-212). Fire contents of each bottle 16 h at 700° before use.

(**b**) *Dichloromethane.*—CH_2Cl_2, distd in glass. (Burdick & Jackson Laboratories, Inc., or equiv.).

(**c**) *Sodium sulfate.*—Anhyd., granular.

(**d**) *Ethanol.*—Anhyd. (National Distillers and Chemical Corp., New York, NY 10016, or equiv.).

(**e**) *NDMA std soln.*—100 μg NDMA/mL alcohol (Thermo Electron Corp., 115 Second Ave, Waltham, MA 02154).

(**f**) *Internal std soln.*—100 ng NDPA/mL alcohol. Dil. 100 μg/mL soln (Thermo Electron Corp.) and dil. aliquot with EtOH to 100 ng/mL.

(**g**) *Boiling chips.*—Carborundum, small size, or equiv.; Boileezers (Fisher Scientific Co. No. B-365), or equiv.

(**h**) *Dry nitrogen.*—Ultra-high purity.

(**i**) *Aqueous ethanol.*—4% v/v, prepd with glass-distd H_2O.

(**j**) *Water.*—Distd in glass. H_2O processed thru deionizer may contain nitrosamines.

10.C05 ***Apparatus***

(a) *Distilling flasks.*—Round-bottom, 1 L with connecting adapter and Graham condenser set vertically.

(b) *Heating mantles.*—For 1 L flasks, with variable transformers.

(c) *Funnels.*—Fritted glass, 60 mL.

(d) *Evaporative concentrator.*—Kuderna-Danish, 250 mL capacity, 24/40 ⧩ column connection, 19/22 lower ⧩ joint. Concentrator tube size 425, 19/22 ⧩ joint, 4 mL capacity, graduated, with 19/22 ⧩ stopper. Snyder distn column, 3 sections, size 121 with 24/40 ⧩ joint (available from Kontes Glass Co., SGA Scientific, and others).

(e) *Tamping rod.*—19 mm diam. disk.

(f) *Glass wool.*—Pyrex, or equiv.

(g) *Chromatographic column.*—Glass, 28 mm id × 400 mm long with stopcock.

(h) *Gas-liquid chromatographs.*—Interfaced with thermal energy analyzer (TEA), or with Hall electrolytic conductivity detector and nitrosamine kit. The following examples of columns and conditions are suitable for nitrosamine sepn. Variations in columns and conditions are acceptable; NDMA response for 0.5 ppb beer std should be ≥5% FSD when recorder is used.

(*i*) Gas chromatograph interfaced with TEA analyzer (Model 502, Thermo Electron Corp.): 6 ft × 6 mm id glass column packed with 10% Carbowax 20M + 5% KOH on 100–120 mesh Anakrom AB; column 145°; injection port 200°; He carrier gas 35 mL/min. TEA conditions: furnace 475°; vac. with O, 1.0 torr; trap −120 to −130°.

(*ii*) Tracor Model 560/700A gas chromatograph equipped with electrolytic conductivity detector and nitrosamine detector kit: 6 ft × 6 mm id glass column packed with 15% LAC-2R-446 on 80–100 mesh Chromosorb W, acid-washed; column 140°; He carrier gas 20 mL/min; injection port 200°; Hall inlet 250°; Hall reactor 700°; H flow 50 mL/min; electrolyte, 50% v/v *n*-propanol; electrolyte flow 0.5 mL/min.

10.C06 ***Calibration Samples***

Prep. beer contg 0, 0.5, 1.0, 2.5, and 5.0 ppb (μg/L) added NDMA as follows:

Decarbonate two 12 oz bottles of beer, contg negligible NDMA content, according to **10.001**, without paper filtration. This is base beer. Prep. NDMA dil. stds from 100 μg/mL std (**e**), using EtOH for diln as follows:

Diln A = dil. 1.0 mL of 100 μg/mL to 10 mL
= 10 μg/mL

Diln B = dil. 5.0 mL of A to 100 mL
= 500 ng/mL

Diln C = dil. 5.0 mL of B to 10 mL
= 250 ng/mL

Diln D = dil. 2.0 mL of B to 10 mL
= 100 ng/mL

Diln E = dil. 1.0 mL of B to 10 mL
= 50 ng/mL

Add 1 mL of the following to sep. 100 mL vol. flasks: EtOH, diln E, diln D, diln C, and diln B; dil. each to vol. with previously decarbonated base beer. These samples contain 0, 0.5, 1.0, 2.5, and 5.0 ppb (μg/L) of added NDMA, resp.

10.C07 ***Celite Separation***

Carry each calibration sample thru entire procedure.

Weigh 25 ± 0.1 g decarbonated beer (**10.001**, without paper filtration) into tared 600 mL beaker. Add 1.0 mL internal std soln and 25 g Celite. Stir mixt. until uniform (ca 30 s). Mixt. will not pour but will appear light and fluffy. Place small glass wool plug in bottom of chromatgc column and cover with 20 g Na_2SO_4. Place tamping rod and powder funnel in column with end of tamping rod extending into column thru funnel. Transfer Celite mixt. to column thru funnel and tamp, a little at a time, to depth of 8–10 cm. Place K-D evaporator with 4 mL concentrator tube under column. Add 75 mL CH_2Cl_2 to beaker, swirl with spatula, and pour thru funnel before removing tamping rod. Adjust stopcock so CH_2Cl_2 flows at 1–2 mL/min into evaporator. Let column run dry (ca 35 mL CH_2Cl_2 will be recovered). Add 3 small boiling chips, fit K-D app. with distg column, and conc. to ca 4 mL in 60° H_2O bath. Let Snyder column drain, remove, and further conc. solv. to 1.0 mL under gentle stream of N at room temp. (this final concn should take ca 30 min). Inject aliquot into gas chromatograph, using either GLC/TEA or GLC/HECD conditions.

Prep. reagent blank by substituting 4% v/v EtOH in H_2O for beer, and carry thru analysis. If reagent blank shows peak for NDMA, check CH_2Cl_2 by concg 95 mL to 1 mL and chromatographing. If CH_2Cl_2 does not show NDMA peak, check other reagents. Do not use reagents showing background nitrosamines.

10.C08 ***Distillation Separation***

Carry each calibration sample thru entire procedure.

Decarbonate ca 55 mL beer by **10.001,** without paper filtration. Transfer 50.0 mL beer to 1 L r-b distn flask contg 8 g $Ba(OH)_2$ and Boileezers. Add 1.0 mL internal std soln. Distill slowly (variable transformer setting 60%), collecting ca 48 mL in ice-cooled 250 mL separator. Add 0.4 g Na_2CO_3. Ext 4 times with 20 mL portions of CH_2Cl_2, shaking each for 1 min. Pool exts in second 250 mL separator. Pass ext thru 30 g Na_2SO_4 (held in 60 mL fritted glass funnel prewetted with CH_2Cl_2) into 250 mL K-D evaporator with 4 mL concentrator tube attached. Wash Na_2SO_4 with 15 mL CH_2Cl_2 and add wash to evaporator flask. Add one Boileezer and Snyder column and carefully conc. to 4 mL in 60° H_2O bath. Remove Snyder column and further conc. to 1.0 mL under gentle stream of N at room temp. (this final concn should take ca 30 min). Inject aliquot into gas chromatograph, using either GLC/TEA or GLC/HECD conditions.

Prep. reagent blank as in **10.C07.**

10.C09 ***Calculations***

Measure peak ht (or area) of NDMA and NDPA (internal std) peaks on chromatograms and det. ratio:

$$R = \frac{\text{peak ht (or area) NDMA}}{\text{peak ht (or area) NDPA}}$$

Subtract R for 0 ppb calibration sample from R values obtained for other calibration samples. Prep. std curve by plotting ppb added NDMA vs R values for each NDMA calibration level (after subtraction of R for 0 ppb).

Calc. slope and intercept of regression line, using method of least squares where X = NDMA (μg/L) and $Y = R$ value.

Calcn of unknowns: Measure peak ht (or area) for NDMA and NDPA peaks and calc. R as above. Det. μg/L in beer by calcn using regression equation and solving for X as follows:

$$\mu g/L = \frac{R - \text{intercept}}{\text{slope}}$$

Report results to one decimal place.

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(3) The following gas-liquid chromatographic-thermal energy analyzer method for the determination of *N*-nitrosodimethylamine (NDMA) in beer was adopted official first action:

N-Nitrosodimethylamine (NDMA) in Beer
Gas-Liquid Chromatographic Method
Official First Action
Method II

10.C10 ***Principle***

Sample is treated with sulfamic acid and HCl, and *N*-nitrosodipropylamine (NDPA) is added as internal std. Soln is made alkaline, and NDMA is sepd by distn and detd by GLC with thermal energy analyzer (TEA) detector.

Caution: *N*-Nitrosamines are potent carcinogens; take adequate precaution to avoid exposure. Carry out all steps, wherever possible, in well ventilated fume hood and wear protective gloves while handling nitrosamine stds. Use mech. pipetting aids for measuring all solns. Use sep. pipetting device for measuring stds and mark it appropriately; do not use it for pipetting other reagents. Because these compds are highly photolabile, all work should be carried out under subdued light. Destroy all nitrosamine stds by boiling with HCl, KI, and sulfamic acid before disposal.

10.C11 ***Reagents***

(**a**) *Dichloromethane.*—Distd in glass. Test each bottle before use: Conc. 200 mL to 1 mL as described under *Concentration* and then analyze 10 μL aliquot by GLC-TEA. Test must show absence of NDMA.

(**b**) *Sodium sulfate.*—Anhyd. (granular). Test each bottle as follows: Dissolve 40 g in 50 mL H_2O, add 2 mL 10N KOH, ext with two 50 mL portions of CH_2Cl_2, dry ext over anhyd. Na_2SO_4 as described later, conc. to 1 mL, and analyze 10 μL aliquot by GLC-TEA. Ext must be free of NDMA.

(**c**) *Distilled or deionized water.*—Test as follows: Take 50 mL H_2O, add 2 mL 10N KOH, ext with two 50 mL portions of CH_2Cl_2, and test for NDMA contamination as under (**b**). If test under (**b**) is neg. (which includes 50 mL H_2O), there is no need to test H_2O sep.

(**d**) *Boiling aids.*—Boileezers® (Fisher Scientific Co., Catalog No. B-365), or equiv.

(**e**) *Sulfamic acid.*—10% in H_2O; store at 4°.

(**f**) *KOH.*—10N and 3N; store in polyethylene bottles.

(**g**) *NDMA.*—(*1*) *Stock soln, 10 mg/mL.*—Accurately weigh (±0.0001 g) ca 100 mg in 10 mL vol. flask (with polyethylene stopper), dil. to mark with CH_2Cl_2, and mix well. Store at −20°, and warm to room temp. in the dark before use.

Prep. fresh stock soln once a year. (*2*) *Dilute solns.*—By serial dilns (using ≥1 mL pipets) (*1*, above), prep. the following NDMA solns in CH_2Cl_2: 500, 200, 100, 40, 20, 10, and 5 ng/mL. Store at −20° and warm to room temp. in dark before use. Prep. fresh dil. stds once a month.

(**h**) *N-Nitrosodi-n-propylamine (NDPA) stds.*—As described above, weigh and prep. soln contg 250 ng NDPA/mL anhyd. EtOH. Note: Dild stds available from com. firms are acceptable. Always use appropriate solvs (CH_2Cl_2 for NDMA and anhyd. EtOH for NDPA) for dilns.

10.C12 *Apparatus*

(**a**) *Graham condenser.*—No substitutes, with $^{24}/_{40}$ joints, jacket length 200 mm (Kontes No. K-439000)

(**b**) *Kuderna-Danish (K–D) evaporative concentrator.*—250 mL capacity, with $^{24}/_{40}$ column connection and $^{19}/_{22}$ lower joint, complete with springs (Kontes No. K-570000).

(**c**) *K–D concentrator tube.*—4 mL capacity, with $^{19}/_{22}$ joint, and 0.1 mL subdivisions from 0 to 2.0 mL (Kontes No. K-570050). Check accuracy of graduations. Use with pennyhead stoppers ($^{19}/_{22}$ joint).

(**d**) *Snyder column.*—3 section, 150 mm, with $^{24}/_{40}$ joints (Kontes No. K-503000).

(**e**) *Micro Snyder column.*—3 chambers, with $^{19}/_{22}$ joint (Kontes No. 569001:3-19).

(**f**) *GLC columns.*—6 ft × $^1/_8$ in. (od) stainless steel column packed with 20% Carbowax 20M and 2% NaOH on 80–100 mesh acid-washed Chromosorb P. Column must be able to handle 10 μL sample ext and must give good resolution of NDMA peak from both solv. (in beer ext) and NDPA peaks. Injector and column temps, 220° and 170°, resp. Carrier gas (Ar) flow, 25–30 mL/min.

(**g**) *GLC–thermal energy analyzer.*—Thermo Electron Corp., Waltham, MA, connected to 1 mV recorder. Operate according to instrument manual and with −110° to −130° slush bath. Adjust instrumental parameters, such as vac. chamber pressure, O flow, calibration knob, etc., to obtain proper sensitivity. Set recorder chart speed at ca 0.5 cm/min.

Note: Thoroly clean all glassware before use. After normal cleaning and washing, wash with chromic acid. If contamination still exists, rinse all glassware with CH_2Cl_2 before use. Let charred residue in distn flask soak with dil. alkali and then wash in normal manner.

10.C13 *Sampling and Storage*

Store beer sample at 4° in dark and analyze as soon as possible. When opening bottle or can, transfer ca 120 mL aliquot into g-s erlenmeyer and store as above. Alternatively, recap bottle, using bottle capper, after taking aliquot. In the latter case, test 4% alc. ext of new cap liner for NDMA contamination before use.

10.C14 *Distillation*

Accurately weigh 50 ± 0.1 g beer into 1 L distn flask and add 1.0 mL each of 10% sulfamic acid, NDPA internal std (250 ng/mL), and 1N HCl. Mix contents by gentle swirling and let stand in dark 10 min. Then add 10.0 mL 3N KOH and 2 small Boileezers, and mix. Set up distn app. so that connecting adapter slopes downward toward vertical Graham condenser. Loosely wrap glass wool around distn flask and connecting adapter. Set up 100 mL graduate under condenser to collect distillate. Cooling H_2O for condenser should be ≤20°.

During initial 10 min of distn, adjust rheostat (usually at 50) so that mixt. boils smoothly without too much frothing or bumping. Watch constantly for excessive foaming and, if necessary, turn off heat for 1–2 min. After 10 min, increase rheostat setting to 60 or 65 and continue distn (watch for foaming) until most of soln is distd. Stop distn when ca 55 mL distillate is collected. *Do not heat distg flask to complete dryness;* this may give erroneous results. Total distn time should be ≤1 h. If any portion of sample foams over during distn, discard experiment and start over with fresh aliquot.

10.C15 *Extraction and Cleanup*

Disconnect adapter after distn; do not rinse adapter. Add 2.0 mL 10N KOH to distillate in graduate and transfer to 250 mL separator. Use same cylinder for all subsequent measuring of CH_2Cl_2. Rinse condenser with 50 mL CH_2Cl_2, and collect rinsing directly into separator contg distillate and KOH. Ext distillate with CH_2Cl_2 by shaking vigorously 2 min and drain off CH_2Cl_2 layer into second separator. Ext aq. layer with 2 addnl 50 mL portions of CH_2Cl_2 and combine all CH_2Cl_2 exts in second separator. Discard aq. layer.

Place 40 g anhyd. Na_2SO_4 in coarse sintered-glass buchner, wash with ca 20 mL CH_2Cl_2, and discard washing. Assemble 250 mL K–D evaporative concentrator with 4 mL concentrator tube at bottom. While connecting bottom tube, wet joint with CH_2Cl_2 and attach springs. Dry combined CH_2Cl_2 ext by passing through Na_2SO_4 bed on buchner and collecting ext di-

rectly in K-D concentrator. Wash Na_2SO_4 bed with further 20 mL CH_2Cl_2 and collect washing in K-D concentrator.

10.C16 ***Concentration***

Add 1 tiny piece (1-2 mm) of Boileezer to contents of K-D flask, attach 3-section Snyder column, and conc. ext by heating flask in H_2O bath (50-60°). Initially maintain outside H_2O level close to level of CH_2Cl_2 inside flask and continue heating until concd ext is ca 4 mL (ca 40 min). (If excessive boiling occurs during concn, control it either by raising flask slightly out of H_2O bath or by decreasing temp. of bath.) Finally raise flask above H_2O and let condensed CH_2Cl_2 in Snyder column drain into flask. Add ca 1 mL CH_2Cl_2 to top of Snyder column and let it drain into flask. Disconnect concentrator tube from flask.

Add another tiny piece of Boileezer to contents and attach micro Snyder column and springs. Conc. ext to ca 0.8 mL by heating concentrator tube in 50-60° H_2O bath. Lift out or immerse tube in H_2O to control boiling rate but *do not* lift tube completely out of H_2O bath; this will stop action of Boileezer. *Avoid overheating and excessive accumulation of CH_2Cl_2 in column chambers.* Stop concn when CH_2Cl_2 level reaches ca 0.8 mL; do not conc. to less than 0.8 mL. Carry out this final concn step slowly, taking at least 30 min. Raise tube (bottom still touching H_2O), let liq. drain, and note vol. to see if it is ca 0.8 mL. If >0.8 mL, continue concn as above. Finally, rinse micro Snyder column with a few drops of CH_2Cl_2, let rinsing drain to tube, disconnect column, and dil. ext to 1.0 or 1.1 mL (not >1.1 mL). (Do not use N stream for concentrating ext at any stage.)

Stopper tube, mix in vortex mixer, and store at 4° in dark until analysis. Let warm to room temp. and note vol. before analyzing ext.

10.C17 ***Reagent Blank***

To ensure absence of contamination, carry out reagent blank taken thru all steps as mentioned above, except use 50 mL 4% alcohol in H_2O instead of 50 g beer. Inject 10 μL ext for GLC-TEA analysis as described below.

10.C18 ***Determination of Standard Curve***

Set attenuation (usually 4) of TEA detector so that injection of 30 pg NDMA gives definite peak with acceptable background. Using this attenuation, analyze 6 μL aliquots, in duplicate, of NDMA stds 5, 10, 20, and 40 ng/mL. Before injection, draw out syringe plunger slightly and note exact vol. of ext to be injected (there must be a small air gap between sample and rinsing solv. already inside needle). During injection, make sure no sample is lost thru back of plunger due to back pressure. After injection, hold needle in septum 5 s before withdrawing.

Next, choose a higher attenuation setting that gives on-scale peak for 6 μL of NDMA std 500 ng/mL. Using this setting, analyze 6 μL aliquots, in duplicate, of NDMA stds 500, 200, 100, and 40 ng/mL.

Accurately measure peak hts (±0.1 cm) and det. av. peak hts of 2 injections at each concn. If exactly 6 μL is not injected, make appropriate corrections and convert all peak hts equiv. to 6.0 μL injections. Draw 2 std curves, one for each attenuation setting, peak hts vs pg injected. Det. std curve weekly.

10.C19 ***Analysis of Beer Extract***

As above, inject 6 μL aliquots of beer ext, in duplicate, using lowest attenuation setting sensitive to 30 pg NDMA. Measure and det. av. peak ht corresponding to 6.0 μL injection. Compare this av. peak ht with std curve and det. which std NDMA soln, when injected under same attenuation setting, produces closest peak ht. Choose that NDMA std soln, inject 6 μL aliquots, in duplicate, and det. av. peak ht.

If sample ext on first injection produces off-scale peak, choose a higher attenuation setting (16 or 32) and carry out analysis, in duplicate, as above. Also analyze corresponding std NDMA soln at same attenuation. For samples giving off-scale peaks at attenuation 32, dil. exts with CH_2Cl_2 to 5.0 mL in a vol. flask and re-analyze. *For accurate results, analyze beer ext and corresponding std under same attenuation setting and all within 60 min.*

If, on the other hand, ext gives neg. result for NDMA or peak is too small to measure, inject 10 μL aliquots, in duplicate (use 25 μL syringe). Similarly, inject duplicate 10 μL aliquots of NDMA std 5 ng/mL for quantitation. To achieve 0.1 ppb detection limit, 10 μL aliquots of beer ext must be analyzed under attenuation setting that gives detectable peak for 30 pg NDMA.

Note: If using 25 μL syringe, which usually has thick needle, watch for septum damage and check for leaks. To be on safe side, *use a new septum* daily.

10.C20 ***Calculation***

Calc. concn of NDMA in beer, using following formula:

Uncorrected ppb NDMA in beer = $(h_1 p v_2)/(h_2 g v_1)$

where h_1 = av. NDMA peak ht (cm) of beer; h_2 = av. peak height (cm) of corresponding NDMA std; p = pg NDMA that produced h_2 peak ht; v_1 = μL beer ext injected; v_2 = final vol. (mL) of beer ext; g = g beer taken for analysis.

Correction for % recovery of NDPA.—Accurately measure peak ht of NDPA peak on each beer chromatogram and calc. av. peak ht of 2 injections. Make appropriate corrections if final vol. of beer ext is not exactly 1.0 mL or injection vol. is not exactly 6.0 μL. Then (within 60 min) inject, in duplicate, 6 μL NDPA std (250 ng/mL) under same attenuation setting. Calc av. peak ht and correct value if exactly 6.0 μL is not injected. Calc. % recovery of NDPA for each sample. If recovery of NDPA is less than 80%, repeat analysis from beginning. Finally, correct results as follows:

Corrected ppb NDMA in beer = (uncorrected ppb/% recovery of NDPA) × 100.

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(4) The following method for the determination of barley extract, Report of Subcommittee on Methods of Barley Analysis (May 1, 1945) American Society of Brewing Chemists, 3340 Pilot Knob Rd, St. Paul, MN, p. 31; Report of Subcommittee on Methods of Barley Analysis (1946) *Am. Soc. Brew. Chem. Proc.*, p. 92; *J. Assoc. Off. Anal. Chem.* **64,** 1138 (1981), was adopted official first action as an ASBC–AOAC method:

Extract of Barley Malt
Official First Action
ASBC–AOAC Method

10.C21 ***Principle***

Ground barley malt is digested with stdzd mixt. of enzymes for stated period at specified temp. Released ext is filtered, and sp. gr. is detd.

10.C22 ***Reagents***

(a) *Malt diastase.*—Anal. grade (Sturge Enzymes, 75C Third Ave, New York, NY 10017, or equiv.).

(b) *α-Amylase.*—Anal. grade (Sturge Enzymes, or equiv.).

10.C23 ***Apparatus***

See **10.106(c)**, (d), (f), (g), (i), (k).

10.C24 ***Preparation of Sample***

Grind sample to pass No. 20 sieve or sieve having circular openings 1 mm (1/25 in.) diam. and mix thoroly.

10.C25 ***Determination***

Weigh 50 ± 0.05 g ground barley into weighed or tared mash beaker, **10.106(c)**. Add 2.5 ± 0.0005 g malt diastase and 0.5 ± 0.0005 g α-amylase. Mix well with stirring rod. Add 200 mL H_2O in 3 portions as follows: Mix meal and first portion of H_2O to form uniform slurry; use second portion to wash sides of beaker and use third portion to rinse stirring rod. Place mash beaker in 20° H_2O bath, **10.106(k)**, and let stand overnight (15–16 h).

Place mash beaker in mashing app., **10.106(d)**, maintained at 70°. Start stirrer in mash beaker and stir continuously 60 min at 70°. Continue as in **10.108(c)**, *Cooling and filtration*, and **10.108(d)**, *Specific gravity*. Det. Plato value from **52.009**.

Det. ext value of enzyme prepd by carrying thru blank of enzymes and H_2O only. Det. sp. gr. and Plato value as with barley mash.

10.C26 ***Calculations***

(a) Calc. ext correction for enzyme blank:

$$E = [Pe(900 - 2D)]/(100 - Pe)$$

where E = ext correction; Pe = Plato value of enzyme blank filtrate; D = g enzyme prepn used = 3.0 g.

(b) Calc. ext in barley, as-is basis:

$$BE, \text{ as-is}, \% = \frac{P(800 + M - 2D)}{100 - P} - E$$

where P = Plato value of barley mash filtrate; M = barley moisture, % (detd as in **10.B01**).

(c) Calc. ext in barley, dry basis:

BE, dry basis, % = BE(as-is, %) × 100/(100 − M)

Report barley ext to 0.1%.

11. BEVERAGES: WINES

No additions, deletions, or changes.

12. BEVERAGES: NONALCOHOLIC AND CONCENTRATES

No additions, deletions, or changes.

13. CACAO BEAN AND ITS PRODUCTS

No additions, deletions, or changes.

14. CEREAL FOODS

(1) The official first action method for the

determination of starch, **14.075-14.080,** was deleted.

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(2) The following recommendations of the Ad Hoc Nitrogen-to-Protein Conversion Factor Committee, *J. Assoc. Off. Anal. Chem.* **65,** 333 (1982), were adopted official first action:

(a) All analyses shall report the percentage nitrogen in the sample.

(b) If the percentage of protein in reported, the nitrogen-to-protein conversion factor used shall be specified in the report.

(c) As desired, the traditional and customary nitrogen-to-protein conversion factors may be used as listed in **14.063** (12th Ed.) or **14.068** (13th Ed.), modified ("Changes in Methods," *J. Assoc. Off. Anal. Chem.* **64,** 505 (1981)) to read as follows:

"See **2.057.** Protein = nitrogen × 6.25 except for wheat and its products in which protein equals N × 5.7."

Other traditional and customary factors are 5.18 for almonds, 5.46 for peanuts and brazil nuts, 5.30 for tree nuts and coconut, 6.38 for dairy products.

Modification of **14.068** was approved interim official first action in March 1980. Committee D gave official first action approval at the October 1980 meeting, and the 6.25 factor approved in October appeared in "Changes in Methods" in March 1981. It should be made clear that this "change" is actually a reversion to the factors as published in the 12th Edition of *Official Methods of Analysis.*

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(3) The following liquid chromatographic method for the determination of fructose, glucose, sucrose, and maltose in presweetened cereals, *J. Assoc. Off. Anal. Chem.* **65,** 256 (1982), was adopted official first action:

Fructose, Glucose, Sucrose, and Maltose in Presweetened Cereal

Liquid Chromatographic Method

Official First Action

14.C01 *Apparatus*

(a) *Chromatography equipment.*—See **13.A01(a)** thru (d), but change capacity factor in **13.A01(b)** to 1.5; include automatic injectors (Waters Associates, Inc., WISP 710B, or equiv.) and use specific injection vol. in 10–50 μL range in **13.A01(c).**

(b) *Filter cartridge.*—Sep-Pak C_{18} (Waters Associates, Inc.), or equiv.

(c) *Guard column packing.*—Optional. C_{18} Corasil, 100 × 2 (id) mm (Waters Associates, Inc.), or equiv., as long as overall LC system meets column criteria of **13.A01(b).**

14.C02 *Reagents*

(a) *Sugar std solns.*—Dry individual sugar stds (fructose, glucose, sucrose, and maltose; available from Sigma Chemical Co.) 12 h at 60° under vac. Dissolve in H_2O or, optionally, alcohol–H_2O (1 + 1) to obtain concns of 3 mg/mL each for fructose, glucose, and maltose and 15 mg/mL for sucrose. After LC injection, compare peak response of sample and std, and adjust concns of std soln proportionately to obtain std response within 10% of sample responses.

(b) *Mobile phase.*—CH_3CN (LC grade) and H_2O (charcoal-filtered) (80 + 20). Filter thru Whatman GF/F 0.7 μm glass fiber filter. Optionally, filter CH_3CN and H_2O separately thru 0.45 μm PTFE and cellulose ester membranes, resp. Degas in ultrasonic bath before use. Vary CH_3CN–H_2O ratio and flow rate if necessary to meet column criteria.

14.C03 *Preparation of Sample*

(a) *Fat extraction.*—Weigh 2.00–10.00 g finely ground cereal into ≥100 mL centrf. bottle. If sample does not warrant fat extn, proceed to step (b). Add 50 mL pet ether and centrf. ca 10 min at 2000 rpm. Aspirate and discard pet ether without siphoning off solid material. Repeat extn. Evap. residual pet ether with gentle stream of N and break up solid material with glass rod.

(b) *Sugar extraction.*—Add 100 mL alcohol–H_2O (1 + 1) and weigh. Place in 80–85° H_2O bath 25 min and stir occasionally. Cool to room temp. and add alcohol to original wt. Centrf. 10 min at ca 2000 rpm. If very cloudy, recentrf. portion of ext 5 min at ca 3500 rpm and filter thru 0.45–0.7 μm Swinney syringe filter. If guard column is used, omit step (c) and save filtered ext for LC analysis.

(c) *Cleanup.*—Fill C_{18} Sep-Pak cartridge with mobile phase and force thru filter, leaving liq. above packing. Repeat with sample ext twice, collecting eluate from second pass for LC analysis. Filter thru 0.45–0.7 μm Swinney syringe filter if necessary.

14.C04 *Determination*

Inject sample soln (10–50 μL) into column with

flow rate of mobile phase at 1.5–2.5 mL/min. Inject same vol. of std soln that will give peak response ±10% of sample peak response. Two injections each of sample and std soln are required for adequate precision.

Measure areas or peak hts of each sugar peak in sample and std, but only measure peak ht for components which are near detection limit and have adjacent interfering peak.

$$\% \text{ Component} = (R/R') \times (C'/W) \times V \times 100$$

where R and R' = area or peak ht of sample sugar and std sugar, resp.; V = mL alcohol-H_2O added to sample = 100; W = g sample; C' = concn of sugar std in g/mL.

15. COFFEE AND TEA

No additions, deletions, or changes.

16. DAIRY PRODUCTS

(1) The following microbiological penicillin-affinity method for the detection of penicillin in milk at ≥0.01 IU penicillin G/mL was adopted official first action:

Penicillin in Milk

Affinity Quantitative Determination

Official First Action

(Applicable to levels ≥0.01 IU penicillin G/mL or β-lactam equiv.).

16.C01 ***Principle***

Assay is based on specific, irreversible affinity of β-lactam antibiotics for certain enzyme sites on cell wall of microorganisms. ^{14}C-labeled penicillin and *Bacillus stearothermophilus* are added to milk sample. Antibiotic in sample competes with ^{14}C-penicillin for binding sites. Amt of bound carbon-14 is counted and compared with control to det. presence of β-lactam antibiotic.

16.C02 ***Reagents***

Note: Stds and reagents are conveniently measured into tubes, stoppered, and frozen at −20° if held >1 day. Keep reagents at <4° when used. Milk should be <15° when test starts.

(**a**) *Penicillin-free milk.*—Reconstitute penicillin-free whole milk powder (Yankee Milk, Newington, CT) with H_2O.

(**b**) *Penicillin-free skim milk powder.*—Test according to **16.131-16.136.**

(**c**) *14-C Penicillin.*—Contg 103 μCi/μmole (Penicillin Assays Inc., 33 Harrison Ave, Boston, MA 02111). Use 0.0027 μCi in each assay.

(**d**) *B. stearothermophilus.*—Use vegetative cells, ca 47 mg wet wt in each test.

(**e**) *Penicillin G stds. (1) Zero std:* Use penicillin-free milk, (**a**). *(2) 0.01 IU/mL std:* Add 1.00 IU Na or K penicillin G USP ref. std to small amt of reconstituted skim milk powder in 125 mL bottle and freeze-dry. Add 100 mL penicillin-free milk (**a**) and mix well.

16.C03 ***Apparatus***

(**a**) *Heater.*—Const temp., dry well tube heater for 13 × 100 mm glass tubes (Constantemp, Roeco Manufacturing Co., Box 357, City, ST zip).

(**b**) *Cold plate.*—Accommodates 13 × 100 mm tubes (Penicillin Assays Inc., or equiv.).

(**c**) *Radiation counter.*—Models 150 or 300 mm (Penicillin Assays Inc., or equiv.).

(**d**) *Pipets.*—Semi-automatic (Absoluter, Tri-Continent Scientific, 12541 Loma Rica Dr, Graff Valley, CA 95945, or equiv.).

16.C04 ***Procedure***

Pipet 5 mL sample into 13 × 100 mm glass tube. Pipet in 200 μL ^{14}C-penicillin and mix. Pipet in 200 μL *B. stearothermophilus* suspension and mix. Incubate 3 min in 90° dry well heater. Centrf. 4 min at 1200 × g.

Decant milk and swab out fat ring, using 2 cotton swabs. Rinse tube twice with H_2O from wash bottle; do not disturb ppt at bottom of tube. Add ca 300 μL H_2O and resuspend ppt, using tube mixer.

Place Al planchet on 400° hot plate. Pour suspension into planchet, touching mouth of tube to planchet to remove last drop. Rinse tube twice with 300 μL H_2O and add washings to planchet. Let planchet dry. Place dry planchet in penicillin analyzer and measure radiation from ^{14}C for 8 min. Compare count with predetd control point to det. whether sample is pos. or neg.

16.C05 ***Control Point Determination***

Analyze 10 zero stds and average. If any zero std falls ±20% from av., replace with new std and det. new av. Control point = 0.80 × av. count. Test samples fall below control point if they contain β-lactam antibiotic or if there is a test failure.

To identify test failure, make second detn of pos. samples, zero std, and 0.01 IU/mL std at same time. Zero std count should be greater than control point, and pos. sample should again be less than control point to confirm pos. detn.

(2) The following *Bacillus stearothermophilus* disc method for the detection of penicillins in milk at ≥0.008 IU penicillin G/mL was adopted official first action:

Penicillins in Milk

Bacillus stearothermophilus Qualitative Disc Method II

Official First Action

(Applicable to levels ≥0.008 IU penicillin G/mL)

16.C06 ***Culture Media***

(**a**) *Agar medium B.*—See **42.196**(b).

(**b**) *Agar medium P.*—Dissolve 3.0 g beef ext, 5.0 g peptone, 1.7 g tryptone, 0.3 g soytone, 5.25 g dextrose, 0.5 g NaCl, 0.25 g K_2HPO_4, 1.0 g polysorbate 80, 0.06 g bromcresol purple, and 15.0 g agar in H_2O and dil. to 1 L. Adjust if necessary so that after sterilization pH is 7.8 ± 0.2. (Difco PM Indicator Agar has been found satisfactory.)

(**c**) *Agar medium M.*—See **16.131**(b).

(**d**) *Broth medium D.*—Dissolve 17.0 g pancreatic digest of casein, 3.0 g papaic digest of soybean, 5.0 g NaCl, and 2.5 g K_2HPO_4 in H_2O and dil. to 1 L. Adjust if necessary so that after sterilization pH is 7.3 ± 0.2. (BBL Trypticase Soy Broth without Dextrose has been found satisfactory.)

16.C07 ***Reagents and Apparatus***

(**a**) *Penicillin stock soln.*—See **16.132**(**a**). (Difco PM Positive Controls, Penicillin G, and Penicillin Assays Inc. penicillin stds have been found satisfactory.)

(**b**) *Penicillinase (beta-lactamase).*—See **16.132**(**b**). (Commercial Penase Discs (12.7 mm) have been found satisfactory.)

(**c**) *Filter paper disc, blank.*—Use S&S 740 E, 12.7 mm discs or discs of equiv. absorption performance qualities and purity.

(**d**) *Control discs.*—Prep. fresh daily from pos. control milks contg 0.008 IU penicillin/mL.

(**e**) *Petri dishes (plates).*—See **42.198**(**b**).

16.C08 ***Stock Culture of Test Organisms***

Maintain *B. stearothermophilus* ATCC 10149 on agar medium M (**c**), transferring to fresh slant weekly. Inoculate slant of agar medium M with test organism and incubate overnight at 55 ± 2° or 64 ± 2°. Inoculate three 300 mL erlenmeyers, each contg 150 mL broth medium D (**d**), with 1 loopful of test organism. Incubate at 55 ± 2° or 64 ± 2° and periodically make spore stains to det. extent of sporulation. When ca 80% sporulation has occurred (usually in 72 h), centrf. cell suspension 15 min at 5000 rpm. Decant supernate, resuspend cells in saline solution, **42.197**(r), and recentrf. Repeat saline washing. Resuspend washed cells in 30 mL saline and store at 4°. Spore suspension will remain viable 6–8 months. Check viability periodically by prepn of trial test plates. (Com. prepd spore suspension has been found satisfactory.)

16.C09 ***Preparation of Plates***

Inoculate aliquot of liquified agar medium B (**a**) or P (**b**), cooled to 55° or 64° with previously prepd spore suspensions. Adjust inoculum level to provide clear, readable zones of inhibition from penicillin-pos. control discs (d) after 3–4 h incubation at 55 ± 2° or 2–3 h incubation at 64 ± 2°. Pour 6 mL inoculated agar medium B or P into each plate, and let harden on flat, level surface. Use within 5 days.

16.C10 ***Assay***

Screening.—With clean, dry forceps, touch paper disc to surface of well mixed milk and let milk be absorbed by capillary action. Drain excess milk by touching disc to inside surface of sample vessel. Immediately place disc on agar surface, pressing gently to ensure good contact. Identify each disc or section on which it is placed. Place control disc contg 0.008 IU penicillin/mL on plate. Invert plate and incubate at 55 ± 2° or 64 ± 2° until well defined zones of inhibition (17–20 mm) are obtained with the 0.008 IU/mL control. Examine plate for clear zone of inhibition surrounding discs. Clear zone of >14 mm indicates presence of inhibitory substances. Zones of ≤14 mm are read as neg. Confirm presence of inhibitor.

Confirming.—Heat test samples to 82° ≥2 min. Cool promptly to room temp. With clean, dry forceps, touch paper disc to surface of well mixed milk and let milk be absorbed by capillary action. Also fill penicillinase-impregnated disc or add 0.05 mL penicillinase to 5 mL sample and fill disc. Drain excess milk by touching disc to inside surface of sample vessel. Immediately place each disc on agar surface, pressing gently to ensure good contact. Place control disc contg 0.008 IU/mL on plate. Invert plate and incubate at 55 ± 2° or 64 ± 2° until well defined zones of inhibition (17–20 mm) are obtained with 0.008 IU/mL control. Examine plate for clear zone of inhibition (>14 mm) surrounding disc, indicating presence of inhibitory substance.

16.C11 ***Interpretation***

Assay of test milk in screening and confirmatory test may produce following results:

(*1*) No zone around disc contg untreated milk in screening test is neg. test for inhibitory substances.

(*2*) Zone around disc contg untreated milk but no zone around disc contg penicillinase-treated milk in confirmatory test is pos. test for β-lactam residue.

(*3*) Clear zone of equal size around both discs in confirmatory test indicates presence of inhibitors other than β-lactam residues.

(*4*) Clear zone around penicillinase-treated milk substantially smaller than around untreated milk disc in confirmatory test indicates presence of β-lactam residues as well as another inhibitor(s).

Penicillin-pos. control soln at 0.008 IU/mL should produce clear, well defined zones of inhibition (17–20 mm). If no zone of inhibition is produced by penicillin-pos. control, test sensitivity is not adequate and test should be repeated.

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(3) The following *Bacillus stearothermophilus* disc method for the quantitative determination of penicillin G residues in milk at ≥0.016 IU penicillin G/mL was adopted official first action:

Penicillin Residues in Milk

Quantitative *Bacillus stearothermophilus* Disc Method

Official First Action

(Applicable to levels ≥0.016 IU penicillin G/mL)

16.C12 ***Apparatus and Reagents***

(**a**) *Microliter pipetter.*—90 μL, with disposable tips (Eppendorf Model, Curtin Matheson Scientific, Inc., PO Box 1546, Houston, TX 77001).

(**b**) *Vernier calipers.*—Readable to 0.1 mm (Curtin Matheson Scientific, Inc.).

(**c**) *Petri dishes.*—Flat type (Falcon Series, Curtin Matheson Scientific, Inc.).

(**d**) *Filter discs.*—Non-sterile, 1/2 in. diam., round 740E (Schleicher and Schuell, Inc., 543 Washington St, Keene, NH 03431).

(**e**) *Penicillinase discs.*—Round, 1/2 in. diam. (Difco Laboratories).

(**f**) *Incubator.*—Capable of maintaining temp. 64 ± 2°.

(**g**) *Assay medium.*—Antibiotic Medium 4 (Difco Laboratories), **42.196**(b).

(**h**) *Spore suspension.*—Stdzd *B. stearothermophilus* spore suspension, ATCC No. 10149 (Difco Laboratories).

(**i**) *Phosphate buffer.*—Dissolve 8 g anhyd. KH_2PO_4 and 2 g anhyd. K_2HPO_4 in 1 L distd or deionized H_2O. Adjust to pH 6.0 if necessary.

(**j**) *Std soln.*—1000 IU USP Na penicillin G Ref. Std/mL phosphate buffer. This stock soln may be frozen for later use. For assay, dil. first to 1.0 IU/mL in phosphate buffer and then prep. 0.016 IU/mL ref. std milk by dilg aliquot with previously tested penicillin-free milk.

16.C13 ***Preparation of Seeded Medium and Culture Plates***

Prep. sterile Antibiotic Medium 4 in flasks in 100 mL lots. Add 1 mL ampule of *B. stearothermophilus* spore suspension to each 100 mL lot previously tempered to 65°. Preferably, seed fresh each day of testing. Work only on absolutely flat surface. Using warm 10 mL pipet, transfer 6 mL seeded medium to center of petri dish. Cover dish and swirl gently to cover entire bottom surface. Let medium solidify at room temp.

16.C14 ***Procedure***

Let stock std and samples thaw (if frozen) at room temp. Prep. 0.016 IU/mL ref. std milk, (**j**). Heat ref. std milk and samples to 82° for 2 min. Cool in ice-H_2O bath.

Using clean, dry forceps, place 6 filter discs evenly spaced around periphery of culture plate, ca 1 cm from outer edge. Touch each disc gently to surface of medium with tips of forceps. Using sep. forceps, place penicillinase disc in center of plate.

Within 45–60 s after disc is placed on culture plate, add 90 μL ref. std or sample. Alternate ref. std with samples on each plate, adding sample to penicillinase disc. Hold pipetter over center and 3–5 mm above disc when adding sample. Eject sample in even flow; avoid splash. Use 1 disposable tip for triplicate ref. stds and 1 for triplicate samples. Prep. replicate plates: 3 plates (total of 9 replicate ref. stds and 9 replicate samples) provide satisfactory discrimination on quantitative basis.

Invert plates and place immediately in 64 ± 2° incubator. Incubate 2 h and 45 min. Remove plates in sets as prepd, and place on laboratory bench at room temp. while reading.

16.C15 ***Measurement of Zone Diameter***

Using vernier calipers, measure zone diam. of ref. std and sample to nearest 0.1 mm. Include both disc and zone of inhibition in this measurement. If present, measure any zone found around penicillinase disc, record as "other inhibitor," and proceed no further with test. If no zone of inhibition is found around penicillinase disc, and if zone diams of sample are larger than those of ref. std, proceed with calcns.

16.C16 ***Calculations***

Det. validity of difference between ref. std and sample zone diam. by paired-t analysis using paired differences that occur between each ref. std and clockwise adjacent unknown. Each plate thereby yields 3 differences for use in paired-t analysis. Calc. t-value, $t = \sqrt{n} \times \bar{d}/S_d$, where n = number of observations, $\bar{d}$ = mean of differences, and S_d = std dev. of differences =

$$\sqrt{\frac{\Sigma(d_i - \bar{d})^2}{n-1}} = \sqrt{\frac{\Sigma d_i^2 - \left(\frac{(d_i)^2}{n}\right)}{n-1}}$$

Simplified formula for calcg t for 9 replicates (3 plates) is as follows:

$$t = \frac{2.828 \times D_1}{\sqrt{9 \times D_2 - (D_1)^2}}$$

where D_1 = sum of differences of 9 replicate detns; and D_2 = sum of squares of differences of 9 replicate detns.

A t-value >1.860 indicates with 95% confidence that sample contains ≥0.016 IU penicillin/mL.

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(4) The following *Bacillus stearothermophilus* color-reaction qualitative method for the detection of penicillins in milk at ≥0.007 IU penicillin G/mL were adopted official first action:

Beta-Lactam in Fluid Milk Products
Qualitative Color Reaction Tests
Official First Action

16.C17 ***Principle***

Test is based on rapid growth rate and acid production of *Bacillus stearothermophilus* var. *calidolactis*. Acid changes color of bromcresol purple to yellow in absence of β-lactam inhibitors. In presence of inhibitors, purple color remains. Applicable for detecting and confirming levels of β-lactam residues ≥0.007 IU/mL processed fluid milk products and raw milk. Test may be performed by using ampule or multitest kits.

16.C18 ***Reagents and Apparatus***

(**a**) *Delvotest®-P-Ampule test kit.*—(GB Fermentation Industries, Inc., One N Broadway, Des Plaines, IL 60016). Contg: 100 test ampules, seeded with *B. stearothermophilus* var. *calidolactis* in plain solid agar medium (store at 4–15°); 100 nutrient tablets contg tryptone (0.5 mg), glucose (5.0 mg), nonfat dry milk (2.0 mg), and bromcresol purple (0.025 mg) (store at 4–15° until opened and then store at room temp.); plastic forceps to transfer nutrient tablets; and plastic syringe with 100 disposable tips for sampling and dispensing 0.1 mL portions of milk.

(**b**) *Delvotest®-P-Multi test kit.*—(GB Fermentation Industries, Inc.). Contg: hermetically sealed Al bag contg 3 plates with 96 cups each of *B. stearothermophilus* var. *calidolactis* in solid medium with bromcresol purple indicator. Each plate can be divided into 6 blocks of 16 cups each. Similar Al bag contains 3 similar plates with 1 nutrient tablet per cup, and sealing tapes to cover all blocks individually. Store at 4–15° until opened; then store at room temp.

(**c**) *Heaters.*—Block heater and H_2O bath, both thermostatically controlled at 65 ± 2°. Check daily.

(**d**) *Dispensing pipet.*—Disposable, as in ampule test, or micropipettor (Micro/Pettor, Scientific Manufacturing Industries, 1399 64th St, Emeryville, CA 94608).

(**e**) *Phosphate buffer.*—1%, pH 6.0. Dissolve 8.0 g anhyd. KH_2PO_4 and 2.0 g anhyd. K_2HPO_4 in H_2O and dil. to 1 L with H_2O.

(**f**) *Penicillinase (beta-lactamase).*—Conc., store at 0–4.4° (Difco, BBL, or Penicillin Assays, Inc., material is satisfactory).

(**g**) *Penicillin stock soln.*—Accurately weigh ca 30 mg USP K penicillin G ref. std and dissolve in pH 6.0 buffer to give known concn of 100–1000 IU/mL. Store at 0–4.4° not >2 days. Stock soln may be dild in inhibitor-free milk to final desired concn, distributed in small quants in tubes, sealed, and frozen ≤6 months.

(**h**) *Inhibitor-free milk.*—Any fluid milk product (butterfat content 0.00–3.50%, total solids <13%) may be used after being tested with this method to verify it is inhibitor-free. Use for dilg stds and as neg. control.

16.C19 ***Check Run Procedure***

Perform on each new lot number of ampule or

test kits and on new batches of prepd reagents.

Prep. 10 mL vols of penicillin stds in inhibitor-free milk, contg concns 0.002, 0.004, 0.006, 0.008, and 0.010 IU/mL, 10 mL H_2O, and 10 mL inhibitor-free milk in test tubes. Transfer 5 mL aliquot from each tube into a corresponding tube. Heat both sets of tubes to 82° for 3 min in H_2O bath. Remove; cool rapidly to room temp. Add 0.2 mL penicillinase to 1 series of tubes. Shake well; let stand 15 min at room temp.

For ampule test, remove and identify 1 ampule for each test tube from test kit. Break off neck of ampule and place ampule in lid of ampule box, or other suitable rack. With clean, dry forceps, place 1 nutrient tablet in each ampule. For multi test, remove 1 plate from each of the 2 foil bags. Score foil covering at cutline on end block of each plate and break off 1 block of agar cups and 1 block of cups contg nutrient tablets. Open both blocks by carefully tearing back foil. Place block contg agar upside down, exactly on top of those with nutrient tablets. Holding both blocks together, invert them and tablets will fall into each corresponding cup. Light tapping of tablet cup may be needed to get all of tablets out. Arrange test tubes of controls according to cup locations in the block. Attach dry sampling pipet to plastic syringe. Completely depress plunger, place end of pipet into tube, ca 1 cm below top of sample level. Allow plunger to return slowly under pressure of spring. Level of sample should reach wide part of pipet. If air bubble appears, slowly expel sample back into tube and let plunger slowly return again. Do not contaminate syringe. If drop of milk clings to outside of pipet tip, gently touch it off on edge of tube. This vol. is ca 0.1 mL. Empty pipet into appropriate ampule or cup. Remove pipet tip and replace with new pipet for each sample, control, or std. If using micropipettor, wipe outside with tissue and rinse 3 times in sample before removing test aliquot. Continue this pipetting procedure until all tubes have been sampled.

For ampule test, place ampules into appropriate holes in heater block. Incubate at 65 ± 2° exactly 2½ h. Remove ampules immediately. Read penicillinase-treated ampule and its corresponding untreated ampule side by side, looking through agar against white reflective background; compare and record colors as yellow, purple, or yellow-purple. Disregard intense color surrounding nutrient tablet.

For multi test, carefully seal block of cups with strip of adhesive tape enclosed in kit. Very carefully float sealed block in 65° H_2O bath. Incubate exactly 2 h and 45 min at 65 ± 2°. Remove block from H_2O bath, read, and record colors developed. Read from bottom side of block. Compare and record colors as yellow, purple, or yellow-purple.

Following colors are satisfactory in check run: yellow for H_2O, inhibitor-free milk, and 0.002 IU/mL std; yellow or yellow-purple for 0.004 IU/mL std; purple for 0.006, 0.008, and 0.010 IU/mL stds.

Note: Occasionally kits of a particular Lot No. may require a longer incubation time for color to fully develop. If H_2O and inhibitor-free milk samples are not truly yellow and/or 0.006 and 0.008 IU/mL stds are not completely purple at end of 2½ h incubation (2 h and 45 min for multi test) continue incubating until proper colors are developed. Check color development at 10 min intervals and record optimum incubation time required for each Lot No.

16.C20 *Screening Procedure*

Use 1 neg. and 1 pos. control (0.008 or 0.010 IU/mL). Samples may be heat-treated, as in check run, if desired.

Prep. test ampules or blocks of cups as in check run and arrange samples in same order as ampules or cups. Shake samples 25 times through arc of 1 ft in 7 s. Using new pipet tip for each sample, control, and std, proceed with sampling and incubation as in check run. Remove ampules or blocks of cups and record colors.

All-yellow samples are neg. and need not be confirmed. Purple or yellow-purple samples must be confirmed before reporting.

16.C21 *Confirmation Procedure*

Heat-treat two 5 mL portions of each sample to be confirmed and the 0.002, 0.004, and 0.006 IU/mL stds. Do not treat neg. control or 0.008 IU/mL std. Add 0.2 mL penicillinase to 1 portion of each heat-treated sample and the 3 low concns of stds. Prep. ampules or blocks of cups, sample, and incubate as in check run. Remove ampules or blocks and read treated and untreated samples side by side and record color results.

16.C22 *Interpretation*

All results are reported as pos. or neg. for β-lactam residue.

Neg.: Solid yellow in screening test.
Neg.: Heated sample yellow, penicillinase-treated sample yellow.
Neg.: Heated sample purple, penicillinase-treated sample purple.

Neg.: Heated sample yellow-purple, penicillinase-treated sample yellow-purple.
Pos.: Heated sample yellow-purple, penicillinase-treated sample yellow.
Pos.: Heated sample purple, penicillinase-treated sample yellow.

Samples contg heat-stable natural inhibitor give true neg. test for β-lactam residues. Samples contg heat-stable natural inhibitor plus penicillin may result in false neg. test for β-lactam residues. Sample contg other inhibitory substances (e.g., tetracycline) will give true neg. test for β-lactam residues. Samples of chocolate-flavored products are difficult to read in the multi test kit because of light-distorting colors from adjacent cubes. They should not be reported as pos. by that method without first confirming with another method.

17. EGGS AND EGG PRODUCTS

No additions, deletions, or changes.

18. FISH AND OTHER MARINE PRODUCTS

(1) The official first action liquid chromatographic fluorometric method for the determination of indole in shrimp, **18.B01-18.B05,** was adopted official final action.

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(2) The official final action gas-liquid chromatographic method for the determination of indole in shrimp, **18.075-18.078,** was modified to improve method performance for samples containing indole in the range 5–50 μg indole/100 g shrimp. The following method was adopted official first action to replace **18.075-18.078** which was repealed official first action:

Indole in Shrimp

Gas Chromatographic Method – Official First Action

18.C01 ***Reagents***

(**a**) *Purified water.*—Distd H_2O treated by Milli-Q water purification system (Millipore Corp., Bedford, MA 01730), or equiv.

(**b**) *Solvents.*—Ether, anhyd. (contg 0.05% alcohol), ethyl acetate, and hexane, distd from glass.

(**c**) *Carbonate buffer.*—pH ca 9.6; 0.2M each Na_2CO_3 and $NaHCO_3$. Dissolve 21.2 g Na_2CO_3 and 16.8 g $NaHCO_3$ in H_2O and dil. to 1 L.

(**d**) *Silica gel.*—Dry 70–230 mesh silica gel 60 (EM Laboratories, Elmsford, NY 10520) 2 h at 125° in open vessel in layers ≤2 cm deep. Add 3.0 g H_2O per 25.0 g dried silica gel and place on wrist-action shaker ≥4 h. Store in air-tight container.

(**e**) *Indole std solns. Stock soln I.*—1.00 mg/mL. Dissolve 100.0 mg indole in alcohol in 100 mL vol. flask and dil. to vol. with alcohol. *Indole std soln II.*—20 μg/mL. Pipet 2.0 mL stock soln into 100 mL vol. flask and dil to vol. with ethyl acetate. *Indole std soln III.*—5 μg/mL. Pipet 25 mL std soln II into 100 mL vol. flask and dil to vol. with ethyl acetate. *Indole std soln IV.*—2 μg/mL. Pipet 10 mL std soln II into 100 mL vol. flask and dil. to vol. with ethyl acetate. *Indole std soln V.*—1 μg/mL. Pipet 5 mL std soln II into 100 mL vol. flask and dil to vol. with ethyl acetate.

(**f**) *2-Methylindole soln. Stock soln I.*—1 mg/mL. Dissolve 100 mg 2-methylindole in alcohol in 100 mL vol. flask and dil. to vol. with alcohol. *Dilute stock soln II.*—50 μg/mL. Pipet 5 mL stock soln into 100 mL vol. flask and dil. to vol. with alcohol. *Working std soln III.*—10 μg/mL. Pipet 20 mL dil stock soln II into 100 mL vol. flask and dil. to vol. with alcohol.

18.C02 ***Apparatus***

(**a**) *Gas chromatograph.*—With N-specific detector, such as Perkin-Elmer Model Sigma 2 (Perkin-Elmer Corp., Norwalk, CT 06856), or equiv., and 6 ft × 2 mm id glass column packed with 10% neopentylglycol adipate (NPGA) or Superpak 20M (Analabs Inc., North Haven, CT 06473) (see (**b**) below), or equiv. Adjust bead current, air flow, and H flow to obtain ≥50% full scale deflection for 5 μg indole (3 μL calibration soln 4, Table **18:C1**). Adjust chromatographic conditions so that retention times for indole and 2-methylindole are ca 6.8 and 9.4 min, resp. Baseline resolution should be obtained for tri-*n*-butyl phosphate (ca 5 min), indole, skatole (ca 8 min), and 2-methylindole.

(**b**) *GLC columns.*—(*1*) or (*2*) below:

(*1*) *NPGA*—Dissolve 1.2 g NPGA (HI-EFF-3a, Applied Science Laboratories, Inc.) in 100 mL $CHCl_3$ in 400 mL beaker. Add 10.8 g 80–100 mesh acid-washed Chromosorb W (Johns-Manville Products Corp.) with stirring. Evap. solv. on steam bath with stirring. Transfer powder to rotary evaporator and remove last of solv. at 40–50° with vac. Fill clean, dry glass column, (**a**), with *5% soln of dimethyldichlorosilane in toluene* and let stand ca 5 min. Rinse column with ca 50 mL MeOH and dry under N. (Caution: See **36.019(d)**. Pack column with coated support and

Table 18:C1. Calibration solutions for GLC determination of indole

Cal. soln	Indole: Std soln	Indole: Concn, μg/mL	Indole: mL	Indole: μg	2-Mel.[a] mL	Ethanol, mL	Total, mL
1	V	1	1	1	1	1	3
2	V	1	2	2	1	0	3
3	IV	2	2	4	1	0	3
4	III	5	1	5	1	1	3
5	III	5	2	10	1	0	3
6	II	20	1	20	1	1	3

[a] 2-Methylindole working std soln. 10 μg/mL

purge ≥2 h with N at room temp. Condition 24 h at 220° with 10 mL N/min.

Representative conditions are: temps (°)—column 195, injector 220, detector 250; carrier gas flow ca 30 mL N/min.

(2) *Super Pak 20M.*—Fill clean, dry glass column with *5% soln of dimethyldichlorosilane in toluene* and let stand ca 5 min. Rinse with ca 50 mL MeOH and dry under N. Pack column with SuperPak 20M and purge ≥3 h with He at room temp. Condition column 24 h at 220° with He flow of 20 mL/min. Typical GLC operating conditions are: temps (°)—column 160, injector 220, detector 250; carrier gas 30 mL He/min.

(c) *Whatman 1PS phase separating filter paper.*

18.C03 *Calibration*

Prep. calibration solns contg vols as indicated in Table **18:C1.** Pipet indicated vols into sep. vials with Teflon-lined screw caps and mix. Store in refrigerator.

Make duplicate injections (ca 3 μL) of each soln. Measure peak hts and calc. peak ht ratio, R = ht indole peak/ht internal std peak. Prep. std curve by plotting R against μg indole in std soln.

18.C04 *Determination*

Weigh 25.0 g well mixed sample into blender bowl, add 100 mL carbonate buffer, and blend 2 min at high speed. Quant. transfer slurry to 500 mL separator. Rinse blender with 25 mL H_2O from squeeze bottle and add rinse to separator. Add 200.0 mL ethyl acetate to separator and shake vigorously 2 min.

Let layers sep. and drain lower layer into beaker. If ethyl acetate (upper layer) is <150 mL, centrf. lower layer, sep., and combine resulting org. layers. Pass org. layer thru Whatman phase-sepg paper. Measure exactly 150 mL filtrate into g-s flask and add 1.00 mL 2-methylindole working soln and 10 g anhyd. Na_2SO_4. Shake 1 min.

Decant ext and conc. to ca 5–10 mL, using rotary evaporator and ca 35–40° H_2O bath. *Do not evap. to dryness.* (Alternatively, conc. under stream of N in 50° H_2O bath.) Transfer ext to vial and conc. to ca 1–1.5 mL under N. Add 2 mL hexane and mix. Add 1.5 g anhyd. Na_2SO_4 and shake vigorously 2 min.

Prep. cleanup column by firmly placing plug of glass wool in bottom of 10 cm × 15 mm or 10.5 mm id glass chromatgc tube. Add 6 g silica gel and tap firmly. Place ca 0.5 cm anhyd. Na_2SO_4 on top of silica gel bed. Pipet ca 2 mL hexane on top of column. Immediately after hexane is absorbed, transfer concd ext (contg added hexane) to column with min. disruption of bed. When liq. level reaches top of Na_2SO_4, add 10 mL hexane. Similarly, add 10 mL ether-hexane (15 + 85) and 40 mL ether-hexane (15 + 85). Collect all column effluent. Conc. to ca 1.5 mL at 50° under N. *Do not evap. to dryness.*

Make duplicate injections, measure peak hts, and calc. R values. Det. I (μg indole) for samples from calibration plot. Calc. amt of indole in each sample:

$$\mu g\ \text{Indole}/100\ g\ \text{sample} = (I \times 100)/[g\ \text{sample} \times (150/200)]$$

Calc. av. amt of indole from duplicate injections.

19. FLAVORS

The following liquid chromatographic method for the determination of glycyrrhizic acid or glycyrrhizic acid salts in licorice products was adopted official first action:

Glycyrrhizic Acid or Glycyrrhizic Acid Salts in Licorice

Liquid Chromatographic Method

Official First Action

19.C01 *Apparatus*

(a) *Liquid chromatograph.*—With Model

M6000A solv. delivery system, Model U6K universal injector, and Model 440 UV absorbance detector set at 254 nm, 0.2–0.1 AUFS range (Waters Associates, Inc.), or equiv.

(b) *Recorder.*—Strip chart 1- or 2-pen recorder (Houston Instrument Omni Scribe Model B5Z17-1, or equiv.). Chart speed 0.2 in./min.

(c) *Column.*—μBondapak C_{18}, 10 μm particle size, 30 cm × 4 mm id (Waters Associates, Inc.), or equiv.

(d) *Solvent and sample clarification kits.*—Org. and aq. (Waters Associates, Inc., or equiv.).

19.C02 *Reagents*

(a) *Mobile phase.*—Use chromatgy grade reagents. H_2O-HOAc-CH_3CN (61 + 1 + 38). Filter and degas mobile phase with solv. clarification kit. Flow rate 2.0 mL/min.

(b) *Glycyrrhizic acid salt std soln.*—0.1 mg/mL. Dissolve 10 mg mono ammonium glycyrrhizinate (available from MacAndrews & Forbes Co., Camden, NJ 08104) in 100 mL mobile phase. Filter thru 0.45 μm filter, using org. sample clarification kit. Prep. fresh std soln daily.

19.C03 *Sample Preparation*

Dissolve 50 mg licorice product in 50 mL H_2O. Use mobile phase if not sol. in H_2O. Vortex-shake until sample is completely dissolved. Filter through 0.45 μm filter, using aq. sample clarification kit.

19.C04 *Determination*

Warm up UV detector 15 min before start-up. Run mobile phase thru column at specified operating conditions ≥20 min before injecting first sample. Using 10 μL syringe, inject accurately measured vol. of std soln in duplicate. Inject similar vol. of sample soln. If samples are analyzed in series, re-inject std soln at regular intervals. Use peak ht (peak area in case of electronic integrator) to calc. concn of glycyrrhizic acid or glycyrrhizic acid salt as follows:

$$\text{Glycyrrhizic acid monoammonium salt, \%} = (C'/C) \times (PH/PH') \times (V'/V) \times 100$$

where C' and C = concn of std and sample soln in mg (dry basis)/mL, resp.; PH' and PH = peak ht of std and sample, resp.; V' and V = vol. of std and sample injected in μL, resp.

20. FOOD ADDITIVES: DIRECT

The official first action differential pulse polarographic method for the determination of saccharin, **20.A06–20.A10,** was adopted official final action.

21. FOOD ADDITIVES: INDIRECT

No additions, deletions, or changes.

22. FRUITS AND FRUIT PRODUCTS

The following carbon ratio mass spectrometric method for the determination of corn syrup in orange juice was adopted official first action:

Corn Syrup in Orange Juice
Carbon Ratio Mass Spectrometric Method
Official First Action

22.C01 *Principle*

Sample is burned completely to CO_2 and H_2O; CO_2 is purified, and $^{13}C/^{12}C$ ratio is measured in isotope ratio mass spectrometer. Difference in $^{13}C/^{12}C$ values for orange juice (av. $\delta\,^{13}C = -24.5 \pm 0.591$‰ (parts per thousand)) and corn syrup (av. $\delta^{13}C = -9.7$‰) provides measure of corn syrup (including high fructose corn syrup (HFCS)) in orange juice.

22.C02 *Apparatus*

(a) *Combustion system.*—Vac.-tight glass manifold including quartz combustion tube ½ filled with CuO in tubular furnace, liq. N trap, automatic Toepler pump, and high-vac. source.

(b) *Purification system.*—Glass manifold interconnected with combustion system including trap, sample collection tube, and manometer (see Figure **31:02** and *Geochimica et Cosmochimica Acta* **3,** 54–55 (1953)).

(c) *Mass spectrometer.*—Micromass 602 (new Model 602E) (Kearns Group, 58 Buckingham Dr, Stamford, CT 06902), Nuclide 6-60-RMS (Nuclide, 642 E College Ave, State College, PA 16801), Varian MAT G D150 (superseded by MAT 250) (Varian MAT Mass Spectrometry, 25 Hanover Rd, Florham Park, NJ 07932), or equiv. instrument designed or modified for isotope ratio measurement and capable of accuracy of 0.01% of abundance at mass 45.

22.C03 *Preparation of Sample*

Orange juice samples may be freeze-dried to thick syrup for stabilization. Place 300–400 mg sample, weighed to nearest 0.1 mg, in ceramic boat, position boat in tube, and evacuate system. Admit to 600 mm Hg, tank O purified over CuO at 700°, followed by liq. N trap. Heat sample to ≥850° in manifold in tubular furnace, condensing CO_2 in liq. N trap. Recirculate gases over CuO 10–30 min at 850°. Isolate collection trap

and purification system from combustion system and Toepler pump by valves, and pump off O. Cool purification trap with solid CO_2-acetone; cool sample tube with liq. N. Let collection trap warm, condensing impurities in solid CO_2 trap and CO_2 in sample tube.

22.C04 ***Determination***

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

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

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

$$\delta_{(X-PDB)} = \delta_{(X-B)} + \delta_{(B-PDB)} + 10^{-3}\,\delta_{(X-B)}\delta_{(B-PDB)}$$

where $(X - B)$ and $(X - PDB)$ refer to analyses of sample (X) relative to std (B) and relative to PDB, and $(B - PDB)$ is analysis of std (B) relative to PDB, all δ values in parts per thousand.

Sample with $\delta^{13}C$ value less negative than −22.1‰ relative to PDB is considered adulterated.

23. GELATIN, DESSERT PREPARATIONS, AND MIXES

No additions, deletions, or changes.

24. MEAT AND MEAT PRODUCTS

The following interim official first action mineral oil distillation-thermal energy analyzer method for the determination of nitrosamines in fried bacon was adopted official first action:

Volatile *N*-Nitrosamines in Fried Bacon

Mineral Oil Vacuum Distillation-Thermal Energy Analyzer Method

Official First Action

Caution: Extreme care should be exercised in handling nitrosamines or solns of nitrosamines. These compds are reported to be potent carcinogens.

24.C01 ***Apparatus***

Thoroly clean all glassware and rinse with CH_2Cl_2 before use.

(**a**) *Boiling flask.*—500 mL with thermometer well and 24/40 ⧩ neck.

(**b**) *Thermometer.*—75 mm immersion, −20 to 150°, 1° subdivisions (Fisher Scientific Co. No. 14-585-5C, or equiv.).

(c) *Vapor traps.*—A. H. Thomas No. 9466-275, or equiv., fitted with "O" ring socket joints size 18/9 (Kontes Glass Co. No. K-671500, or equiv.).

(d) *Adapter.*—Kontes Glass Co. No. K-183000 with ⧩ 24/40 inner joint at bottom fitted with 18/9 "O" ring ⧩ ground ball joint (Kontes Glass Co. No. K-671300, or equiv.).

(**e**) *Vacuum pump.*—25 L/min free air displacement, Welch Duo Seal 1400 B (Fisher Scientific Co. No. 1-096), or equiv.

(f) *Vacuum tubing.*—$^5/_{16}$ in. bore (Fisher Scientific Co. No. 74-175D).

(g) *Vacuum controller.*—Cartesian-type (Cole Parmer Instrument Co. No. C-909-00), or equiv.

(h) *McLeod gage.*—Shielded (Cole Parmer Instrument Co. No. C-903-00), or equiv.

(**i**) *Heating mantle.*—500 mL (Kontes Glass Co. No. K-72600, or equiv.).

(**j**) *Heating tape.*—Double element, type 12, $^1/_2$ in. wide × 2 ft long (Kontes Glass Co. No. K-729500, or equiv.).

(**k**) *Dewar flask.*—350 mm deep × 110 mm id (Kontes Glass Co. No. K-611785, or equiv.).

(**l**) *Laboratory jack.*—Lab Lift (Fisher Scientific Co. No. 14-673-10), or equiv.

(**m**) *Auto transformer.*—Power stat type 3PN117B (A. H. Thomas. No. 9461-F-15), or equiv.

(n) *Funnel.*—Buchner, 60 mL, coarse porosity fritted disk (Kontes Glass Co. No. K-955000, or equiv.).

(**o**) *Evaporative concentrator.*—Kuderna-Danish (K-D), 250 mL 24/40 ⧩ top joint, 19/22 ⧩ lower joint (Kontes Glass Co. No. K-850500, or equiv.).

(p) *Concentrator tube.*—Size 425, 19/22 ⧩ top joint, 4 mL (Kontes Glass Co. Cat. No. K-570050) with 19/22 ⧩ stopper (K-850500).

(q) *Distillation column.*—Snyder, with 24/40 ⧩ joints, 3 sections, size 121 (Kontes Glass Co. No. K-503000, or equiv.).

(**r**) *Concentration equipment.*—N-Evap with H_2O bath No. A-11151, Teflon-covered needles No. 10603, and thermometer No. 1111 (Organomation Associates), or equiv.

(s) *Gas chromatograph.*—Shimadzu GC 4C PR 6, with automatic cooling, temperature programmer, injection port temp. programmer, automatic door opener, glass-lined heated transfer line from gas chromatograph to TEA, dual column system with differential flow controllers, pressure gauges, and rotometers; recorder R 11M, single pen, 1 mV full scale, or equiv. (Shimadzu Scientific Instruments, Inc., Oakland Ridge Industrial Center, Columbia, MD 21045).

Glass column, 2.7 m × 3.0 mm id (5.0 mm od) packed with 100–120 mesh Analab AB (Analabs, Inc.) coated with 10% Carbowax 20M and 5% KOH. To prep. packing, dissolve 5.0 g Carbowax 20M and 2.5 g KOH in 100 mL MeOH contained in 500 mL r-b flask, ⅁ 24/40 neck. Slowly add 42.5 g Anakrom AB, 100–120 mesh, while gently swirling flask. Place flask on rotary evaporator ca 5 min. Apply vac. slowly at first and then increase to ca 26 in. Hg as foaming or bubbling subsides.

Allow support to rotate until all MeOH is drawn off as indicated by tumbling of support. Release vac. slowly and remove flask from system. Place flask contg support in 100° gravity convection oven to remove traces of MeOH. Transfer coated support to jar with screw-cap lid until used. Pack column by incremental addn under light vac. while gently tapping with rod. When filled, insert glass wool plugs in ends. Condition packed column, unconnected from heated transfer line, 24 h at 225° with 40 mL N carrier gas/min. Conditions: injector 185°, column 165° isothermal, interface line from gas chromatograph to TEA 230°, N carrier gas 40 mL/min.

Operating parameters: Recorder response should be >10% for 1.5 ng *N*-nitrosopyrrolidine. Resolution (R) between *N*-nitrosopiperidine and *N*-nitrosopyrrolidine should not be <0.8 when calcd as follows:

$$R = (T_2 - T_1)/{}^1\!/_2\,(W_1 + W_2)$$

where T_1 and T_2 = retention times (mm) of *N*-nitrosopiperidine and *N*-nitrosopyrrolidine; W_1 and W_2 = peak width at base (mm) of *N*-nitrosopiperidine and *N*-nitrosopyrrolidine.

(t) *Thermal energy analyzer (TEA).*—Model 502 (Thermo Electron Corp., 85 First Ave, Waltham, MA 02154). Operate according to manufacturer's instructions except use liq. N as trap coolant.

24.C02 *Reagents*

(a) *Paraffin oil.*—Heavy, Saybolt viscosity 335/350, laboratory grade (Fisher Scientific Co. No. 0-120, or equiv.).

(b) *Dichloromethane.*—Distd in glass (Burdick & Jackson Laboratories, Inc., or equiv.). Conc. 100 mL of each lot to 1.0 mL and check for interfering peaks on GLC/TEA system.

(c) *Sodium sulfate.*—Anhyd. (Mallinckrodt Chemical Works, or equiv.).

(d) *Carborundum.*—No. 12 granules (A. H. Thomas Co. No. 1590-D30, or equiv.).

(e) *Mixed N-nitrosamine reference std.*—Stock std soln of 5 μg each/mL isooctane: *N*-nitrosodimethylamine (NDMA), *N*-nitrosodiethylamine (NDEA), *N*-nitrosodipropylamine (NDPA), *N*-nitrosodibutylamine (NDBA), *N*-nitrosopiperidine (NPIP), *N*-nitrosopyrrolidine (NPYR), and *N*-nitrosomorpholine (NMOR) (Chemical Repository, Illinois Institute of Technology Research Institute (IITRI), 10 W 35th St, Chicago, IL 60616).

(f) *Working mixed std soln.*—0.25 μg each nitrosamine/mL. Dil. stock soln 1:20 with CH_2Cl_2.

(g) *N-Nitrosodipropylamine (NDPA) internal std solns.*—*Stock soln:* 5 μg/mL isooctane (IITRI). *Working std soln:* 0.5 μg/mL. Dil. stock soln 1:10 with CH_2Cl_2. Use as internal std soln.

24.C03 *Sample Preparation*

Store fried bacon in −18° freezer overnite or in dry ice. Grind frozen sample thru ⅜ in. plate, mix thoroly, add sample thru grinder second time, and repeat mixing. Store sample in −18° freezer until analysis.

24.C04 *Distillation*

Assemble app. as in Figure **24:C1.**

Weigh 25.0 g sample in 500 mL r-b flask with thermometer well, add 2.0 mL 0.2N NaOH, 0.500 mL NDPA internal std soln, and 25.0 mL mineral oil. Place flask in heating mantle. Use adapter to connect boiling flask to prewet trap (2 mL H_2O) equilibrated in liq. N. Connect to vac. and check for leaks as system comes to operating pressure (<2 torr). Maintain vac. 10 min before applying heat. Wrap adapter with heating tape. Increase temp. in boiling flask, as indicated by thermometer in oil-filled well, from ambient to 120° in 55–60 min. Internal temp. of adapter is 170–175°. At end of heating period, lower heating mantle away from flask. Let flask cool while adapter remains heated 15 min (170–175°) with vac. maintained.

Carefully release vac. and lower Dewar flask away from trap. Disconnect flask. Invert

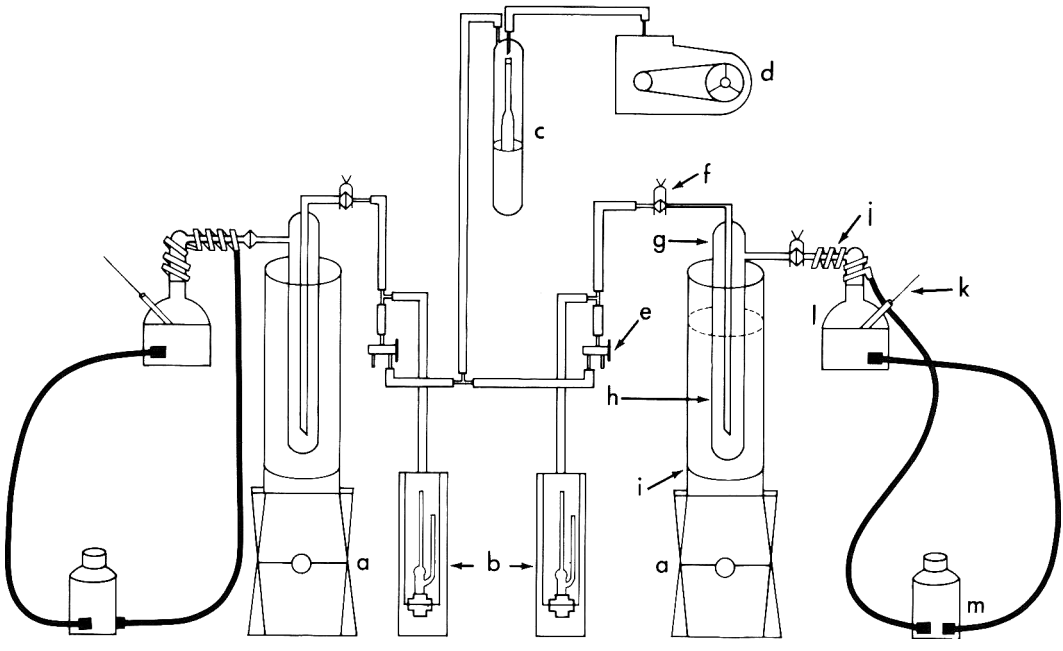

Figure 24:C1. Pumping and distillation assembly. (Dual system. Single distillation setup may be used.) a, laboratory jack; b, McLeod gage; c, vacuum controller; d, vacuum pump; e, 3-way stopcock; f, pinch clamp; g, vapor trap; h, liquid nitrogen; i, Dewar flask; j, heating tape; k, thermometer (−20° to 150 °); l, 500 mL boiling flask with thermometer well; m, power stat.

adapter. Place trap with adapter attached in hood until contents are liq.

24.C05 *Transfer, Extraction, and Drying*

Rinse adapter with 10.0 mL CH_2Cl_2, collecting rinsing in trap. Remove adapter. Transfer distillate and CH_2Cl_2 washing to 125 mL separator. Wash trap by adding 15 mL CH_2Cl_2, 5 mL through stem and 10 mL to body. Rinse by shaking 1 min. Transfer CH_2Cl_2 rinsing to separator and ext by shaking 1 min. Let stand until phases sep.

Drain lower CH_2Cl_2 layer into second separator. Repeat trap washing and extn twice. Drain pooled CH_2Cl_2 thru 30 g anhyd. Na_2SO_4 (held in 60 mL coarse fritted glass funnel prewetted with 25 mL CH_2Cl_2) into 250 mL K-D flask with 4 mL concentrator tube attached. Rinse second separator with 25 mL CH_2Cl_2 and drain rinse into K-D flask.

24.C06 *Concentration*

Place one carborundum grain in concentrator tube, attach 3-ball Snyder column to K-D flask, and carefully conc. solv. to ca 4 mL on 60° H_2O bath in ca 1.5 h. Remove concentrator, wipe dry, and air-cool to ambient temp. Let remaining solv. in Snyder column drain into concentrator tube.

Transfer concentrator tube to N-Evap system. Reduce vol. to 1.0 mL in ca 30 min under gentle stream of N.

24.C07 *Determination*

Inject 6.0 μL working mixed std soln contg 0.25 μg *N*-nitrosoamines/mL, into GLC app. coupled to TEA. Obtain retention time and response in ng/mm for each compound (see Figure **24:C2**).

Inject 6.0 μL concd sample ext. Obtain retention time and response of each compd present. (*Note:* Single std solns must be used to establish relative retention patterns.) If recovery of NDPA internal std soln is <70 or >110%, repeat analysis of sample.

Calc. amt of each nitrosamine in sample ext and det. amt in original sample, as follows:

For each nitrosamine, ng/g sample = $(PH/PH') \times 10$, where PH and PH' = peak ht in sample and std, resp.

25. METALS AND OTHER ELEMENTS

(1) The official first action rapid screening method for the detection of acid-extractable

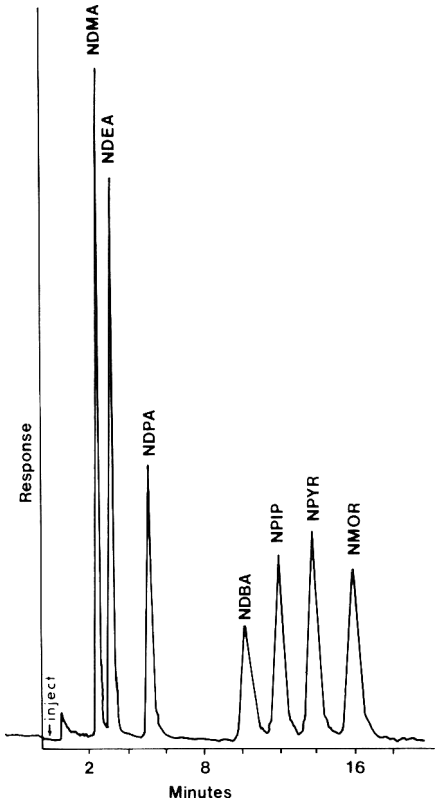

Figure 24:C2. Liquid chromatogram of *N*-nitrosamines mixed standard solution: 1.50 ng NDMA; 1.58 ng NDEA; 1.46 ng NDPA; 1.59 ng NDBA; 1.64 ng NPIP; 1.67 ng NPYR; 1.80 ng NMOR.

cadmium and lead in earthenware, **25.035-25.037**, was declared surplus.

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(2) The following dry ash anodic stripping voltammetric method for the determination of lead and cadmium in foods other than fats and oils was adopted official first action:

Lead and Cadmium – Anodic Stripping Voltammetry

Official First Action

25.C01 ***Principle***

Sample is dry-ashed with K_2SO_4 and HNO_3 at ca 500°. Pb and Cd are detd by anodic stripping voltammetry (ASV). Estd quantitation limits, based on 10 g sample, are 0.005 ppm Cd and 0.010 ppm Pb.

25.C02 ***Apparatus***

(Thoroly soak all glassware and plasticware in 20% (v/v) HNO_3 for ≥24 h and rinse with distd, deionized H_2O.)

(**a**) *Voltammetric analyzer.*—Capable of ASV and equipped with necessary accessories, i.e., cells, electrodes, recorders, Hg capillaries, micrometer or similar device for adjusting drop size, stirring motor, etc. (EG&G Princeton Applied Research Corp., Princeton, NJ 08540, Models 174A, 315A, and 303, or equiv., for differential pulse anodic stripping voltammetry (DPASV) at hanging Hg drop electrode; Environmental Sciences Associates, Bedford, MA 01730, Model 2014, or equiv., for linear sweep anodic stripping voltammetry (LSASV).

(**b**) *Ashing vessels.*—150-250 mL quartz, Vycor, or Pyrex beakers equipped with suitable glass covers (Fisher Scientific Co., No. 2-609A, or equiv.). Quartz is preferred. Vycor or Pyrex may be used if quartz beakers are not available. Note: For best results, quartz beakers should be fire-polished to retard etching.

(**c**) *Drying oven.*—Controllable within range 50–150° with <5° variation.

(**d**) *Furnace.*—Controllable within range of 100–1000° with <5° variation. Check calibration of oven temp. control to ensure accurate temps. Furnace must be operated in suitable fume hood.

(**e**) *Controllable hot plate.*—Corning Glass Works, Corning, NY, PC-35, or equiv.

(**f**) *Micropipets.*—10 thru 100 μL (Eppendorf, or equiv.).

25.C03 ***Reagents***

Note: Use only distd, deionized H_2O.

(**a**) *Nitric acid.*—J.T. Baker Chemical Co. No. 9598, or equiv.

(**b**) *Potassium sulfate ashing soln.*—10 g/100 mL. Dissolve 50.0 g K_2SO_4 (J.T. Baker Chemical Co. No. 3278, or equiv.) in 400 mL H_2O contg 10 mL HNO_3. Dil. to 500 mL with H_2O.

(**c**) *Nitrogen.*—Prepurified, H_2O-pumped.

(**d**) *Electrolyte soln.*—1.7M in HOAc, 1.25M in Na acetate trihydrate, and 0.01M in tartaric acid. Dissolve 170.0 g $NaOAc{\cdot}3H_2O$ (ACS) in 300 mL H_2O. Add 97 mL glacial HOAc and 1.5 g tartaric acid (ACS). Dil. to 1 L with H_2O. pH should be 4.7 ± 0.1.

(**e**) *Cadmium std soln.*—1.0 mg/mL. Dissolve 1.000 g Cd (99.99%) in 10 mL HNO_3 in 1 L vol. flask. Dil. to vol. with H_2O.

(**f**) *Lead (Pb) std soln.*—1.0 mg/mL. Dissolve 1.000 g Pb (99.99%) in 10 mL HNO_3 in 1 L vol. flask. Dil. to vol. with H_2O.

(**g**) *Working std solns.*—Prep. either sep. or mixed working std soln for Cd and Pb in the

range 0.1–10 μg/mL from std solns (e) and (**f**) by dissolving appropriate aliquots in 1% (v/v) HNO_3.

Note: Electrolyte soln (**d**) and K_2SO_4 soln (**b**) may require further cleanup for sufficiently low reagent blanks. For stated quantitation limits, analyte concns in final cell solns (electrolyte and sample solns) of reagent blank should not be >0.5 ng Cd/mL and >1 ng Pb/mL. Controlled potential elecrolysis is recommended means of cleaning reagents.

25.C04 *Preparation of Sample*

Note: Laboratory contamination control is important. Take all precautions possible to avoid contamination of samples, reagents, and equipment. Prep. at least 3 control reagent blanks which include any addnl H_2O and HNO_3 used for sample ashing. Carry control reagent blanks thru entire method.

Weigh 5.0–10.0 g homogenized sample into ashing vessel (**b**). Use 5.0 g for dry materials such as cereals. Add 5.0 mL K_2SO_4 ashing soln (**b**) and mix thoroly, using glass stirring rod. If needed, add H_2O to ensure sample and ash aid are well mixed. Cover ashing vessel with glass cover and dry in 110–120° oven (c) until thoroly dry (usually 2–3 h or, if desired, overnight). Place vessel in cold furnace (**d**) and set temperature at 500–550°. Caution: Do not heat >500° if using Pyrex beakers, and avoid excessive overshooting of temp. Maintain set temp. ≥4 h (may be ashed overnight). Remove vessel from furnace, and cool. Ash should be white and essentially carbon-free. Brownish-red color in the ash (possible Fe_2O_3) is acceptable and does not require the following HNO_3 treatment.

If ash contains C particles (i.e., ash is grey or black instead of white), wash down sides of vessel with H_2O and add 2.0 mL HNO_3. Use glass stirring rod to break up solid particles. Dry thoroly on hot plate (e) at low setting. If samples such as sugars and cereals splatter on hot plate during HNO_3 treatment, dry under IR lamp instead. Increase hot plate setting to medium for several minutes to ensure dryness. Return vessel to 500° furnace 30 min. Cool; if necessary, repeat HNO_3 treatment using 1 mL increments of HNO_3, until white, C-free ash is obtained.

Add 1.0 mL HNO_3 and ca 10 mL H_2O to vessel and, if necessary, heat on hot plate at low heat until sample ash is dissolved. Small amt of white, siliceous-like ppt may remain undissolved. Cool, and quant. transfer sample to 50 mL vol. flask with aid of H_2O. Dil. to vol. with H_2O and mix well. Let stand to allow any ppt present to settle. Do not filter. Use clear supernate to det. analytes by either DPASV or LSASV below.

25.C05 *Differential Pulse Anodic Stripping Voltammetry*

Transfer 5.0 mL aliquot of sample soln to electrolysis cell containing Teflon-coated stirring bar and add 5.0 mL electrolyte soln (**d**) to cell. (Aliquot vol. may be varied as long as 1:1 ratio is maintained between sample soln and electrolyte.) pH of cell soln should be 4.3 ± 0.3. Room temp. should be constant (±1°/2 h) and between 20 and 30°. Purge soln 5 min with N (c). Adjust gas inlet to let N flow gently above and across soln surface. If hanging Hg drop electrode is used, add fresh drop of Hg to capillary tip with micrometer or similar device to ensure reproducibility of drop. Turn on stirrer motor and electrolyze soln at −0.8 V vs satd calomel electrode (SCE) or Ag/AgCl electrode. Deposition time may vary with instrument (see manufacturer's instructions). When using PAR 174 polarographic analyzer, 1–2 min is sufficient, depending on level of analytes of interest in cell soln. Stop stirring and let soln equilibrate 30 s. Linearly increase applied voltage anodically. Follow manufacturer's instructions for rate of scan, e.g., 2–6 mV/s. Measure wave ht at peak potentials for Cd at −0.62 ± 0.05 V and for Pb at −0.45 ± 0.05 V vs SCE or Ag/AgCl. For widely varying concns of Cd and Pb, change current sensitivity to appropriate range by momentarily stopping stripping scan at end of Cd peak, switching to appropriate sensitivity setting for Pb, and then continuing scan before Pb peak begins.

Quantitate total amts of Pb and Cd in cell soln by using method of std addns in cell as follows: Record voltammogram from known vol. of cell soln. From working std soln (g), add known amts of Pb and Cd, using appropriate micropipets (**f**) and being certain to add amt of each element sufficient to generate peak hts ca twice those given by sample cell soln. Repeat with 2 more similar addns of working std soln to cell soln. For each analyte, plot μg added on x-axis vs peak ht in μA current on y-axis. Extrapolate linear plot to x-axis intercept to det. total amt of analyte in sample aliquot. If available, use computer program based on method of least squares to calc. regression line and det. amt of analyte in sample aliquot. Similarly, det. amt of each analyte in reagent blank aliquots, using same vol. of aliquots for reagent blank as for sample.

Calc. ppm analyte in sample as follows:

$$\text{ppm } (\mu g/g) = [(B - C)/A] \times (50/W)$$

where A = mL sample soln taken for analysis; B = μg analyte in sample soln aliquot; C = av. μg analyte in reagent blank soln aliquots; and W = total g sample.

25.C06 *Determination by Linear Sweep Anodic Stripping Voltammetry*

Transfer 2.0 mL aliquot of sample soln to electrolysis cell and add 3.0 mL electrolyte (**d**). pH of cell soln should be 4.3 ± 0.3. Deposit elements of interest onto composite Hg graphite electrode (CMGE) at −0.9 V vs Ag/AgCl ref. electrode for 30 min. Bubble N through cell soln during entire deposition period. Linearly increase applied voltage anodically at 60 mV/s from −0.9 to −0.2 V vs Ag/AgCl ref. electrode. Measure peak current (μA) for each analyte.

Run reagent blank in same manner using same size aliquot as for sample and det. peak current (μA) for each analyte. For each analyte, make std addn to cell soln and measure peak current (μA). Calc. conversion factor, $\mu g/\mu A$, for each analyte as μg of addn divided by diff. between peak current before and after addn of analyte std. Verify conversion factors periodically. Multiply sample peak current (μA) by conversion factor to det. μg of each analyte in sample soln aliquot. Calc. ppm, using equation in **25.C05.**

25.C07 *Interference*

Tl may interfere with Pb detn, but its occurrence in food is unlikely. If Tl interference is suspected, treat as follows: Transfer 5.0 mL aliquot of sample soln to electrolysis cell and make basic with 3.0 mL NaOH. Det. elements of interest in this soln by ASV in the usual manner. Plating potential is −1.0 V vs SCE or similar ref. electrode. Strip deposited elements by anodically scanning from −1.0 to −0.3 V vs SCE. In this manner, Cd and Pb peaks shift to −0.78 ± 0.05 V and −0.73 ± 0.05 V vs SCE, resp. Tl peak remains at −0.47 V vs SCE.

26. NATURAL POISONS

(1) The official first action method for the determination of patulin in apple juice, **26.111-26.116,** was adopted official final action.

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(2) The official first action method for the confirmation of aflatoxin M_1 identity in dairy products by derivative formation on a TLC plate, **26.A15,** was extended to include confirmation of M_1 in liver.

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(3) The following method for the determination of aflatoxins B_1 and M_1 in liver was adopted official first action:

Aflatoxins B_1 and M_1 in Liver
Official First Action

26.C01 *Apparatus*

(**a**) *Wrist-action shaker.*—Burrell, or equiv.

(**b**) *Meat grinder.*—Waring blender, Model EP-1, and any manual food grinder.

(c) *Chromatographic columns.*—Glass column 30 × 1.0 (id) cm with porous polyethylene frit (35 μm) and Luer nylon stopcock (Bio-Rad Econo-columns No. 737-2260 and 732-9009, resp., or equiv. glass column).

(**d**) *Filter paper.*—32 cm, S & S No. 588, or equiv. rapid flow, high wet-strength paper; and 24 cm, S & S No. 560, or Whatman 2V or equiv., medium flow paper.

(**e**) *Thin layer plates.*—10 × 10 cm com. prepoured, 0.25 mm thickness, glass plates (hand-cut from 20 × 20 cm) (E. Merck silica gel 60, No. 5763 or Macherey, Nagel Sil G-25 HR), or prep. in laboratory as follows: 10 × 10 or 20 × 20 cm plates coated with 0.25–0.5 mm (wet thickness) layer of Macherey-Nagel GHR silica gel for TLC (Macherey, Nagel & Co., D-5160 Duren, GFR; distributed by Brinkmann Instruments, Inc.) dried 1 h at 105° or Adsorbosil-1 silica gel for TLC (Applied Science Laboratories, Inc.), or equiv.

26.C02 *Reagents*

(**a**) *Solvents.*—Reagent grade, distd in glass. Glacial HOAc, acetone, CH_3CN, benzene, $CHCl_3$ (0.75% EtOH), CH_2Cl_2, ether (≤0.1% EtOH, peroxide-free), hexane (68–69°), isopropanol, and toluene.

(**b**) *Citric acid soln.*—20%. Dissolve 200 g citric acid monohydrate in 1 L H_2O.

(c) *Silica gel for column chromatography.*—E. Merck silica gel 60 (No. 7734), 0.063–0.200 mm (70–230 mesh), or equiv. Stir 1 h in MeOH, filter, and treat similarly with $CHCl_3$. Activate by drying 1 h at 105°. Add H_2O, 1 mL/100 g, seal, shake until thoroly mixed, and store ≥15 h in air-tight container.

(**d**) *Sodium sulfate.*—Anhyd., granular.

(**e**) *Diatomaceous earth.*—Hyflo Super-Gel.

(f) *Aflatoxin reference stds.*—Prep. as in **26.004-26.011** to contain 0.25 μg aflatoxin B_1 and M_1/mL in benzene-CH_3CN (9 + 1) for either visual or densitometric analysis. If aflatoxins G_1, B_2, and/or G_2 are needed, prep. G_1 at 0.25 μg/mL and B_2 and G_2 at 0.05 μg/mL. Store stds in 1 dram vials fitted with Teflon-lined screw caps at 0°F when not in use.

26.C03 ***Extraction***

Blend or grind meat tissue until homogeneous. Weigh 100 g mixt. into 500 mL wide-mouth, g-s erlenmeyer (or equiv.). Add 10 mL citric acid soln and mix thoroly with 30 cm × 1 cm glass stirring rod. After 5 min, stir again, and mix with 20 g diat. earth. Add 200 mL CH_2Cl_2 and stir to remove excess solids from rod. Shake flask vigorously on wrist-action shaker (setting 5 on a Burrell) for 30 min. Filter mixt. thru fast flow paper into 300 mL erlenmeyer contg 10 g Na_2SO_4. Close filter top and compress entire filter against funnel to obtain max. filtrate vol. Gently swirl flask intermittently ca 2 min and refilter contents thru medium flow paper into 250 mL graduate and record vol. (cover funnel with watch glass to prevent evapn of solv). Evap. filtrate in 500 mL r-b flask, under vac., to near dryness and save for column chromatgy.

26.C04 ***Column Chromatography***

Fill column half full with CH_2Cl_2 and add 2.0 g silica gel. Add 3–4 mL CH_2Cl_2 and slurry silica with stainless steel rod (ca 0.32 cm diam.). Drain CH_2Cl_2 to settle silica and rinse silica off column sides with CH_2Cl_2. Add 2 g Na_2SO_4 to supernate solv. above silica gel to cap column and drain excess CH_2Cl_2 to ca 1 cm above column packing.

Redissolve concd filtrate in ca 25 mL CH_2Cl_2, add to column, rinse r-b flask and column with addnl CH_2Cl_2, and drain entire soln thru column by gravity. If flow rate slows, stir Na_2SO_4 gently. When filtrate reaches Na_2SO_4, rinse column sides with CH_2Cl_2 and drain similarly. Wash column with 25 mL toluene-HOAc (9 + 1), 25 mL hexane, and 25 mL hexane-ether-CH_3CN (6 + 3 + 1) and discard washes. Elute aflatoxins with 40 mL CH_2Cl_2-acetone (4 + 1) and evap. eluate to near dryness in vac. or on steam bath. Quant. transfer ext with $CHCl_3$ or CH_2Cl_2 rinses to 1-dram vial with Teflon-lined screw cap. Evap. to dryness under N on heat source, but avoid overheating of dry ext. Save for TLC.

26.C05 ***Visual and Densitometric Analysis***

Add 100 μL benzene-CH_3CN (9 + 1) to sample residue in vial from **26.C04**, cap vial, and mix vigorously ca 1 min, preferably on vortex mixer. After TLC analysis, reserve remaining ext in freezer for confirmation of identity.

See Figure **26:01** for spotting and scoring patterns of 2-dimensional TLC plates, except dimensions for 20 × 20 cm plate, direction 1, bottom to top are as follows: 2, 11, 1, 1, 1, 4 cm and dimensions for direction 2, left to right are 2, 12, 6 cm; similarly for 10 × 10 cm plate: direction 1: 1.5, 4.5, 1, 1, 1, 1 cm, and direction 2: 1.5, 6.5, 2 cm. Spot 20 μL aliquot of sample ext on sample spot and either 1.5, 0.5, 1.0, and 1.5 ng of ref. std (visual) or 2.5, 1.25, 1.25, and 2.5 ng of ref. (densitometric). Develop plate in ether-MeOH-H_2O (95 + 4 + 1) in first direction (see also **26.013**). When solv. reaches score line, remove plate, air-dry, heat in forced air oven at 50° (ca 2 min), cool plate, and redevelop in second direction in $CHCl_3$-acetone-isopropanol (87 + 10 + 3) to score line. Quantitate visually or densitometrically as in **26.074** and calc. concn of B_1 or M_1 as:

$$\mu g/kg = (S \times Y \times V)/(X \times W)$$

where S = μL aflatoxin ref. std equal to unknown; Y = concn of ref. std, μg/mL; V = μL of final diln of sample ext; X = μL sample ext spotted giving fluorescent intensity equal to S (ref. std); W = (100 g or mL × filtrate vol.)/200.

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(4) The following method for the confirmation of aflatoxins B_1 and M_1 in liver was adopted official first action:

Confirmation of Aflatoxins B_1 and M_1 in Liver

Official First Action

26.C06 ***Reagents***

(a) *Solvents.*—$CHCl_3$ (≤0.75% EtOH), acetone, isopropanol, and hexane.

(b) *TFA-hexane spray.*—(1 + 4). Mix 1 vol. of trifluoroacetic acid (TFA, ≥95% pure) with 4 vols of hexane. Prep. fresh daily.

(c) *Aflatoxin std solns.*—Prep. sep. std solns of aflatoxins B_1 and M_1 (0.25 μg/mL each) in CH_3CN-benzene (1 + 9) or $CHCl_3$.

(d) *TLC plates.*—0.25 mm thick layer of Macherey-Nagel Sil-G-25HR silica gel (Macherey, Nagel & Co., D-5160, Duren, GFR, distributed by Brinkmann Instruments, Inc.) or Merck Kieselgel 60 on 10 × 10 cm plates, self-cut from 20 × 20 cm plates.

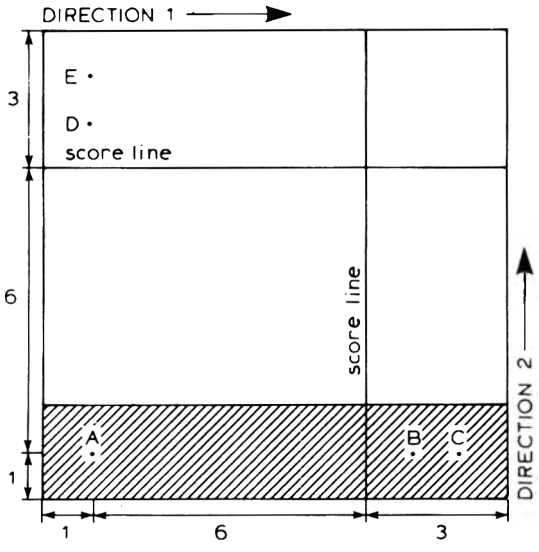

Figure 26:C1. Schematic representation of thin layer chromatogram for confirmation of identity of aflatoxins B_1 and M_1. A = spotting place for sample extract; B and D = spotting place for M_1 standard; C and E = spotting place for B_1 standard.

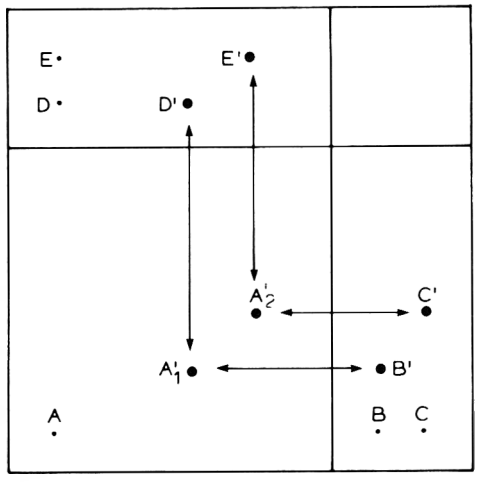

Figure 26:C2. Schematic representation of thin layer chromatogram after confirmation chromatography. A'_1 = M_1 derivative from sample; A'_2 = B_1 derivative from sample; B' = M_1 derivative from standard; C' = B_1 derivative from standard; D' = M_1 from standard; E' = B_1 from standard.

26.C07 ***Apparatus***

(a) *UV illumination cabinet.*—365 nm.

(b) *Disposable capillary pipets.*—10 and 20 μL, or microsyringes.

(c) *Spray unit for thin layer chromatography.*—Low vol. capacity (5-20 mL).

(d) *Air dryer.*—Unit capable of providing stream of warm air (40-50°) to evap. solv. from TLC plates.

26.C08 ***Thin Layer Chromatography***

Score 2 straight lines on 10 × 10 cm TLC plate at right angles (3 cm in from each edge) (see Figure **26:C1**) to limit migration of developing solv. fronts. Spot following solns on plate, using capillary pipets or microsyringes:

(a) Vol. of sample ext equal to vol. used for quantitation on point A (normally ca 20 μL).

(b) Vol. of std soln contg ca 2.5 ng M_1 on points B and D.

(c) Vol. of std soln contg ca 2.5 ng B_1 on points C and E.

Develop plate in first direction with isopropanol-acetone-$CHCl_3$ ((3 + 10 + 87) for Macherey-Nagel TLC plates or (8 + 10 + 82) for Merck TLC plates) (see Figure **26:C1**), until solv. front reaches solv. limit line. Dry plate after development 5-10 min with stream of warm air to evap. solv. completely (check odor). Spray TFA-hexane soln from a distance of 5-10 cm along band (ca 2 cm wide), covering points A, B, and C (indicated by hatched area in Figure **26:C1**) until plate is thoroly sprayed (ca 2 mL spraying reagent). After hexane has evapd, cover TLC plate with warm, clean, glass plate (75°) and immediately heat 6-8 min in 75° oven with TLC plate on oven floor. Cool 1 min on cold surface, evap. excess TFA with stream of air, and develop in second direction with isopropanol-acetone-$CHCl_3$ ((6 + 10 + 84) for Macherey-Nagel TLC plates or (12 + 10 + 78) for Merck TLC plates).

Examine plate under longwave UV light (365 nm) and check for following fluorescent zones (see Figure **26:C2**):

(a) Appearance of blue fluorescent spots D' and E' of std aflatoxins M_1 and B_1, resp., originating from D and E (migration in direction 1).

(b) Appearance of blue fluorescent spots B' and C' of TFA derivatives of aflatoxins M_1 and B_1, resp., originating from std solns spotted at B and C (migration in direction 2).

(c) Appearance of blue fluorescent spots A'_1 and/or A'_2 originating from ext spotted at A, with R_f values matching those of spots B' and C', resp.

Identity of aflatoxin B_1 in ext is confirmed when R_f values of B_1 derivative from Sample (A'_2) and B_1 std (C') match. Similarly, identity of aflatoxin M_1 in ext is confirmed when R_f values of M_1 derivative from Sample (A'_1) and M_1 std (B') match.

27. NUTS AND NUT PRODUCTS

No additions, deletions, or changes.

28. OILS AND FATS

(1) The following official first action methods were adopted official final action:

(a) Isolated trans isomers, **28.075-28.080.**

(b) 1-Monoglycerides, **28.139-28.147.**

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(2) The following interim official first action IUPAC-AOAC method for the determination of polar components in frying fats, *Fette Seifen Anstrichm.* **80,** 106 (1978); *J. Assoc. Off. Anal. Chem.* **64,** 1329 (1981), has been adopted official first action:

Polar Components in Frying Fats – Official First Action
IUPAC-AOAC Method

28.C01 ***Principle***

Method assesses deterioration of used frying fats, and is applicable to all fats and oils. Polar components are those components of fats detd by column chromatgy under conditions specified, and include polar substances such as monoglycerides, diglycerides, free fatty acids that occur in unused fats, as well as polar transformation products formed during frying of foodstuffs and/or during heating. Nonpolar components are mostly unaltered triglycerides. Frying fats are sepd by column chromatgy on silica gel into nonpolar and polar components. Polar components are detd indirectly by subtracting concn of nonpolar components. Quality of sepn can be checked by thin layer chromatgy.

28.C02 ***Apparatus***

(**a**) *Column.*—Glass, 2.1 cm id × 45 cm, with Teflon stopcock and ground-glass joint.

(**b**) *TLC plates.*—Pre-coated silica gel (without fluorescence indicator), 20 × 20 cm, layer thickness = 0.25 mm.

28.C03 ***Reagents***

(**a**) *Adsorbent.*—Silica gel 60, particle size 0.063-0.200 mm (70-230 mesh ASTM), Merck No. 7734, or equiv., adjust to H_2O content of 5% as follows: Dry silica gel ≥4 h in porcelain dish in 160° oven; cool in desiccator to room temp. Adjust H_2O content to 5%, e.g., weigh 152 g silica gel and 8 g H_2O in 500 mL r-b flask with ground-glass stopper and mech. shake 1 h.

(**b**) *Eluting solvent mixture.*—Petroleum ether (bp 40-60°)-ether (87 + 13).

(c) *Sea-sand.*—Anal. reagent grade; purified by acid and calcined.

(**d**) *Spray reagent.*—Molybdophosphoric acid, 10% in alcohol.

28.C04 ***Preparation of Sample***

Warm semi-liq. and solid samples to temp. slightly above mp and mix thoroly; avoid overheating. Remove visible impurities by filtration; if H_2O is present, use hydrophobic filter.

28.C05 ***Preparation of Column***

Fill column with ca 30 mL petroleum ether-ether (87 + 13). Place wad of cotton wool in bottom of column and remove air by pressing with glass rod.

In 100 mL glass beaker, prep. slurry of 25 g silica gel and ca 80 mL petroleum ether-ether (87 + 13) and pour slurry into column thru 8 cm glass funnel. Rinse beaker, funnel, and sides of column with same solv. Open stopcock and drain solv. to 10 cm above silica gel. Level silica gel by tapping column.

Add ca 4 g sea-sand thru funnel into column. Drain solv. to sand layer.

28.C06 ***Chromatography***

To det. polar components by diff., only nonpolar fraction is used. However, if sepn is controlled by TLC, both polar and nonpolar fractions are required. Sepn may also be controlled by checking recovery of sample, but for samples contg substantial amts of polar material, recovery may be incomplete because small amts of highly polar material, generally 1-2%, are not eluted under conditions specified.

Accurately weigh 2.5 ± 0.1 g (to 0.001 g) sample into 50 mL vol. flask, and dissolve in ca 20 mL petroleum ether-ether (87 + 13) while warming slightly. Let cool to room temp. and dil. to vol. with same solv. Using vol. pipet, transfer 20 mL sample aliquot to column, without disturbing surface.

Dry two 250 mL r-b flasks in 103 ± 2° oven, cool to room temp., and accurately weigh to 0.001 g. Place one flask under column, open stopcock, and let sample soln drain to level of sand layer. Elute nonpolar components with 150 mL petroleum ether-ether (87 + 13) contained in 250 mL dropping funnel. Adjust flow rate so that 150 mL passes thru column within 60-70 min. After elution, wash any substance adhering to outlet of column into r-b flask with petroleum ether-ether (87 + 13).

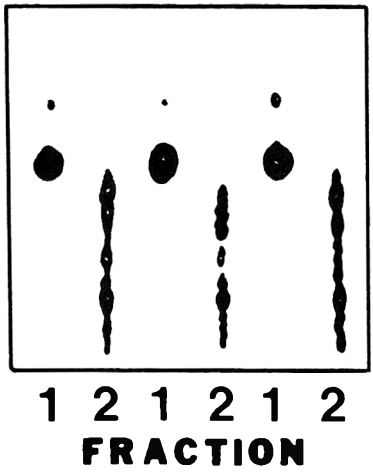

Figure 28:C1. Evaluation of efficiency of fractionation by TLC separation of polar and non-polar fraction; Fraction 1 contains non-polar components, Fraction 2 contains polar components.

In same manner, elute polar components into second 250 mL r-b flask with 150 mL ether. Discard silica gel.

Remove solv. from each fraction with a rotary evaporator and 60° H_2O bath or with N stream in 250 mL flask on steam bath. Avoid losses due to foaming. If rotary evaporator is used, shortly before end of evapn, introduce N into system from rubber bulb. Cool residue to ambient temp. and introduce N into flask. Weigh flasks.

28.C07 ***Calculations***

Calc. polar components, as % (w/w) by formula:

$$\text{Polar components, \%} = [(E - A)/E] \times 100$$

where A = g nonpolar fraction; E = g sample in 20 mL aliquot (ca 1 g). Report result to 1 decimal place.

28.C08 ***Check of Column Chromatography Efficiency by Thin Layer Chromatography***

Dil. polar and nonpolar fraction (1 + 9) in $CHCl_3$. Apply 2 μL spots using capillary dispensing pipet. Develop plate with petroleum-ether-HOAc (70 + 30 + 2) in tank lined with filter paper for ca 35 min (ca 17 cm). Remove plate and let solv. evap.

Spray plate with 10% molybdophosphoric acid. After evapn of alcohol, heat plate in 120-130° drying oven. Fraction 1 (nonpolar) should be free of polar substances (see Fig. **28.C1**).

29. PESTICIDE RESIDUES

The following official first action methods were adopted official final action:

(a) Polychlorinated biphenyls in poultry fat, fish, and dairy products, **29.001-29.018**

(b) Polychlorinated biphenyls in paper and paperboard, **29.035-29.038,** including both the total peak area or peak height method and the individual peak area method for quantitation of PCBs by electron capture gas-liquid chromatography, **29.018,** and Table **29:02**

(c) Hexachlorobenzene (HCB) and mirex in adipose tissue, **29.A01-29.A04**

30. SPICES AND OTHER CONDIMENTS

No additions, deletions, or changes.

31. SUGARS AND SUGAR PRODUCTS

No additions, deletions, or changes.

32. VEGETABLE PRODUCTS: PROCESSED

(1) The official first action method for the determination of pH of acidified foods, **32.B01-32.B08,** was adopted official final action.

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(2) The following official final action methods were declared surplus:

(a) Sodium chloride (Method I), **32.023**

(b) Sodium chloride (Method II, rapid method), **32.024**

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 Red No. 40, **34.B01-34.B06,** was adopted official final action.

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(2) The following liquid chromatographic method for the determination of intermediates and reaction by-products in FD&C Yellow No. 5 was adopted official first action:

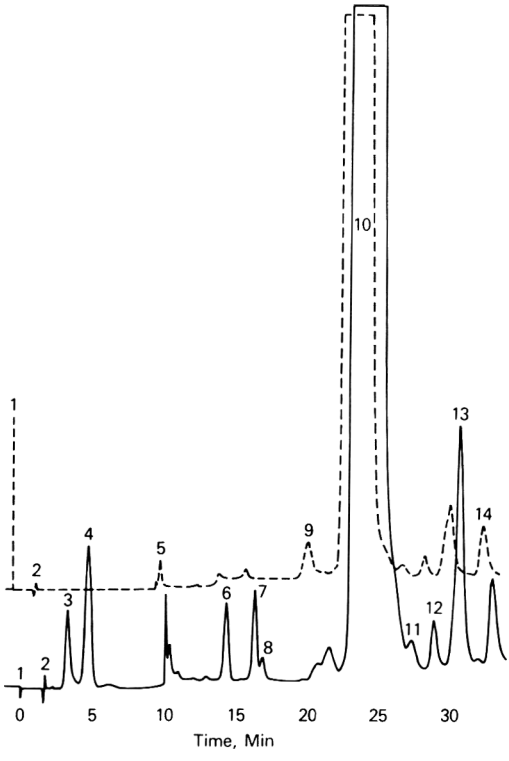

Figure 34:C1. Chromatogram of FD&C Yellow No. 5 (peak 10). 1 = injection; 2 = t_0; 3 = PHSA; 4 = SA; 5 = gradient front peak; 6 = EEpyT; 7 = PyT; 8 = unknown; 9 = DAADBSA; 11, 12, 13, and 14 = unknowns. Top tracing from 358 nm detector; bottom tracing from 254 nm detector; DuPont 850 chromatograph used for both tracings.

Intermediates and Reaction By-Products in FD&C Yellow No. 5

Liquid Chromatographic Method

Official First Action

34.C01 *Principle*

Aq. soln of dye is analyzed by ion exchange LC, using gradient elution.

34.C02 *Apparatus*

(a) *Liquid chromatograph.*—With gradient elution capability, e.g., DuPont 830 or DuPont 850 with 50 μL sampling loop. Operating conditions: chart speed 0.5 cm/min; eluant flow rate 1.0 mL/min; temperature ambient. Gradient profile for Model 830: initial composition—eluant 0% B, final composition—95% B; gradient rate 4%/min, function—nonlinear, slow start mode 2. Gradient profile for Model 850: segment 1, 0 to 7% B eluant in 6 min; segment 2, 7 to 26% B in 12 min; segment 3, 26 to 35% B in 4 min; segment 4, 35 to 95% B eluant in 8 min; segment 5, hold on 95% B eluant until end of chromatogram (see Figure **34:C1**); all segments are linear. Equilibration period (only A or primary eluant flowing) between gradient runs is detd by ability of column to sep. first 2 eluting peaks, phenylhydrazine-*p*-sulfonic acid (PHSA) and sulfanilic acid (SA). For Model 830, use 8 min initially; for Model 850, use 13 min initially. If resolution is not adequate (see Figure **34:C1**), increase length of equilibration time.

(b) *Detectors.*—(*1*) DuPont 254 nm detector (low pressure Hg source) with attenuation set at 0.04 AUFS for Model 830 and 0.05 AUFS for Model 850. (*2*) DuPont Model 835 multiwavelength detector (medium pressure Hg lamp source and 325–385 nm filter) with attenuation set at 0.08 AUFS or DuPont UV spectrophtr detector set at 358 nm and attenuation at 0.08 AUFS.

(c) *Column.*—DuPont Zipax SAX column 100 cm × 2.1 mm id (Cat. No. 882950-403). Condition new column by heating 5 h at 50° with 0.01M $Na_2B_4O_7$ flowing through column at 1000 psi. Check resolution of FD&C Yellow No. 5 contaminants, and continue 5 h segments of heating with 0.01M $Na_2B_4O_7$ flowing until good resolution, Figure **34:C1,** is obtained.

(d) *Spectrophotometer.*—Visible and UV range.

34.C03 *Reagents*

(a) *Eluants.*—(*1*) Primary or A eluant, 0.01M aq. $Na_2B_4O_7$. (*2*) Secondary or B eluant, 0.1M $NaClO_4$ in 0.01M aq. $Na_2B_4O_7$. Filter eluants thru 0.45 μm filter (Cat. No. HAWP 047 00, Millipore Corp., Bedford, MA 01730, or equiv.) before use.

(b) *Std solns.*—(*1*) PHSA, ca 0.05 mg/mL 0.02M HCl; (*2*) SA, ca 0.08 mg/mL H_2O; (*3*) 1-(4-sulfophenyl)-3-ethylcarboxy-5-hydroxypyrazolone (EEpyT), ca 0.08 mg/mL H_2O; (*4*) 1-(4-sulfophenyl)-3-carboxy-5-hydroxypyrazolone (PyT), ca 0.08 mg/mL H_2O; (*5*) 4,4′-(diazoamino)-dibenzenesulfonic acid (DAADBSA), ca 0.08 mg/mL 0.004M aq. NaOH. Det. exact concns of stds 1–4 from UV spectra of dild aliquots in H_2O; det. concn of std 5 from visible spectrum of dild aliquot in 0.02M aq. NaOH. PHSA std soln is reliable only ca 3 days. Approximate absorptivities (mg/mL, 1 cm) are (*1*) 72.8 at 250 nm, (*2*) 81.6 at 250 nm), (*3*) 57 at 257 nm, (*4*) 57.7 at 257 nm, (*5*) 86.5 at 410 nm.

Table 34:C1. Preparation of calibration solutions

Soln No.	Std soln added, mL				
	PHSA	SA	EEpyT	PyT	DAADBSA
1					
2	5				
3		5			
4			5		
5				5	
6					5
7	1	2	3	4	1
8	2	3	4	1	2
9	3	4	1	2	3
10	4	1	2	3	4

34.C04 ***Suitability Test***

Prep. test soln contg 150 mg FD&C Yellow No. 5/100 mL 0.01M $Na_2B_4O_7$ and, relative to FD&C Yellow No. 5, 0.1% each of PHSA, SA, EEpyT, PyT, and DAADBSA. Set parameters and run blank gradient (no injection) and test soln. Compare chromatogram of test soln to that of Figure **34:C1.** If similar resolution is not attained, adjust operating parameters to those needed to resolve these compds.

34.C05 ***Calibration***

For each calibrating soln, dissolve 0.150 g FD&C Yellow No. 5 (free of PHSA SA, EEpyT, PyT, and DAADBSA) in ca 50 mL H_2O. Add 10 mL 0.1M $Na_2B_4O_7$ first to 100 mL vol. flask, then add aliquots as indicated in Table **34:C1** to flask, add soln of FD&C Yellow No. 5, and dil to 100 mL with H_2O. Prep. calibration solns within 13 min before injection. Samples of FD&C Yellow No. 5 will change on standing. Calc. concns of compds being detd as wt % of FD&C Yellow No. 5. Calc. % concn in calibration soln as:

$$C = V \times C' \times 0.667$$

where V = vol. std soln taken; C' = concn std soln as detd spectrophtric in mg/mL; and 0.667 = 100(%)/150 mg. Measure peaks for PHSA, SA, EEpyT, and PyT, using 254 nm detector, and for DAADBSA, using 358 nm detector. Obtain areas from integrator or by multiplying peak ht by peak width at one-half ht. Plot % concn of each compd against peak areas. Use calibration solns 1–6 to det. retention times of compds.

34.C06 ***Determination***

Weigh 0.150 g sample, dissolve in ca 50 mL H_2O, add 10 mL 0.1M $Na_2B_4O_7$, and dil. to 100 mL with H_2O. Prep. soln within 13 min before injection. Chromatograph sample solns interspersed with calibrating solns, and compare chromatograms. Identify by retention times, and measure areas of peaks corresponding to 5 compds. Use calibration plots to det. % PHSA, SA, EEpyT, PyT, and DAADBSA.

35. COSMETICS

No additions, deletions, or changes.

36. DRUGS: GENERAL

The official first action method for the determination of chlorinated hydrocarbons, **36.013-36.017,** was declared surplus.

37. DRUGS: ACIDIC

No additions, deletions, or changes.

38. DRUGS: ALKALOID AND RELATED BASES

(1) The following official first action methods were adopted official final action:

(a) Phenothiazine, **38.185-38.186**

(b) Procainamide hydrochloride, spectrophotometric method, **38.224-38.227**

(c) Ephedrine in solid dosage forms, **38.A01-38.A05,** *J. Assoc. Off. Anal. Chem.* **63,** 692 (1980)

(d) Chlorpheniramine maleate tablets, semiautomated method, **38.A06-38.A11,** *J. Assoc. Off. Anal. Chem.* **62,** 552, 1197 (1979)

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(2) The official final action fluorometric method for the determination of quinacrine hydrochloride, **38.231-38.235,** was editorially revised so that full baseline scans are obtained. Change **38.234** to read:

38.234 ***Determination***

Adjust spectrophotofluorometer to ca 80% fluorescence intensity (F) at 500 nm with std

soln. Transfer ca 3 mL 0.1N HCl to clean 10 × 10 mm cell and record the blank scan between 350 and 650 nm. Repeat with std and sample solns. In each case, draw baseline from 350 to 650 nm. Det. %*F* at peak max. (ca 500 nm) of sample and std solns relative to 0.1N HCl blank.

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(3) The official first action spectrophotometric method for the determination of amphetamine, **38.122-38.126,** was deleted.

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(4) The following method for the liquid chromatographic determination of physostigmine salicylate and physostigmine sulfate in solutions, *J. Assoc. Off. Anal. Chem.* **65,** 132 (1982), was adopted official first action:

Physostigmine Salicylate and Physostigmine Sulfate in Solutions
Liquid Chromatographic Method
Official First Action

38.C01 *Principle*

Physostigmine salicylate or sulfate is dild with CH_3CN and detd by LC with UV (254 nm) detector and with flurazepam as internal std.

38.C02 *Apparatus*

(**a**) *Liquid chromatograph.*—Model 204 equipped with 2 Model 6000 pumps, Model 660 solv. programmer, 254 nm UV detector, Model U6K injector (Waters Associates, Inc.) and Model 3380A integrator (Hewlett-Packard). Equiv. LC system with strip chart recorder may be used.

(**b**) *LC column.*—μBondapak C_{18}, 3.9 mm id × 30 cm (Waters Associates, Inc.) or equiv. reverse phase column providing appropriate retention times and sepn for physostigmine and internal std.

38.C03 *Reagents*

(**a**) *Ammonium acetate.*—0.05M. Dissolve 3.85 g NH_4OAc in H_2O and dil. to 1 L. Filter thru 4.7 cm Whatman GF/F glass microfiber filter, or equiv., in Millipore-type filter holder. Adjust filtrate to pH 6.0 ± 0.1 with HOAc or NH_4OH.

(**b**) *Solvents.*—UV grade hexane and CH_3CN (Burdick & Jackson Laboratories, Inc., or equiv.); filtered thru same filter as in (**a**).

(**c**) *Mobile phase.*—CH_3CN-0.05M NH_4OAc (1 + 1) at flow rate of ca 2.0 mL/min. Mobile phase ratio and flow rate may be varied to give retention time of ca 3–4 min for physostigmine peak (first) and sepn of flurazepam internal std peak (second).

(**d**) *Internal std soln.*—Dissolve 50 mg flurazepam HCl in MeOH and dil. to 100 mL with MeOH.

(**e**) *Physostigmine std solns.*—3.0 mg/100 mL. Transfer 60 mg accurately weighed USP Physostigmine, Physostigmine Salicylate, or Physostigmine Sulfate to 100 mL vol. flask and dil. to vol. with CH_3CN. Transfer 5.0 mL aliquot to 100 mL vol. flask contg 5.0 mL internal std soln and dil. to vol. with CH_3CN. Use physostigmine and salicylate stds without drying. Dry sulfate std 2 h at 105°C.

38.C04 *Sample Preparation*

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.

38.C05 *System Suitability (Chromatographic System) Check*

(**a**) *Reproducibility.*—Let system equilibrate with flow rate of ca 2 mL/min. Then make four 10.0 μL injections of any std soln. Measure coeff. of variation of peak response for 4 injections by following formula:

$$\text{CV, \%} = 100 \sqrt{\frac{\Sigma(x - \bar{x})^2}{n - 1}} \div \bar{x}$$

where x = ratio of area of physostigmine peak divided by area of internal std peak, $\bar{x}$ = mean of these ratios, and n = number of injections.

Coeff. of variation should be ≤2%. If reproducibility is unsatisfactory, let system equilibrate longer and repeat test.

(**b**) *Resolution.*—Retention time for physostigmine peak should be 2.5–4.5 min. Resolution factor, *R*, for physostigmine peak and internal std peak should be ≥3.0, using following formula:

$$R = 2(t' - t)/(PW + PW')$$

where t and t' = mm retention of physostigmine and internal std peaks, respectively; and PW and PW' = mm peak widths measured at baseline of physostigmine and internal std, respectively.

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:

Physostigmine (or salt), mg/mL

$$= 100 \times (RR/RR') \times (C/V)$$

where RR and RR' = response ratio of sample and std; C = concn of std (mg/100 mL); V = mL sample. Identification is based on same retention times for samples and stds.

39. DRUGS: NEUTRAL

(1) The official first action semiautomated method for the determination of nitroglycerin in sublingual tablets, **39.A01-39.A07,** *J. Assoc. Off. Anal. Chem.* **63,** 696 (1980), was adopted official final action.

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(2) The following interim official first action protein nitrogen unit precipitation method for allergenic extracts, *J. Assoc. Off. Anal. Chem.* **64,** 1435 (1981), was adopted official first action:

Allergenic Extracts – Protein Nitrogen Unit Precipitation Method Official First Action

39.C01 ***Principle***

Protein is pptd from allergenic ext by phosphotungstic acid, and N in ppt is detd by appropriate Kjeldahl procedure. Protein nitrogen unit (PNU) is equiv. to 1×10^{-5} mg N.

39.C02 ***Reagent***

Phosphotungstic acid (PTA) *precipitating solution.*—Dissolve 15.0 g PTA in ca 70 mL H_2O. Add 22.2 mL HCl and dil. to 100 mL with H_2O.

39.C03 ***Determination***

Combine vol. of allergenic ext indicated below with 0.25 mL HCl in 12 mL conical centrf. tube. Use 2 mL sample when approx. PNU value of ext is not known. When approx. PNU value of ext is known, analyze following vols:

Allergenic ext, PNU/mL	*Vol., mL*
>35 500	1
15 500–35 500	2
<15 500	3

Add 1 mL PTA pptg soln. Mix thoroly. Let stand 1 h at room temp. (22 ± 3°).

Centrf. mixt. at room temp. at 2700 rpm (rotor radius = 10.80 cm) for 10–15 min (rel. centrifugal force measured to tip of sample tube = g = 880).

Test for completeness of pptn by adding 5 drops PTA soln. Check visually for turbidity in supernate. If turbidity develops, add addnl 0.5 mL PTA soln. Let mixt. stand 1 h at room temp. Recentrf. at 2700 rpm for 10–15 min (room temp.).

Pour off supernate. Invert centrf. tube to drain ppt. Do *not* wash ppt.

Dissolve ppt in 10 mL 2% NaOH by first adding 3 mL 2% NaOH with vol. pipet. Use vortex mixer to loosen ppt. Add 7 mL 2% NaOH (vol. pipet). Mix thoroly. Det. N by appropriate Kjeldahl method. PNU/mL = 10^5 × mg N/mL.

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(3) The following differential pulse polarographic method for the determination of iodine in thyroid tablets was adopted official first action:

Iodine in Thyroid Tablets – Polarography Official First Action

39.C04 ***Apparatus***

Polarograph.—Model 174 (Princeton Applied Research Corp., Princeton, NJ 08540) or equiv., with dropping Hg electrode. Typical operating parameters: scan rate 5 mV/s; scan direction "–"; potential scan range 1.5 V; initial potential −0.9 V; modulation amplitude 50 mV; differential pulse operating mode; display direction "+"; drop time, 1 s; low pass filter off; push-button, initial; offset, off; current range 1–10 μamp, or as needed.

39.C05 ***Reagents***

Use anal. reagents and glass-distd H_2O thruout.

(a) *Bromine water.*—Br-satd H_2O. Prep. fresh daily.

(b) *Potassium carbonate.*—If reagent grade K_2CO_3 gives high blank, purify as follows: Dissolve ca 200 g K_2CO_3 in 400 mL H_2O, add 50 g 20–50 mesh Amberlite IRA-400 ion exchange resin (Mallinckrodt Chemical Works), and agitate 30 min. Filter thru glass wool plug into porcelain crucible, evap. to dryness on hot plate, and heat at 675° in muffle 25 min. Cool to room temp., and grind to fine powder with mortar and pestle.

(c) *Reagent blank.*—Dissolve 8 g K_2CO_3 in ca 70 mL H_2O in 100 mL vol. flask. Add 1 mL Br-satd H_2O and 20 mg Na_2SO_3. Mix, dil. to vol. with H_2O, and mix.

(**d**) *Standard solns.*—(*1*) 1 mg I/mL: Dissolve 1.686 g KIO_3 in ca 200 mL H_2O in 1 L vol. flask. Dil. to vol. and mix. (*2*) 32 μg I/mL: Pipet 8 mL std soln (*1*) into 250 mL vol. flask, dil. to vol., and mix.

(**e**) *Working soln.*—Pipet aliquot (*V*) of std soln (2) contg same amt of I contained in one tablet (see below) into 100 mL vol. flask contg 8 g K_2CO_3 dissolved in 70 mL H_2O.

Tab. strength, gr. thyroid	*I content, μg*	*Std soln 2, mL*
1/4	32.4	1
1	128.6	4
2	259.2	8
5	643.0	20

Add 1 mL Br-satd H_2O and mix. Add Na_2SO_3 (ca 20 mg) until soln becomes colorless; mix. Dil. to vol. H_2O and mix.

39.C06 ***Sample Preparation***

(**a**) *Composite assay.*—Weigh and finely powder ≥20 tablets. Weigh portion of powder equiv. to 1 tablet into porcelain crucible that has been washed with HNO_3 (1 + 1), rinsed with H_2O, and wiped dry. Mix with 4 g K_2CO_3 and overlay with addnl 4 g K_2CO_3. Place crucible in preheated 675° muffle 25 min. Cool, add 30 mL H_2O, carefully heat on hot plate to dissolve residue, and filter thru funnel with glass wool plug into 100 mL vol. flask. Repeat heating with 2 addnl 30 mL portions of H_2O, and add these exts to vol. flask. Add 1 mL Br-satd H_2O, mix, add Na_2SO_3 (ca 20 mg) until soln becomes colorless. Dil. to vol. with H_2O and mix.

(**b**) *Individual tablet assay.*—Crush 1 tablet in porcelain crucible with glass rod. Remove any sample adhering to glass rod with spatula, and add to crucible. Proceed as in Composite Assay, (**a**), beginning "Mix with 4 g K_2CO_3 . . ."

39.C07 ***Determination***

Add ca 10 mL working soln to dry polarographic cell. Bubble N thru cell 5 min; then direct stream of N above soln. Using typical operating parameters as guide, switch selector to external cell and wait until pen becomes stationary; then depress scan button. Similarly, using same settings, analyze sample soln followed by reagent blank. From baseline established by reagent blank, measure peak hts of std and sample solns at ca −1.18 V vs SCE. Calc. as follows:

$$\text{I as \% of declared thyroid} = (PH \times V \times W_t \times 3.2)/(PH' \times W_s \times TH)$$

where PH and PH' = peak ht of sample and std, resp.; V = mL of 32 μg/mL std used to prep. working std soln; W_t and W_s = av. wt of tablet and wt of sample, g, resp.; and TH = declared thyroid per tablet, mg.

40. DRUGS: ILLICIT

No additions, deletions, or changes.

41. DRUGS AND FEED ADDITIVES IN ANIMAL TISSUES

(1) The following interim official first action gas-liquid chromatographic-mass spectrometric method for the determination of sulfamethazine in swine tissues, *J. Agric. Food Chem.* **29,** 727 (1981); *J. Assoc. Off. Anal. Chem.* **64,** 1386 (1981), was adopted official first action:

Sulfamethazine in Swine Tissue

Gas-Liquid Chromatographic-Mass Spectrometric Method

Official First Action

(Applicable to residues at 0.05–0.20 ppm)

41.C01 ***Principle***

Sulfamethazine is extd from tissue with CH_3Cl-acetone. Ext is filtered and solv. is removed by evapn. Residue is redissolved in hexane and partitioned against 1N HCl. Acid phase is neutzd and sulfamethazine is extd with CH_2Cl_2. Solv. is removed and residue is methylated using diazomethane. Sulfamethazine is identified and quantitated using electron impact gas-liq. chromatgy/mass spectrometry (EI-GLC/MS) in selected ion mode. Six ions, m/z 92, 98, 227, 228, 233, and 234 are monitored. Ion current from each is accumulated thruout GLC run, stored on mag. tape, and plotted as ion current vs time. Peaks appearing in ion current profiles are identified and retention times and areas for each peak are calcd. Sulfamethazine is quantitated from std curve prepd by least squares linear regression using data from analysis of known std solns. Identity of sulfonamide residues is confirmed by presence of significant ions appearing at proper retention time in proper relative abundances.

Procedure gives quant. results as well as data for confirmation of residues detected. Procedure is accurate at 0.1 ppm level with expected coefficient of variation of 4.6%. Min. detectable level is 0.002 ppm. To accommodate residues >0.2 ppm, reconstruct std curve as follows:

Expected Concn Range	*Use Stds (in ppm)* A	B	C
0 -0.2 ppm	0.05	0.10	0.20
0.2-2.0 ppm	0.50	1.0	2.0
2.0-20.0 ppm	5.0	10.0	20.0

41.C02 ***Apparatus***

(a) *Blender.*—Virtis Model 45, or equiv., with 500 mL flasks.

(b) *Evaporator.*—N-Evap Model III (Organomation Associates).

(c) *Gas-liquid chromatograph-mass spectrometer.*—Hewlett-Packard Model 5992 quadrupole operated in multiple ion monitoring under following conditions: electron energy, 70 eV; electron multiplier, 2000-2800 eV; source temp., 140°; integration time, 200 ms/mass monitored. Column: 2 mm id × 3 ft glass, packed with 3% OV-17 on 80-100 mesh Gas-Chrom Q (Supelco, Inc., Bellefonte, PA 16826). GLC conditions: injection port 230°; column 220°, He flow 30 mL/min; GLC/MS interface, silicone membrane separator. Total analysis time is ca 17 min. Sulfamethazine retention time is 9-12 min.

41.C03 ***Reagents***

(a) *Solvents.*—Distd in glass, or equiv.: acetone; CH_2Cl_2; MeOH (shake with and store over anhyd. Na_2SO_4); CH_3Cl (no preservatives).

(b) *Trisodium citrate soln.*—Add 720 g trisodium citrate dihydrate to 1 L H_2O.

(c) *Diazomethane.*—Aldrich Chemical Co. No. Z210-159-1. Prep. according to manufacturer's instructions. *Caution:* Prep. diazomethane in hood behind protective screen or shield. Wear gloves to prevent skin contact with reagents. Observe caution when handling diazomethane. It is toxic and under some conditions explosive. See **41.013.** Recommended conditions and total vol. of diazomethane generated minimize instability of compd. Freshly made diazomethane soln is golden yellow and can be used for max. of 1 week. Store in freezer.

(d) *Sulfamethazine stock std soln.*—100 μg/mL. Accurately weigh 10.0 mg sulfamethazine into 100 mL vol. flask. Dissolve in and dil. to vol. with anhyd. MeOH

(e) *Fortification std soln.*—5 μg/mL. Pipet 5.0 mL stock std soln into 100 mL vol. flask and dil. to vol. with H_2O. Prep. std fresh weekly.

(f) *GLC/MS std soln.*—50 μg/mL. Accurately weigh 10.0 mg unlabeled sulfamethazine into 200 mL vol. flask. Dissolve in and dil. to vol. with anhyd. MeOH.

(h) *^{13}C-Labeled sulfamethazine std soln.*—50 μg/mL. Accurately weigh 10.0 mg ^{13}C-labeled sulfamethazine into 200 mL vol. flask. Dissolve in and dil. to vol. with anhyd. MeOH.

41.C06 ***GLC/MS Quantitation/Confirmation***

Weigh 50.0 g (±0.1 g) ground, frozen tissue into 500 mL blender flask. Select blank tissue as control. Fortify second blank tissue sample at 0.1 ppm level with unlabeled sulfamethazine (100 μL GLC/MS std). Spike all samples at 0.10 ppm level with ^{13}C-labeled sulfamethazine std soln (100 μL).

Add 100 mL $CHCl_3$-acetone (1 + 1) to flask. Blend 1 min at low speed. Decant and filter liq. (no vac.) thru 24 cm Whatman 2V fluted paper into 1 L r-b flask.

Repeat extn and filtering twice more. Transfer all tissue to filter paper after third extn.

Rinse flask with ca 25 mL $CHCl_3$-acetone (1 + 1), transfer rinsing to filter paper, and let drain. Rinse filter paper and contents with three 20 mL aliquots of $CHCl_3$-acetone (1 + 1). If combined filtrates are not clear, refilter and wash filter paper with ca 20 mL $CHCl_3$-acetone (1 + 1).

Evap. on rotary evaporator at 55° (±5°) to oily residue (ca 1-2 mL). Remove from rotary evaporatory promptly. Quant. transfer residue to 250 mL separator using, in order, four 25 mL portions of hexane, two 3 mL portions of acetone, and two 25 mL portions of hexane. Add 10 mL 1N HCl to separator. Shake gently 2 min and let phases sep. Emulsions may be eliminated by placing separator in 60° H_2O bath. Repeat extn 3 times with 5 mL portions of 1N HCl, drawing off acid phase and combining filtrates in the 125 mL separator. Add 3.0 mL 10N NaOH to 125 mL separator and mix. Det. pH, using pH paper. If pH is not 12-13, add addnl 10N NaOH with mixing to attain this pH.

Add 25 mL $CHCl_3$ to basic soln and shake 1 min. Let phases sep. completely, and discard $CHCl_3$. Repeat $CHCl_3$ extn a second time, discarding $CHCl_3$. Quant. transfer aq. phase to small beaker (ca 100 mL). Buffer by adding 25 mL satd aq. trisodium citrate. Adjust pH with pH meter to 5.55-5.65 by adding NaOH or HCl as required.

Quant. transfer contents of beaker to 125 mL separator, add 15 mL CH_2Cl_2, and shake 1 min. Let phases sep. and transfer CH_2Cl_2 to 50 mL conical centrf. tube. Check pH of aq. phase and re-adjust to 5.55-5.65 if necessary. Repeat CH_2Cl_2 extn twice.

Evap. contents of centrf. tube to dryness at 45° under stream of N on N-Evap. Dissolve residue in 1 mL anhyd. MeOH. Add 1 mL diazomethane

soln, mix with vortex mixer, and let stand at room temp. 5 min. Transfer soln from tube to 15 mL or smaller concentrator tube and evap. to dryness at 45° under stream of N on N-Evap. Dissolve methylated residue in 200 μL anhyd. MeOH.

41.C07 ***Preparation of Standard Curve***

Add 100 μL ^{13}C-sulfamethazine std soln (50 μg/mL) to each of three 15 mL concentrator tubes. Label tubes A, B, and C. Add 50 μL GLC/MS std soln (50 μg/mL) to tube A (equiv. to 0.05 ppm ^{12}C). Add 100 μL GLC/MS std soln (50 μg/mL) to B (equiv. to 0.10 ppm ^{12}C). Add 200 μL GLC/MS std soln (50 μg/mL) to tube C (equiv. to 0.20 ppm ^{12}C). Add 1 mL freshly prepd diazomethane soln to each concentrator tube and mix with vortex mixer, let stand at room temp. 5 min. Evap. to dryness at 45° under stream of N on N-Evap. Dissolve each methylated residue in 200 μL anhyd. MeOH.

Set selected ion monitor (SIM) data acquisition program area as follows:

a Mass 1 = 227, dwell time = 200 ms
b Mass 2 = 228, dwell time = 200 ms
c Mass 3 = 233, dwell time = 200 ms
d Mass 4 = 234, dwell time = 200 ms
e Mass 5 = 92, dwell time = 200 ms
f Mass 6 = 98, dwell time = 200 ms
g Solvent elution time = 1.1 min

Make injections in order given below.

a Inject 2.0 μL std A
b Inject 2.0 μL std B
c Inject 2.0 μL std C

For each std injection, det. ratio of areas of m/z 227 peak to area of m/z 233 peak. Using method of least squares, calc. std curve for 227/233 mass ratio vs amt of unlabeled sulfamethazine added to each std (amt may be expressed as ppm based on 50 g sample). Similarly for each std, calc. following confirmation ratios: 228/227 and 234/233. Mean 228/227 and 234/233 ratios computed from 3 std injections will be used for confirmation of identity of sulfamethazine detected in processed samples.

41.C08 ***Determination***

With SIM program set as for *Preparation of Standard Curve*, inject 2.0 μL from each sample to be analyzed. Plot reconstructed ion current profiles at end of each run and calc. 227/233 ion mass ratio. Read sulfamethazine content of sample from std curve.

For identification purposes: (a) Sample unlabeled sulfamethazine must co-elute with added ^{13}C-labeled sulfamethazine. (b) The m/z 92, 227, and 228 from unlabeled sulfamethazine and m/z 98, 233, and 234 from ^{13}C-labeled sulfamethazine must all be present. (c) Ratios of 228/227 and 234/233 ions in sample should be within 10% of mean ratio detd for stds.

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(2) The following interim official first action gas-liquid chromatographic method for the determination of sulfamethazine in swine tissues, *J. Assoc. Off. Anal. Chem.* **64**, 794, 1386 (1981), was adopted official first action:

Sulfamethazine in Swine Tissues
Gas-Liquid Chromatographic Method
Official First Action

41.C09 ***Principle***

Tissue is extd with acetone-$CHCl_3$, 1N HCl is added, and solv. is evapd. Aq. soln is washed with hexane, pH is adjusted to 5.55–5.65, and sulfamethazine is extd with CH_2Cl_2, methylated with diazomethane, and detd by electron capture GLC.

41.C10 ***Reagents and Apparatus***

Rinse all clean glassware thoroly with MeOH and let dry. Use distd in glass solvs suitable for pesticide analyses (Burdick & Jackson Laboratories, Inc., or equiv.).

(**a**) *Sulfamethazine std solns.*—Prep. std solns contg 1, 2, and 10 μg sulfamethazine USP/mL acetone.

(**b**) *Diazomethane derivatizing reagent.*—Caution: Diazomethane is toxic, can cause specific sensitivity, and is potentially explosive. Prep. diazomethane reagent, methylate, and evap. in hood. Avoid ground glass joints, etched or scratched glassware, and sharp edges. Store diazomethane solns in freezer; do not expose to direct sunlight or strong artificial light. Prep. diazomethane by ethereal basic distn of 21.5 g *N*-methyl-*N*-nitroso-*p*-toluenesulfonamide (Diazald, Aldrich Chemical Co.), with 200 mL ether as described in Diazald kit, Cat. No. Z10, 025-0. (*Note:* Read Diazald kit instructions carefully for safe handling of diazomethane.) After distn of second portion (40 mL) of ether, transfer ether soln of diazomethane thru funnel to narrow-mouth bottle and cap tightly with a polyseal cap. When stored in freezer, soln retains its efficiency as methylating agent ≥1 month.

(c) *Gas chromatograph.*—Tracor Model 222, or equiv., with ^{63}Ni linearized electron capture

detector and 6 ft × 2 mm id glass column packed with 5% OV-7 on 100–120 mesh Gas-Chrom Q (Applied Science Division, Milton Roy Co. Laboratory Group, PO Box 440, State College, PA 16801). Operating conditions: injector 290°, column oven 260°; detector 290°; argon–methane (90 + 10) carrier gas at 30 mL/min; detector purge flow 20 mL/min. Retention time for methylated sulfamethazine is 4–4.5 min. Alternative column: 6 ft × 4 mm id, packed with 5% OV-25.

(**d**) *Food chopper.*—Model 84142D (Hobart Manufacturing Co.), or equiv.

(**e**) *Flasks.*—100 mL pear-shaped, 24/40 joint (Kontes Glass Co. No. K-608700).

41.C11 ***Procedure***

Cut tissue into 1.5 cu. in. pieces and freeze in plastic bags. Pulverize enough dry ice in bowl of Hobart food chopper to chill bowl and grater thoroly. Slowly add small portions (ca 50 g) of sample and continue chopping until complete sample is chopped. If necessary, add more dry ice to maintain sample in frozen state during chopping procedure. Store sample in a freezer (−20°F) until dry ice has dissipated.

Transfer 15 g sample to blender and blend 5 min at medium speed with 150 mL acetone–$CHCl_3$ (1 + 1). Filter thru glass-fiber paper, collecting first 100 mL filtrate in 100 mL graduate. Transfer 100 mL aliquot (equiv. to 10 g sample) to 250 mL r-b flask with 24/40 joint and add 10 mL 1N aq. HCl. Evap. org. solvs on rotary vac. evaporator with flask submerged in 32 ± 4° H_2O bath. (For muscle and fat, some extd fat will prevent complete removal of solvs.) Add 50 mL *n*-hexane to 1N HCl phase and quant. transfer both phases to 125 mL separator. Rinse evapn flask with addnl 5 mL 1N HCl and transfer rinse to 125 mL separator. Shake contents of separator gently by inverting funnel and returning to upright position once a second for 50 s. Let funnel sit until phases sep. (ca 10 min). Draw off lower phase (1N HCl) into second 125 mL separator. (Centrifugation may be required to avoid transfer of emulsified solv. which tends to bump during hydrolysis step.) Rinse r-b flask with 5 mL 1N HCl, transfer to first separator, contg hexane, mix, and sep. as above. Drain HCl phase into second separator and discard hexane. For high fat samples only, add 15 mL CH_2Cl_2 to HCl phase in second separator, shake 30 s, and let phases sep. Draw off and discard lower CH_2Cl_2 phase.

Buffer aq. HCl ext in separator by adding 25 mL satd aq. trisodium citrate; then adjust pH with pH meter to 5.55–5.65 by adding 3N NaOH (ca 2.5 mL). Add 15 mL CH_2Cl_2 to separator and shake vigorously for 90 s. Let phases sep. and transfer lower CH_2Cl_2 layer to 100 mL pear-shaped flask. After first extn, check pH of aq. phase and re-adjust to 5.55–5.65 if necessary. In similar manner, ext with 3 addnl portions of CH_2Cl_2, combining exts in 100 mL pear-shaped flask. Evap. solv. in rotary vac. evaporator with H_2O bath at 25–30°. Do not exceed 30°. Sample may be kept overnight at this stage.

Dissolve residue in 1.0 mL acetone. Swirl flask to dissolve any residue on walls of flasks. Add 1 mL diazomethane derivatizing reagent and let stand 15 min with intermittent gentle swirling. Evap. solv. under gentle stream of N. Dissolve residue in 1.0 mL acetone.

Prep. methylated stds by pipetting 1.0 mL aliquots of each sulfamethazine std soln into sep. 100 mL pear-shaped flasks. Add 1 mL diazomethane derivatization reagent and treat in same manner as sample.

41.C12 ***Gas Chromatography***

Inject 2–8 μL methylated 1 μg/mL sulfamethazine std into gas chromatograph. (Resulting peak ht should be 30–40% FSD.) Inject up to 3 samples followed by std which approx. matches sample. If sample peak goes off scale, quant. dil. methylated sample soln with acetone to give response that is 30–60% FSD. Correct results for diln.

Sulfamethazine, ppm = $1.5 (A \times C \times V')/(A' \times W \times V)$

where A, A' = peak area of sample and std, resp.; V, V' = GLC injection vol. (μL) of sample and std, resp.; C = concn of std (μg sulfamethazine/mL); W = wt sample (g); 1.5 = 150/100 to correct for 100 mL aliquot of 150 mL sample ext taken for analysis.

42. DRUGS IN FEEDS

(1) The following spectrophotometric method for the determination of arprinocid in premixes, *J. Assoc. Off. Anal. Chem.* **65,** 52 (1982), was adopted official first action:

Arprinocid in Premixes
Official First Action

42.C01 ***Principle***

Arprinocid is extd from premix into $CHCl_3$ and transferred into 0.1N HCl. Interferences are removed by partitioning with CCl_4 and arprinocid is detd by direct spectrophtric measurement at 258 nm.

42.C02 ***Reagents and Apparatus***

(**a**) *Spectrophotometer.*—Suitable for measuring *A* at 258 nm.

(**b**) *Hydrochloric acid.*—0.1N. Dilute 8.3 mL HCl to 1 L with H_2O.

(**c**) *Arprinocid std soln.*—Accurately weigh 0.12 ± 0.01 g arprinocid std and dissolve in 100.0 mL $CHCl_3$.

42.C03 ***Extraction***

Accurately weigh 1.00 ± 0.05 g premix and transfer to 125 mL g-s erlenmeyer. Add by pipet 100.0 mL $CHCl_3$ and mech. shake 20 min. Filter ca 20 mL ext thru Whatman No. 42 paper, covering funnel to prevent evapn of solv. Transfer by pipet 3.00 mL filtrate and 3.00 mL arprinocid std soln to sep. 50 mL centrifuge tubes. Place tubes in ca 50° H_2O bath and evap. solv. to dryness under N stream.

42.C04 ***Partitioning***

Add by pipet 5.0 mL CCl_4 followed by 25.0 mL 0.1N HCl to the tube and mix 20 min in ultrasonic bath. If residue is not dissolved, addnl shaking is required. Centrifuge 5 min at 2000–2500 rpm.

42.C05 ***Determination***

Transfer by pipet 5.00 mL upper aq. phase to 100 mL vol. flask, dil. to vol. with 0.1N HCl, and mix. Measure absorbance of the sample (*A*) and std (*A'*) at 258 nm vs 0.1N HCl in ref. cell.

$$\text{Arprinocid, \%} = (A/A') \times (W'/W) \times 100$$

where W = g sample; and W' = g std.

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(2) The following microbiological method for the determination of bacitracin in premixes at ≥10 g/lb was adopted official first action:

Bacitracin in Premix Feeds

Cylinder Plate Assay

Official First Action

(Applicable to premixes contg ≥10 g bacitracin/lb)

42.C06 ***Principle***

Bacitracin is extd from feeds into acidified org. solv. system. Ext is centrfd, and supernate is dild in phosphate buffer and analyzed by cylinder plate assay with *M. flavus* as detection organism.

42.C07 ***Reagents and Apparatus***

(**a**) *Microorganism.*—*Micrococcus flavus* ATCC 10240. Maintain culture as indicated in **42.199**(**a**).

(**b**) *Extracting solv.*—Mix, by vol., 27% CH_3CN, 27% MeOH, 3% pH 6.0 phosphate buffer, (**42.197**)(**f**), 41% H_2O, and 2% H_3PO_4 (85%); add 0.5 g EDTA/L. (Extg solv. is satd with EDTA.).

(**c**) *Phosphate buffer.*—5%, pH 6.5. See **42.197**(**d**).

(**d**) *Diluting solvent.*—Methanol–5% pH 6.5 phosphate buffer (12 + 88).

(**e**) *Dilute HCl.*—Carefully add 89 mL HCl to H_2O and dil. to 1 L (1N). Further dil. soln 1:100 (0.01N).

(**f**) *Cylinders.*—See **42.198**(**a**).

(**g**) *Cylinder dispenser.*—Optional: see **42.198**(**c**).

42.C08 ***Standard Solutions***

See **42.202**(**a**) and (**b**). Also prep. 0.30 and 0.16 unit/mL solns to be plated as samples to monitor assay.

42.C09 ***Preparation of Plates***

Use one layer (ca 15 mL) of agar antibiotic medium 1, **42.196**(**a**). Det. by trial plates optimum concn (usually 0.02–0.05%) of *M. flavus* ATCC 10240 to be added to agar to obtain zones of inhibition 15–17 mm for 0.2 unit bacitracin/mL. Pour 4 plates for each point on std curve (i.e., 16 plates) and 4 plates for each sample soln. Std curve will be plated twice (i.e., 32 plates) as will check samples 0.30 and 0.16 unit/mL. Therefore, total of 48 plates will be needed for 2 curves and check samples, plus 4 addnl plates for each sample.

Let agar harden on level surface. Transfer to refrigerator and cool ≥1 h before dosing. Use plates same day prepd.

42.C10 ***Extraction***

Accurately weigh amt feed contg ca 4600 units of bacitracin into 300 mL erlenmeyer flask, or equiv.

Add 100 mL extg solv. with 100 mL vol. pipet and ext feeds ≥5 min by shaking flask or mixing on mag. stirrer.

Transfer supernate to plastic centrif. tubes and centrif. 10 min at 2000 rpm. Filter supernate thru glass wool into graduate. Use vol. glassware and dilg solv. to prep. final diln 0.2 ± 0.05 unit/mL.

42.C11 ***Plating***

Use 16 seeded plates for first curve. Use 0.20 unit/mL as plate ref. On each plate, fill 3 alternate stainless steel cylinders with plate ref. and the 3 remaining cylinders with 1 std. Be sure all cylinders are filled with const vol. (i.e., 0.25 mL). Preset Eppendorf pipet is best for this purpose. Use 4 plates for each sample, including 0.3 and 0.16 unit/mL check samples.

Use 16 seeded plates for second curve, to be plated after all samples are plated. Use 8 plates for second plating of 0.3 and 0.16 unit/mL check samples.

Incubate dosed plates 16–18 h at 37 ± 2°. Read zones of inhibition to nearest mm, using Fisher–Lily zone reader.

42.C12 ***Determination***

Det. corrected av. zone diams for std (Z') and sample (Z) solns according to **42.200.** Det. response line as least squares linear regression of following equation:

$$Z' = m\log P' + b$$

where P' = potency in unit/mL of std soln associated with Z'; m, b = are least squares fitted slope and intercept parameters. Calc. potency of sample by following equation:

$$\text{g bacitracin/lb} = [\text{antilog}(Z - b/m) \times D \times 0.0108]/\text{sample wt}$$

where D = total sample diln; 0.0108 = 453.6 (g/lb)/42 000 (units/g bacitracin).

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(3) The following liquid chromatographic method for the determination of bacitracin in premixes at ≥10 g/lb was adopted official first action:

Bacitracin in Premix Feeds

Liquid Chromatographic Method

Official First Action

42.C13 ***Principle***

Bacitracin is extd from feed into acidified org. solv. system. Ext is centrfgd, and supernate is analyzed by ion-suppressed reverse phase LC with photometric detection at 254 nm.

42.C14 ***Reagents and Apparatus***

(**a**) *Liquid chromatograph.*—Hewlett-Packard Model 1084-A, equipped with UV photometric detector. Operating conditions: flow rate 2.0 mL/min; detector wavelength 254 nm; 20 μL loop injection valve (Valco Instruments Co., Inc., Houston, TX 77055); ambient temperature.

(**b**) *Chromatographic column.*—15 cm × 3.0 mm id, containing 5 μm Supel Cosil LC-8 reverse phase packing (Supelco, Inc., Bellefonte, PA 16823). Use column for bacitracin analysis only.

(c) *Phosphate EDTA buffer.*—pH 4.5. Dissolve 13.6 g KH_2PO_4 and 2.5 g EDTA in 1 L H_2O.

(**d**) *Phosphate buffer.*—pH 6.0. Dissolve 1.5 g K_2HPO_4 and 8.5 g KH_2PO_4 in 1 L H_2O.

(**e**) *Solvent systems.*—Measure vol. indicated below with graduate (except where noted otherwise) into 100 mL vol. flask and d:l. to vol. with H_2O:

	Vol. %		
Solvent	*A Solv.*	*B Solv.*	*Extg Solv.*
CH_3CN	0	40	28
MeOH	0	12	28
Phosphate-EDTA buffer	20	20	0
Phosphate buffer[a]	0	0	3
Concd phosphoric acid[a]	0	0	1.2

[a] Use vol. pipet.

(**f**) *Mobile phase.*—Mix 59% (v/v) B solv. with 41% (v/v) A solv. Mix, and adjust pH to 6.8 with NaOH. Slight adjustment to % vol. of B solv. may be required to obtain desired sepn.

42.C15 ***Preparation of Standard***

(**a**) *Drying of std.—Caution:* Bacitracin is very hydroscopic. Dry std day before use and store in desiccator overnight. Accurately weigh 130–140 mg bacitracin ref. std (IMC, Terre Haute, IN 47808; 56.3 units/mg) into tared (= A) 50 mL vol. flask. Dry std 3 h at 60° under vac. at <5 mm pressure. Remove from oven and place in desiccator to cool. Reweigh (= B). Amt bacitracin std = B − A.

(**b**) *Preparation of std soln.—Note:* Store stds under refrigeration if not analyzed within 3 h of prepn. Preferably, prep. std, store in refrigerator >30 min before analysis, and remove from refrigerator just before analysis. *Std soln 1:* Dissolve bacitracin std in 50 mL vol. flask with ca 20 mL extg solv. and dil. to vol. Prep. following dilns from this soln. *Std soln 2:* Pipet 20 mL std soln 1 into 25 mL vol. flask; dil. to vol. with extg solv. *Std soln 3:* Pipet 15 mL std soln 1 into 25 mL vol. flask; dil. to vol. with extg solv.

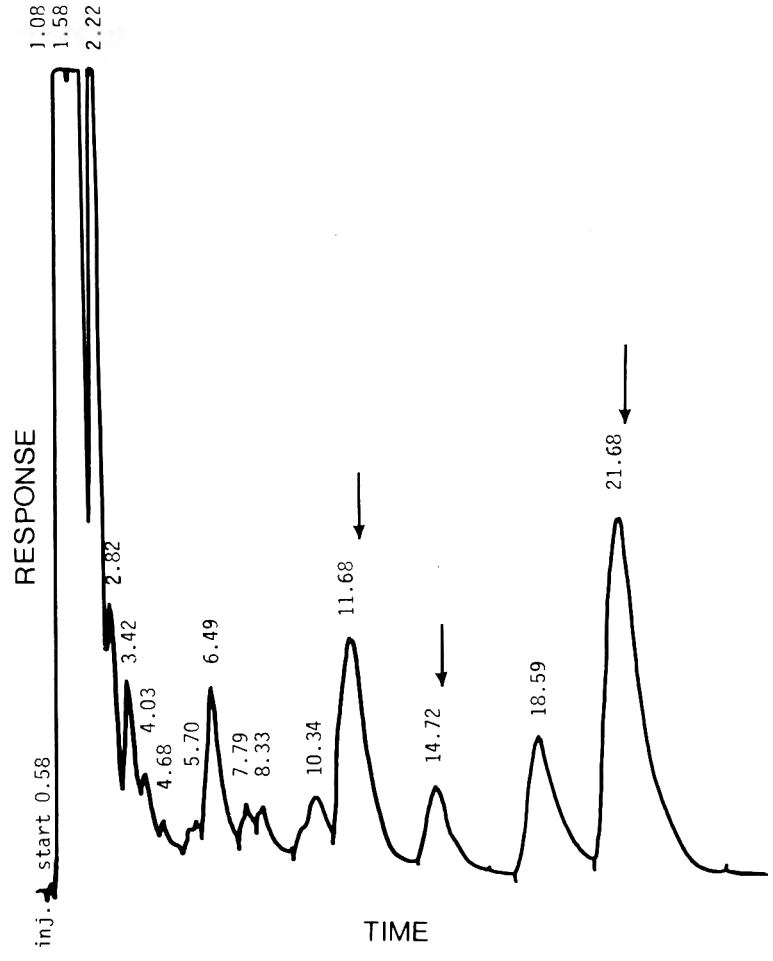

Figure 42:C1. Liquid chromatogram of bacitracin active components (indicated by arrows).

42.C16 *Extraction*

Accurately weigh amt of feed contg ca 6000 units bacitracin activity into 125 mL erlenmeyer. Add 50 mL extg solv. with vol. pipet and ext with wrist-action shaking >5 min. Centrifuge 10 mL portion of ext 2–3 min at 2000–3000 rpm. Use clear supernate for assay. *Note:* Store extd sample soln under refrigeration if not analyzed within 3 h. Preferably, prep. sample solns, store in refrigerator >30 min before analysis, and remove from refrigerator just before analysis.

42.C17 *Determination*

Inject clear supernate from centrfgd feed and std solns into chromatograph, starting with std soln, then 2 sample solns, and then another std soln, until all samples and stds have been injected. Measure and total peak hts of the 3 active component peaks (Figure **42:C1**) for sample (PH) and std (PH') solns.

Calc. response line for stds, using least squares linear fitting of following equation:

$$PH' = m(P') + b$$

where PH' = peak hts of std solns 1, 2, and 3; P' = potency of std soln in units/50 mL for std solns 1, 2, and 3; m, b = least squares detd slope and intercept.

Det. bacitracin content of feed from:

$$\text{g bacitracin/lb} = \frac{0.01080\,(PH - b)}{m \times \text{sample wt}}$$

where 0.01080 = 453.6 (g/lb)/42 000 (units/g bacitracin).

43. VITAMINS AND OTHER NUTRIENTS

(1) The following official first action methods were adopted official final action:

(a) Riboflavin, AACC-AOAC automated method, **43.B01-43.B04**

(b) Niacin and niacinamide, AACC-AOAC automated method, **43.B05-43.B08**

(c) Liquid chromatographic method for vitamin D in fortified milk and milkpowder, **43.B09-43.B15**

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(2) The following liquid chromatographic method for the determination of vitamin D in mixed feeds, premixes, and pet foods was adopted official first action:

Vitamin D in Mixed Feeds, Premixes, and Pet Foods

Liquid Chromatographic Method

Official First Action

(Applicable to products contg <200 IU and >2 IU vitamin D/g. For products contg ≥200 IU vitamin D/g, use **43.A01-43.A08.**)

43.C01 *Principle*

Samples are saponified and extd, and unsaponifiable material is chromatographed successively on alumina to remove tocopherols and carotenes, if present, and on LC cleanup column to sep. from interfering substances. Second LC column packed with silica seps vitamin D from impurities. Vitamin D is corrected for amt previtamin D formed during saponification. Vitamin D is sum of vitamin D and previtamin D.

43.C02 *Reagents*

(a) *Solvents.*—MeOH, alcohol, CH_3CN, toluene, peroxide- and acid-free ether, *n*-hexane (spectroquality). Dry *n*-hexane by passing thru column 60 × 8 cm diam. contg 500 g 50–250 μm silica dried 4 h at 150°.

(b) *Sodium ascorbate soln.*—Dissolve 3.5 g ascorbic acid in 20 mL 1N NaOH. Prep. fresh daily.

(c) *Antioxidant solution.*—1 mg butylated hydroxytoluene (BHT)/mL hexane.

(d) *Petroleum ether.*—Reflux over KOH pellets and collect fraction distg between 40° and 60°.

(e) *Ether petroleum ether eluants.*—8 + 92 and 40 + 60.

(f) *Alumina.*—Neut., type 1097 (E. Merck, Darmstadt, GFR).

(g) *Mobile phase (for cleanup column).*—CH_3CN-MeOH-H_2O (50 + 50 + 5).

(h) *Mobile phase (for analytical column).*—*n*-Hexane contg 0.35% (v/v) *n*-amyl alcohol.

(i) *Vitamin D std solns.*—USP Ref. Std Ergocalciferol (if sample labeled as contg vitamin D_2) or Cholecalciferol (if labeled as contg vitamin D or D_3). Accurately weigh ca 12.5 mg vitamin D std (*W'*) in 100 mL amber vol. flask. Dissolve without heat in toluene and dil. to vol. with toluene (125 μg/mL, soln A). Dil. 10 mL soln A to 100 mL with mobile phase (**h**). Dil. 10 mL of this soln to 100 mL with toluene–mobile phase (**h**) (5 + 95) for vitamin D std (soln B) (1.25 μg/mL; 50 IU/mL). Also dil. 10 mL soln A to 100 mL with mobile phase (**g**); dil. 20 mL of this soln to 100 mL with mobile phase (**g**) (2.5 μg/mL, soln C). Prep. fresh daily.

(j) *System suitability std soln.*—Use USP Vitamin D Assay System Suitability Ref. Std, or prep. soln contg 2 mg vitamin D_3 and 0.2 mg trans-vitamin D_3/g in vegetable oil. Peaks of trans-vitamin D_3 and previtamin D_3 must have ca same peak hts. If necessary, increase previtamin D_3 content by warming oil soln ca 45 min at 90°. Store soln at 5°.

43.C03 *Alumina Column*

Seal coarse fritted glass disk in lower end of 150 × 20 mm (id) tube and 250 mL bulb at upper end. Fit constricted portion at lower end with Teflon stopcock.

Heat 250 g alumina overnight at 750°. Cool and store in vac. desiccator. Weigh 30 g dried alumina into 100 mL erlenmeyer. Pipet 2.7 mL H_2O into flask, and stopper. Heat 5 min on steam bath. Vigorously shake warm flask until powder is free-flowing. Cool and let stand 30 min.

Add 40 mL pet ether to deactivated alumina, swirl, and transfer to tube, using pet ether. Let packing settle. Maintain head of >0.5 cm liq. on column thruout assay (alumina column can be used for only 1 assay).

43.C04 *Liquid Chromatography*

(a) *Liquid chromatograph.*—Hewlett-Packard 1010A, or equiv., with 254 nm UV detector with 2 columns: cleanup and analytical.

(b) *Cleanup column.*—Stainless steel, 250 × 4.6 (id) mm, packed with 10 μm particle size Li-Chrosorb RP-18. Typical operating conditions: chart speed, 1 cm/min; eluant flow rate, 1.4

mL/min; detector sensitivity, 0.08 AUFS; temp., ambient; valve injection vol., 500 μL; solv. system, CH_3CN-MeOH-H_2O (50 + 50 + 5).

(c) *Analytical column.*—Stainless steel, 250 × 4.6 (id) mm, packed with 5 μm particle size Partisil-5, passing system suitability test. Typical operating conditions: chart speed, 1 cm/min; eluant flow rate, 2.6 mL/min (ca 1500 psi); detector sensitivity, 0.008 AUFS; temp., ambient; valve injection vol., 200 μL; solv. system, *n*-hexane contg 0.35% (v/v) *n*-amyl alcohol.

43.C05 *System Suitability Test for Analytical Column*

Dissolve 0.1 g system suitability std soln in 100 mL toluene–mobile phase (5 + 95) and inject 200 μL. Det. peak resolution between previtamin D_3 and trans-vitamin D_3 as: $R = 2D/(B + C)$; where D = distance between peak max. of previtamin D_3 and trans-vitamin D_3; B = peak width of previtamin D_3; and C = peak width of trans-vitamin D_3. Performance is satisfactory if R is ≥ 1.0.

43.C06 *Calibration*

Inject 500 μL vitamin D std soln (soln C) onto cleanup column thru sampling valve and 200 μL soln B onto analytical column, and adjust operating conditions of detector to give largest possible on-scale peaks of vitamin D. Det. retention time of vitamin D on cleanup and analytical columns and peak ht of vitamin D on analytical column. Retention time of vitamin D on cleanup column should be between 15 and 25 min; adjust H_2O content of mobile phase, if necessary, to achieve this situation. Retention time of vitamin D on analytical column should be between 15 and 20 min; adjust amyl alcohol content in mobile phase, if necessary, to achieve this situation.

43.C07 *Preparation of Sample*

Isolation of unsaponifiable matter from powder.—Accurately weigh ca 25 g powdered sample (preferably particle size <1 mm) into saponification flask. Add 80 mL alcohol, 2 mL Na ascorbate soln, a pinch of Na_2EDTA, and 10 mL 50% aq. KOH soln. Reflux 30 min on steam bath under N with mag. stirring. Cool and ext with five 60 mL portions of ether in saponification flask; decant each time and transfer ether layer to 1 L separator contg 100 mL H_2O. Shake ether layer in separator (A), let sep., and transfer aq. phase to 500 mL separator (B). Ext aq. phase with 60 mL ether and transfer ether layer to separator (A). Wash combined ether exts with 100 mL 0.5N KOH soln and then 100 mL portions of H_2O until last washing is neut. to phthln. Add 150 mL pet ether, wait ½ h, sep. from last drops of H_2O, and add 2 sheets of 9 cm filter paper in strips to separator. Shake, add 1 mg BHT, and transfer to r-b flask, rinsing separator and paper with pet ether.

Evap. soln by swirling (Rotavapor) under N stream in 40° H_2O bath. Dissolve residue immediately in 5 mL hexane.

43.C08 *Alumina Column Chromatography*

Transfer sample soln to column with aid of three 10 mL portions of hexane. Discard eluate (contains carotenoids). Elute column with seven 10 mL portions of ether–hexane (8 + 92) and discard eluate (contains tocopherols and ethoxyquin).

Elute column with seven 10 mL portions of ether–hexane (40 + 60), discard first 20–25 mL, collecting rest of eluate in r-b flask (contains vitamins A and D) when front of fluorescent vitamin A band is located 3 cm from bottom of column. Examine column <1 s under UV light (360 nm) with portable UV lamp to verify elution of vitamin A. Evap. soln by swirling (Rotavapor) under N stream in 40° H_2O bath. Transfer to centrf. tube, rinsing flask with 2–3 mL ether, evap. ether, and dissolve in 1.0 mL MeOH with warming. Add 1.0 mL CH_3CN and cool. Centrf. and use clear supernate for injection onto cleanup column.

43.C09 *Determination*

(a) *Cleanup.*—Inject 500 μL sample soln onto cleanup column thru sampling valve and adjust operating conditions of detector to give largest possible on-scale peaks for vitamin D. Collect fraction between 3 min before and 3 min after vitamin D peak in 10 mL vol. flask. Add 1 mL antioxidant soln and evap. to dryness under N stream. Dissolve residue immediately in 2.0 mL toluene–mobile phase (5 + 95). Use this soln for injection onto analytical column.

(b) *Assay.*—Inject 200 μL soln (a) onto analytical column thru sampling valve, and adjust operating conditions of detector to give largest possible on-scale peaks of vitamin D. Measure peak ht of vitamin D. Use same operating conditions and inject std soln B. Measure peak ht of vitamin D.

(c) *Calculation.*—

Vitamin D potency in IU/g sample

$$= \frac{1.25 \times P \times W' \times V \times 40\,000}{P' \times W \times V'}$$

where P = peak ht of vitamin D in sample soln; 1.25 = correction factor for previtamin D formed during refluxing for saponification; P' = peak ht of vitamin D in ref. soln; W = g sample weighed; W' = mg ref. std; V = total mL sample soln; V' = total mL ref. std soln; 40 000 = IU vitamin D/mg USP Ref. Std.

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(3) The following in vitro assay for predicting protein efficiency ratio (PER) as measured by rat bioassay was adopted official first action:

Calculated Protein Efficiency Ratio (C-PER and DC-PER) Official First Action

43.C10 *Principle*

Protein efficiency ratio is calcd from the essential amino acid composition of sample protein (DC-PER) or from both essential amino acid composition and enzymatic digestibility of sample protein (C-PER). Used together, C-PER and DC-PER models are capable of providing reliable ests of protein quality for majority of foods and food ingredients currently in use. Rat bioassay, **43.195-43.208,** remains official method for detg protein quality; C-PER and DC-PER assays are alternative methods for routine quality control screening of foods and food ingredients. Use of both assays is recommended when estg protein quality to provide internal check. Experience indicates that, in rare cases, the 2 models will report quite different ests of protein quality. When this occurs, it should be regarded as a warning that the sample under analysis is probably:

(*1*) single-cell protein or protein surrounded by heavy cell walls (e.g., yeast or wheat bran), where DC-PER will overest. protein quality, or

(*2*) partially or completely predigested proteins (e.g., liq. protein supplements), where C-PER will underest. protein quality, or

(*3*) protein sources known to possess significant quantities of proteolytic inhibitors (e.g., improperly heat-treated soy protein), where DC-PER will overest. protein quality.

For major discrepancies in PER predictions of the 2 models, use rat assay as assay of choice to est. protein quality.

Computational procedures for obtaining C-PER and DC-PER ests are too lengthy for repetitive hand calcn. For routine use of assay, it is recommended that algorithm be placed on computer.

43.C11 *Apparatus*

(**a**) *Amino acid analyzer.*—Able to accurately measure individual amino acids at concns as low as 20 nmolar. Must be stdzd using known amino acid stds at least once every 24 h.

(**b**) *Hydrolysis tubes.*—Any std Kimax/Pyrex text tube or ampule ≥15 mL capacity.

(c) *Water-jacketed chamber.*—To fit on stir plate and connected to 37° circulating H_2O bath.

(**d**) *Water bath.*—55°.

(**e**) *pH meter.*—Having combination pH electrode and capable of reading to 0.01 pH unit.

43.C12 *Reagents*

(**a**) *ANRC reference casein.*—Available from Teklad Test Diets, PO Box 4220, Madison, WI 53711.

(**b**) *Amino acid stds.*—ASP, THR, SER, GLU, PRO, GLY, ALA, VAL, MET, ILE, LEU, TRY, PHE, LYS, HIS, AMM, ARG, CYS, and TRP. Available from any amino acid analyzer supply house (e.g., Beckman Instruments, Fullerton, CA 92634; Pierce Chemical Co.).

(**c**) *Performic acid.*—Add 1 mL 30% H_2O_2 to 9 mL formic acid (88%). Let stand 1 h and cool to 0°.

(**d**) *Buffer soln.*—Use buffer recommended for sample diln for amino acid analyzer.

(**e**) *Enzyme solns.*—Use the following enzymes (Sigma Chemical Co.) or their equiv.: porcine pancreatic trypsin (Type IX), porcine intestinal peptidase (Grade I), bovine pancreatic α-chymotrypsin (Type II), bacterial protease (Pronase P or E). *Soln A.*—Dissolve 227 040 BAEE units of trypsin + 1860 BAEE units of α-chymotrypsin + 0.520 L-leucine β-naphthylamide units of peptidase in 10 mL H_2O. *Soln B.*—Dissolve 65 casein units of bacterial protease in 10 mL H_2O. Store both solns on ice.

(**f**) *Control protein.*—Suspend 10 g ANRC Na caseinate (**a**) in 200 mL H_2O and adjust to pH 8 with NaOH. Maintain at pH 8 ≥1 h. Freeze-dry and det. N content by Kjeldahl method.

43.C13 *Nitrogen Determination*

Det. N by **2.057, 7.057, 7.021,** or other appropriate Kjeldahl method.

43.C14 *Sample Hydrolysis*

(**a**) *Acid hydrolysis.*—Place ca 0.1 g (weigh to 0.1 mg accuracy) sample in hydrolysis tube, add 10 mL 6N HCl, and mix. Freeze in dry ice-alcohol bath. Draw and hold vac. of ≤50 μ for 1 min; seal tube under vac. Hydrolyze 24 h at 110 ± 1°. Cool, open tube, and filter hydrolysate thru

Whatman No. 1 paper; rinse tube 3 times with H_2O and filter each rinse. Dry filtrate at 65° under vac. Dissolve dry hydrolysate in vol. of buffer appropriate for amino acid analyzer. Store hydrolysate not >1 week before analysis. Use this hydrolysate to det. all amino acids except methionine, cystine and/or cysteine, and tryptophan.

(**b**) *Performic acid oxidation followed by acid hydrolysis.*—Place ca 0.1 g (0.1 mg accuracy) sample in hydrolysis tube, add 2 mL cold performic acid, and let sit overnight at 0–5°. Add 3 mL cold HBr + 0.04 mL 1-octanol (antifoam); immediately mix contents 30 s in ice–H_2O bath and evap. to dryness at 40° under vac. Add 10 mL 6N HCl to tube and perform acid hydrolysis as described above. This treatment will quant. convert methionine to methionine sulfone and cystine and/or cysteine to cysteic acid. Use this hydrolysate to det. methionine (MET) and cystine/cysteine (CYS).

(c) *Alkaline hydrolysis.*—Place ca 0.1 g (0.1 mg accuracy) sample into glass hydrolysis tube having Na Pergene polypropylene centrifuge tube as internal liner. Add 25 mg hydrolyzed potato starch (omit if sample is high in starch). Add 0.6 mL fresh 4.2N NaOH + 0.04 mL 1-octanol. Mix contents 2 min under partial vac. Freeze tube contents in dry ice–alcohol bath. Draw and hold vac. ≤50 μ 1 min; seal tube while under vac. Hydrolyze 22 h at 110 ± 1°. Cool, open tube, and transfer contents to 5 mL vol. flask contg sufficient cold 6N HCl to neutze hydrolysate; dil. to vol. using buffer appropriate for amino acid analyzer. Centrf. or filter hydrolysate and store frozen. Use this hydrolysate to det. tryptophan (TRP).

43.C15 *Amino Acid Analysis*

Analyze each of the 3 hydrolysates using parameters optimal for amino acid analyzer being used. Use std amino acid solns to calibrate analyzer at least every 24 h. Each amino acid peak should have ≥85% resolution. When alkaline hydrolysate is analyzed, tryptophan must be sepd from lysinoalanine. Compute for each of the following amino acids, the uncorrected g/16 g N: ASP, THR, SER, GLU, PRO, GLY, ALA, VAL, MET, ILE, LEU, TRY, PHE, LYS, HIS, AMM, ARG, CYS, and TRP according to:

g amino acid (uncorrected)/16 g spl. N = (η moles aa × initial spl. vol. (mL) × MW aa)/(vol. spl. injected (mL) × spl. wt (g) × %N for spl. × 6.25 × 10^5). Compute percentage recovery by detg N content for each amino acid:

g N contributed by each aa/16 g spl. N = (14 × No. of N atoms in aa/MW aa) × (uncorrected g aa/16 g spl. N)

% Recovery = Σ(g aa N for each aa/16 g spl. N) × 100

Note: If percent recovery is <86 or >105, error was made in hydrolysis procedure (weighing errors, diln, instrument calibration) or in computational process of percent recovery. Hydrolysis, analysis, and/or computation of percent recovery must be repeated until percent recovery falls within 86–105 tolerance *before* proceeding further. Adjust amino acid profile to normalize to 95% hydrolysis:

Correction factor = 95%/% recovery

Note: For each amino acid, compute the corrected g/100 g protein by:

g amino acid/16 g N (corrected) = correction factor × g aa/16 g N

43.C16 *In Vitro Protein Digestion – For C-PER*

Use sample or control wt contg 10 mg N.

Place appropriate quantity of control protein, ANRC Na caseinate (**f**) or sample, in labeled vial contg mag. stirring bar. Add 10 mL H_2O and let soak 1 h. Using pH meter, 37° bath, and stirrer, equilibrate sample and control to pH 8 ± 0.03 at 37° by addns of dil. HCl and NaOH. At this time also equilibrate enzyme solns to pH 8 ± 0.03 at 37°. Replace enzymes on ice; hold sample and control at 37°.

To equilibrated control vial, add 1 mL enzyme soln A while stirring. Exactly 10 min after addn of soln A, add 1 mL enzyme soln B, and then transfer vial to 55° H_2O bath. Exactly 19 min after adding soln A, transfer vial back to 37° bath, insert pH electrode, and read pH at 20 min. pH of casein control should read 6.42 ± 0.05 at 20 min. After proper pH reading is obtained for control, carry each sample thru identical procedure and read 20 min pH (X) for each. Calc. % protein digestibility as

$$\% \text{ Digestibility} = 234.84 - 22.56(X)$$

43.C17 *Computing the C-PER*

Compute C-PER using % digestibility and g amino acid/16 g N of: LYS, MET + CYS, THR, ILE, LEU, VAL, PHE + TYR, and TRP. When combining MET + CYS and PHE + TYR, the CYS and TYR can be no >50% of MET + CYS and PHE + TYR totals, resp. For example, if g amino acid/16 g N were: MET = 2 and CYS = 3, use 4 for MET + CYS total, because max. CYS can only be 50% of MET + CYS total.

Step 1: Express each essential amino acid as percentage of FAO/WHO std:

$$\% \text{ FAO} = [(\text{g aa}/16 \text{ g N})/\text{FAO/WHO std}] \times \% \text{ digestibility}$$

where FAO/WHO std is assumed to be: LYS = 5.44, MET + CYS = 3.52, THR = 4.00, ILE = 4.00, LEU = 7.04, VAL = 4.96, PHE + TYR = 6.08, TRP = 0.96.

Step 2: Examine each percentage of FAO/WHO std and adjust as follows: (a) If *all* percentages are >90% (before rounding to nearest integer) of FAO/WHO std, *and* LEU is <135% (before rounding to nearest integer) proceed to Step 3; otherwise, (b) if any percentage is >100, *reduce* to 100 and proceed to Step 3.

Step 3: Compute the following for sample protein and reference casein:

$$X = \Sigma [(1/\% \text{ FAO/WHO for each aa})(\text{wt})]$$

$$Y = \Sigma \text{ wts used}$$

Wts to be used in Step 3 computations:

*% FAO/WHO**	*Wt*
≥100	1
91–99	2
81–90	2.83
71–80	4
61–70	5.66
51–60	8
41–50	11.31
31–40	16
21–30	22.63
11–20	32
0–10	45.25

* Round to nearest integer.

Step 4: Divide the sum of wts (Y) by sum of reciprocols (X) for both sample protein and ref. casein. Results will be termed essential amino acid scores for sample and casein.

Step 5: Divide score of sample by score of ref. casein. Result expresses sample as the ratio of ref. casein, and is termed RATIO. If RATIO is >0.99 and <1.01, then PER of sample is 2.5 and program should terminate at this point, i.e., the sample is casein or its equiv.

Step 6: Compute the following: Z = RATIO × 2.5.

Step 7: Compute 4 discriminant values to det. group into which sample is to be classified. Discriminant equations are:

Group 1 = −671.8418 − 6.57689(LYS) + 3.56696(MET + CYS) + 13.10145(THR) + 2.54503(ILE) + 16.99881(LEU) − 0.43395(VAL) − 11.5244(PHE + TYR) + 31.55321(TRP) + 14.59278(Digestibility)

Group 2 = −666.4492 − 2.78584(LYS) + 5.17441(MET + CYS) + 13.08564(THR) + 4.61808(ILE) + 16.22603(LEU) − 1.63223(VAL) − 10.13673(PHE + TYR) + 32.60196(TRP) + 14.11668(Digestibility)

Group 3 = −619.0813 − 3.13909(LYS) + 4.26918(MET + CYS) + 10.00988(THR) − 1.42144(ILE) + 15.7547(LEU) + 5.6604(VAL) − 11.28705(PHE + TYR) + 30.49168(TRP) + 13.79953(Digestibility)

Group 4 = −744.7122 − 0.37674(LYS) + 6.03697(MET + CYS) + 11.51527(THR) + 1.63251(ILE) + 17.29687(LEU) + 3.0294(VAL) − 11.5033(PHE + TYR) + 37.88725(TRP) + 14.68169(Digestibility)

Step 8: Compute C-PER by examining the 4 group values computed in Step 7. Choose group number that has largest value and use that number to pick correct C-PER equation. For PER predictions, use following group equations when digestibility was estd by the 4 enzyme procedure:

Group 1: C-PER = 1.12683 − 1.61426(Z) + 0.99306(Z^2)

Group 2: C-PER = −7.25391 + 8.14063(Z) − 1.79517(Z^2)

Group 3: C-PER = 4.30469 − 1.99609(Z) + 0.45996(Z^2)

Group 4: C-PER = 12.75 − 8.21484(Z) +1.66016(Z^2)

43.C18 *Computing the DC-PER*

DC-PER is computed using steps just described in computing C-PER, with one additional step—percent protein digestibility is computed from amino acid profile instead of being detd via in vitro procedure. Coefficients for discriminant equations (Step 7) and PER predictive equations (Step 8) are also changed.

Compute digestibility from amino acid profile as follows:

Step 1: Compute the 3 group discriminant values for sample *and* ref. casein.

Group 1 = −203.7537 − 2.59402(LYS) + 9.27153(LEU) + 19.36964(ASP) + 4.19676(PRO) + 12.46035(CYS) + 34.3075(AMM)

Group 2 = −150.3707 − 0.78115(LYS) + 7.6239(LEU) + 15.46558(ASP) + 3.8947(PRO) + 12.79949(CYS) + 29.74493(AMM)

Group 3 = −155.9532 + 4.61135(LYS) + 7.85429(LEU) + 13.25949(ASP) + 4.68431(PRO) + 13.2907(CYS) + 19.89403(AMM)

Examine resulting discriminant values for sample protein and ref. casein, and choose group number associated with highest discriminant value. Use group number to det. which digestibility equation to use. If for sample, group equation No. 3 has highest value, then use digestibility equation No. 3 below to compute sample digestibility. If ref. casein had highest value from group No. 2 equation, then use digestibility equation No. 2 below to compute digestibility for casein.

Group 1

Digestibility = 67.8263 + 0.60144(LYS) − 1.73309(LEU) + 2.48377(ASP) + 2.03523(PRO) − 0.97312(CYS) − 6.44299(AMM)

Group 2

Digestibility = 160.5607 + 5.7998(LYS) − 2.20744(LEU) − 7.35627(ASP) − 0.85275(PRO) + 6.11058(CYS) − 14.54944(AMM)

Group 3

Digestibility = 116.5451 + 0.99537(LYS) − 4.37473(LEU) − 0.10243(ASP) − 0.06304(PRO) − 0.14005(CYS) + 3.48679(AMM)

Previous Step 1 (C-PER procedure) now becomes 1-A. Steps 2–6 remain as before.

Step 7: Substitute following discriminant group equations:

Group 1 = −350.9675 + 2.34642(LYS) − 8.60862(MET + CYS) − 13.80721(THR) + 11.71013(ILE) + 11.7984(LEU) − 12.10787(VAL) + 9.68089 (PHE + TYR) + 46.88927(TRP) + 7.291(Digestibility)

Group 2 = −454.6516 + 7.83575(LYS) − 14.3054(MET + CYS) − 15.64592(THR) + 13.32306(ILE) + 14.1817(LEU) − 17.40405(VAL) + 12.36894(PHE + TYR) + 64.39914(TRP) + 8.00712(Digestibility)

Group 3 = −405.9275 + 5.01252(LYS) − 8.46439(MET + CYS) − 15.014(THR) + 10.1986(ILE) + 11.91023(LEU) − 9.50181(VAL) + 9.46879(PHE + TYR) + 49.43095(TRP) + 7.78124(Digestibility)

Group 4 = −488.5569 + 9.3207(LYS) − 11.36379(MET + CYS) − 15.24675(THR) + 10.60119(ILE) + 13.93578(LEU) − 12.14625(VAL) + 10.15707(PHE + TYR) + 63.1489(TRP) + 8.22588(Digestibility)

Step 8: Substitute the following predictive equations:

Group 1: DC-PER = 1.254 − 2.04932(Z) + 1.30629(Z^2)

Group 2: DC-PER = −4.08594 + 5.125(Z) − 1.08398(Z^2)

Group 3: DC-PER = 4.66406 − 2.29297(Z) + 0.50586(Z^2)

Group 4: DC-PER = 10.44141 − 5.93359(Z) + 1.13281(Z^2)

44. EXTRANEOUS MATERIALS: ISOLATION

(1) The following interim official first action method for the determination of internal insect infestation of wheat, *J. Assoc. Off. Anal. Chem.* **64,** 1408 (1981), was adopted official first action to replace **44.037** which was deleted:

Wheat – Internal Insect Infestation

Official First Action

44.C01 ***Reagent***

Tween 80-Na_4EDTA [(ethylenediamine)-tetraacetic acid tetrasodium salt] premix soln.—Measure 420 mL 40% isopropanol in 500 mL graduate. Add 80 mL Tween 80 (polysorbate 80) to 100 mL g-s graduate. Invert 100 mL graduate over 2 L glass beaker and drain briefly. Rinse 100 mL graduate with several portions of the 420 mL 40% isopropanol, pouring each rinse into beaker. Add rest of 40% isopropanol to beaker, add mag. stirring bar, and start mag. stirrer. Add 10 g Na_4EDTA to beaker while stirring rapidly. Add 500 mL 40% isopropanol and stir until uniform. Mixed reagent stored in g-s flask is stable 1 week.

44.C02 ***Preparation of Sample***

Mix grain by passing 6 times thru Jones sampler, recombining sepns before each pass. Sep. slightly >50 g and weigh 50 g. Transfer weighed sample, small amt at a time, to 5 or 8 in. No. 12 sieve, and with stiff bristle brush, work insects thru sieve as completely as possible.

Grind screened sample in cutting-type mill set at 0.061 in. (An electric coffee grinder, Norelco "Dial-a-Bean" Model HB5115, North American Philips Corp., or equiv., using coarsest grinding position, can be used.) Dry damp or tempered grain in forced-draft oven 1 h at 70–80° or 2 h in oven without draft.

44.C03 ***Isolation***

Transfer cracked grain, including any residue in mill, to 2 L glass beaker contg mag. stirring bar, **44.002(p),** and mixt. of 600 mL H_2O + 50 mL HCl. Stir *gently* while boiling 15 min on hot plate.

Transfer sample to No. 100 sieve, **44.002(u),** with gentle stream of hot tap H_2O. Wash material on sieve with *very gentle* stream of hot (55–70°) tap H_2O until washings show no acidity when tested with blue litmus paper.

Add mag. stirring bar, **44.002(p),** to 2 L trap flask, **44.002(i)**(3). Place wide-stem funnel in flask opening and quant. transfer residue on sieve to flask with 40% isopropanol. Add 40% isopropanol to total vol. of 800 mL.

Clamp stirring rod so stopper or wafer is above liq. in flask. (Trap flask may stand overnight at this point.) Stir *gently* while boiling 7 min ± 10 s on mag. stirring hot plate. Remove flask from hot plate and wash down sides with min. of 40% isopropanol and immediately add 100 mL Tween 80–Na_4EDTA soln slowly down rod. Hand-stir *gently* 1 min and let stand 3 min.

Add 50 mL mineral oil, **44.003(y),** down stirring rod. Stir mag., **44.004(b),** 5 min on cool mag. stirrer, and let stand 3 min.

Fill flask with 40% isopropanol, added slowly down stirring rod to avoid mixing or agitation of flask contents, and let stand 20 min *undisturbed.* Trap off, rinsing neck of flask with 40% isopropanol, and add rinse to trappings in beaker.

Add 35 mL mineral oil to flask and hand-stir 1 min. Clamp stirring rod so stopper or wafer is at midpoint of flask. Let stand 5 min, spin stirring rod to free settlings from stopper or wafer, and adjust oil level with 40% isopropanol to ca 1 cm above fully raised stopper. Let stand *undisturbed* 15 min. Trap off, and combine trappings in beaker. Rinse neck of flask well with isopropanol, adding rinsings to beaker. Transfer trappings to ruled filter paper, rinsing beaker well with isopropanol. Examine papers at 15X, counting only whole insects, insect heads, cast skins, and head capsules.

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(2) The following method for the determination of light filth in rice flours (powders), extruded rice products, and rice paper was adopted official first action:

Light Filth in Rice Flours (Powders), Extruded Rice Products, and Rice Paper

Official First Action

44.C04 ***Sample Preparation***

(a) *Rice flours (powders).*—Preheat hot plate to max. heat. Add mag. stirring bar to 2 L beaker, and tare. Add 100 g sample. With forceful stream, add ca 100 mL hot tap H_2O. Add 75 mL HCl and fill to 800 mL mark with hot tap H_2O. Place hot mixt. on hot plate and, with mag. stirring, bring mixt. to vigorous boil. Boil 5 min. In small increments, transfer hot mixt. to No. 230 sieve. Reserve 2 L beaker. Wash residue with forceful stream of hot tap H_2O until foaming has subsided and H_2O is clear. Transfer residue to reserved 2 L beaker with 40% isopropanol. Add mag. stirring bar. Fill with 40% isopropanol to 800 mL mark. With mag. stirring, bring to boil on hot plate. Add 95 mL mineral oil, **44.003(y),** and boil and stir 3 min.

(b) *Extruded rice products and rice paper.*—Preheat hot plate to max. heat. Add mag. stirring bar to a 2 L beaker, and tare. Break up 225 g extruded product or paper while weighing into tared beaker. Add ca 1450 mL hot tap H_2O mixed with 150 mL HCl. Hand-stir with glass rod while heating to vigorous boil. When product is fluid enough, use mag. stirring. Boil 10 min. In small increments, transfer hot mixt. to No. 230 sieve. Reserve 2 L beaker. Wash residue with forceful stream of hot tap H_2O until foaming has subsided and H_2O is clear. Transfer residue to reserved 2 L beaker with 40% isopropanol. Add mag. stirring bar. Fill with 40% isopropanol to 800 mL mark. With mag. stirring, bring to boil on hot plate. Add 95 mL mineral oil, **44.003(y),** and boil and stir 3 min.

44.C05 ***Filth Analysis***

(a) *Rice flours (powders).*—Clamp off rubber hose on percolator, **44.002(i)**(2). Add 300 mL 40% isopropanol. Transfer hot sample mixt. from above to percolator. Rinse 2 L beaker with

40% isopropanol and pour rinse into percolator. With same beaker, add enough 40% isopropanol (ca 1 L) to fill percolator within 3 cm of top. Let stand 5 min and drain contents to 5 cm from bottom of oil layer. Repeat fill and drain steps at 2 min intervals with hot tap H_2O until aq. phase is clear. Drain as above. Drain oil layer into 1 L beaker. Rinse percolator sides with several alternate washes of H_2O, 40% isopropanol, and isopropanol, collecting rinsings in same 1 L beaker. A 1% sodium lauryl sulfate soln may also be used if needed for final rinse. Filter onto ruled filter paper and examine microscopically at ca 30×.

(**b**) *Extruded rice products and rice paper.*—Proceed as in (**a**), except for second cycling. Refill percolator with 20% isopropanol. Let stand addnl 5 min. Drain oil layer into 1 L beaker and rinse percolator as above.

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(3) The following method for the determination of *Geotrichum* mold in comminuted fruits and vegetables was adopted official first action:

Geotrichum Mold in Comminuted Fruits and Vegetables

Official First Action

44.C06 ***Sample Preparation***

(**a**) *Fruit nectars.*—Add 40 mL nectar and 10 drops of crystal violet stain, **44.003(p),** to 40 mL centrf. tube (Corning, Pyrex No. 8340, or equiv.). Mix well and proceed as in **44.C07.**

(**b**) *Purees with no added starch.*—Add 20 mL puree and 10 drops of crystal violet stain, **44.003(p),** to 40 mL centrf. tube. Mix well. Bring vol. to 40 mL with H_2O and mix well. Proceed as in **44.C07.**

(**c**) *Purees with starch added.*—Add 50 mL HCl soln (5 + 45) to 50 g fruit puree. Mix well and heat with mag. stirring until starch clears. Neutze soln with 50% KOH or 50% NaOH to pH 7.0 ± 1.0. Transfer 40 mL soln to 40 mL centrf. tube and add 20 drops of crystal violet stain, **44.003(p).** Mix well and proceed as in **44.C07.**

(**d**) *Pastes.*—Disperse 1 part paste in 3 parts H_2O. If necessary, warm gently to break gel. Transfer 40 mL soln to 40 mL centrf. tube and add 10 drops of crystal violet stain, **44.003(p).** Mix well and proceed as in **44.C07.**

44.C07 ***Centrifugation***

Centrf. 10 min as in **44.082.** Immediately after centrf. comes to rest, decant aq. layer and read vol. of sediment. Dil. sediment 1 + 3 (v/v) with stabilizer soln, **44.003(gg).**

Pipet and count as in **44.079(d).** Express results in mycelial fragments per 100 mL prepn.

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(4) The following method for Howard mold count in fruit nectars, purees, and pastes was adopted official first action:

Howard Mold Count of Fruit Nectars, Purees, and Pastes

Official First Action

44.C08 ***Sample Preparation***

(**a**) *Fruit nectars.*—Measure 40 mL well mixed sample into 40 mL graduated, thick-wall centrf. tube (Corning, Pyrex No. 8340, or equiv.) and proceed as in **44.C09.**

(**b**) *Fruit purees with no added starch.*—Dil. sample 1 + 1 with H_2O, measure 40 mL well mixed sample into 40 mL graduated, thick-wall centrf. tube, and proceed as in **44.C09.**

(**c**) *Fruit purees with added starch.*—Weigh 50 g fruit puree into beaker and add 50 mL HCl soln (5 + 45). Mix well and heat on steam bath 15 min. Measure 40 mL well mixed, hydrolyzed sample into 40 mL graduated, thick-wall centrf. tube and proceed as in **44.C09.**

(**d**) *Fruit pastes.*—Disperse 1 part paste in 3 parts H_2O. If necessary, warm gently to break gel. Measure 40 mL well mixed sample into 40 mL graduated, thick-wall centrf. tube and proceed as in **44.C09.**

44.C09 ***Centrifugation and Concentration Adjustment***

Centrf. 10 min at 2200 rpm as in **44.082.** Gradually let centrf. come to complete stop. Remove tubes and immediately decant supernate without disturbing sediment. Gently tap centrf. tube to level top of sediment. Dil. sediment with stabilizer soln, **44.003(gg),** as follows: (*1*) peach, apricot, mango, and papaya: 1 + 1; (2) pear and guava: 1 + 3; (3) strawberries, blackberries, raspberries, and blueberries: 1 + 6.

Proceed with Howard mold count as in **44.096,** beginning "Clean Howard cell . . ."

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(5) The following method for the determination of light filth in ground turmeric by using a solvent saver apparatus was adopted official first action as an alternative to **44.122(a):**

Solvent Saver Technique
Official First Action

44.122 ***Pretreatment***

(e) *Alternative solvent saver technic solvent reflux for ground turmeric.*—Weigh sample into 1 L beaker. Add 400 mL isopropanol and boil gently on hot plate 30 min with solv. saver app. inserted into beaker top. Solv. saver app. consists of support stands with clamped 1 L r-b flasks which are stoppered with 2-hole rubber stoppers. Each hole has glass tube and rubber hose attached. One hose is connected to cold H_2O source, the other to drain outlet. With cold H_2O circulating thru flask, which is inserted into 1 L beaker contg sample, solv. is heated to boil and allowed to reflux back into sample for set period of time. Multiple units should be set up in parallel, using "T" connectors, rather than in series, because increased temp. at end of series may affect efficiency of solv. reflux process. See Figure **44:C1.**

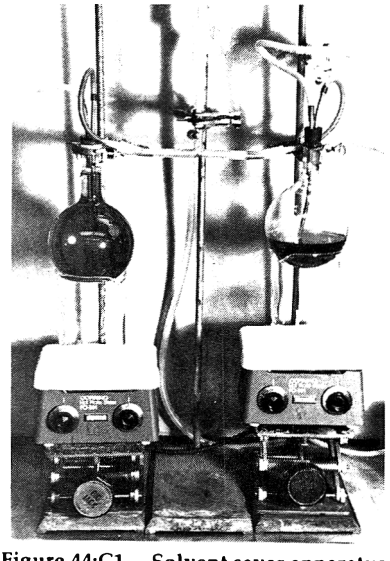

Figure 44:C1. Solvent saver apparatus.

(Precaution: Only use isopropanol as solv. with this technic.)

Pour sample into No. 230 sieve and wash with gentle stream of hot tap H_2O. Proceed with extn as in **44.123(b).**

45. FORENSIC SCIENCES

No additions, deletions, or changes.

46. MICROBIOLOGICAL METHODS

(1) The official first action revision, "Changes in Methods," *J. Assoc. Off. Anal. Chem.* **64,** 529 (1981), of **46.054-46.067** for the detection and identification of *Salmonella* to delete the combination of brilliant green, *Salmonella-Shigella* (SS), and bismuth sulfite agars and replace them by a combination of bismuth sulfite, xylose lysine desoxycholate, and Hektoen enteric agars was adopted official final action.

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(2) The official first action revision, "Changes in Methods," *J. Assoc. Off. Anal. Chem.* **64,** 529 (1981), of **46.054-46.067** for the detection and identification of *Salmonella* to extend the applicability of the method to edible casein and milk chocolate was adopted official final action.

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(3) Section **46.058(b)** in method **46.054-46.067** for the detection and identification of *Salmonella* was revised to clarify directions for the treatment of typical or suspicious triple sugar iron (TSI) agar cultures. Change **46.058(b)** to read:

(b) *Presumptive reactions.*—Incubate TSI and LIA slants at 35° for 24 ± 2 h and 48 ± 2 h, resp. Cap tubes loosely to maintain aerobic conditions while incubating slants to prevent excessive H_2S production. *Salmonella* cultures typically have alk. (red) slant and acid (yellow) butt, with or without H_2S (blackening of agar) in TSI agar. In LIA, *Salmonella* cultures typically have alk. (purple) reaction in butt. Consider only a distinct yellow coloration in butt of tube as an acidic (neg.) reaction. Do not eliminate cultures that produce discoloration in butt solely on this basis. Most *Salmonella* cultures produce H_2S in LIA. Retain all presumptive pos. *Salmonella* cultures on TSI (alk. slant and acid butt) agar for biochem. and serological tests whether or not corresponding LIA reaction is pos. (alk. butt) or neg. (acid butt). Do not exclude a TSI culture that appears to be non-*Salmonella* if the reaction in LIA is typical (alk. butt) for *Salmonella.* Treat these cultures as presumptive pos. and submit them to further examination. LIA is useful in detection of *S. arizonae* and atypical *Salmonella* strains that utilize lactose and/or sucrose. Discard only apparent non-*Salmonella* TSI agar cultures (acid slant and acid butt) if corresponding LIA reactions are not typical (acid butt) for *Salmonella.* Test retained presumptive pos. TSI agar cultures as directed in **46.058(c)** to det. if they are *Salmonella* sp., **46.062(e)**(*1*), or *S. arizonae* organisms, **46.062(e)**(*2*).

If TSI slants fail to give typical *Salmonella* reactions, pick addnl suspicious colonies from se-

lective medium plate not giving presumptive pos. culture and inoculate TSI and LIA slants as in (**a**).

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(4) The following method for the detection of *Escherichia coli* invasiveness by using monolayer HeLa cells prepared in antibiotic-free minimal essential medium with fetal bovine serum was adopted official first action:

Escherichia coli
Detection of Invasiveness
Official First Action

46.C01 ***Principle***

Invasiveness is detected by intracellular growth on monolayer of HeLa cells on slides. To minimize extracellular bacterial multiplication, host-pathogen interaction is resolved into 2 phases, infective and intracellular, using appropriate substrates and the following protocols: growth of monolayer in chamber slides, using controlled inoculum and period of incubation; detn of optimal pre-infection growth conditions for pathogen; washing pathogen to remove toxic end products; infection of host cell under controlled conditions of number and multiplicity of infection, and medium and length of incubation; subsequent removal of unattached bacteria; use of post-infection medium to permit only intracellular bacterial growth for limited period.

46.C02 ***Culture Media***

(**a**) *Minimal essential medium (MEM).*—Eagle-type with Earle's salts. Dissolve 126.4 mg L-arginine.HCl, 24 mg L-cystine, 292 mg L-glutamine, 41.9 mg L-histidine.HCl.H_2O, 52.5 mg L-isoleucine, 52.4 mg L-leucine, 73.1 mg L-lysine.HCl, 14.9 mg L-methionine, 33.0 mg L-phenylalanine, 47.6 mg L-threonine, 10.2 mg L-tryptophan, 36.2 mg L-tyrosine, 46.8 mg L-valine, 1 mg D-calcium pantothenate, 1 mg choline chloride, 1 mg folic acid, 2 mg isoinositol, 1 mg pyridoxal HCl, 1 mg nicotinamide, 0.1 mg riboflavin, 1 mg thiamine.HCl, 1 g glucose, 265 mg $CaCl_2$. $2H_2O$, 400 mg KCl, 200 mg $MgSO_4.7H_2O$, 6.8 g NaCl, 2.2 g $NaHCO_3$, 140 mg $NaH_2PO_4.H_2O$, and 10 mg phenol red in 1 L H_2O. Sterilize by filtration. Final pH should be 7.2 ± 0.2. Check sterility of all culture fluids before use. Store at 4–8°.

(**b**) *Fetal bovine serum (FBS).*—Sterile, virus-screened, mycoplasma-free, obtained aseptically during slaughter (Flow Laboratories, Inc., McLean, VA 22102). Store at 4–8°.

(**c**) *Antibiotic concentrate (AC).*—Dissolve 500 000 international units (IU) penicillin G and 500 mg streptomycin (Flow Laboratories, Inc.) in 100 mL H_2O and sterilize by filtration. Store at −10°.

(**d**) *MEM-FBS-AC medium.*—Routine medium for cultivation of HeLa mammalian cells. Mix 90 mL MEM (**a**), 10 mL FBS (**b**), and 1 mL AC (**c**). Store at 4–8°.

(**e**) *MEM-FBS medium.*—Medium for cultivation of HeLa cells before infection. Mix 90 mL MEM (**a**) and 10 mL FBS (**b**). Store at 4–8°.

(**f**) *Earle's salts.*—Prep. without phenol red as follows: Dissolve 6.8 g NaCl, 400 mg KCl, 265 mg $CaCl_2$, 200 mg $MgSO_4.7H_2O$, 140 mg $NaH_2PO_4.H_2O$, 1.0 g glucose, and 2.2 g $NaHCO_3$ in 1 L H_2O. Sterilize by filtration. Final pH should be 7.2 ± 0.2.

(**g**) *Veal infusion broth.*—Dissolve 500 g veal (infusion) and 10 g proteose peptone in 1 L H_2O with gentle heating. Dispense 5 mL portions into 13 × 100 mm screw-cap tubes. Autoclave 15 min at 121°. Final pH should be 7.3 ± 0.2.

(**h**) *Brain heart infusion (BHI).*—Dissolve 12.5 g BHI (powder) in 1 L Earle's salts (**f**). Sterilize by filtration. Final pH should be 7.2 ± 0.2.

(**i**) *Bile salts No. 3.*—Dissolve 5 g bile salts No. 3 formulation in 1 L Earle's salts (**f**). Sterilize by filtration.

(**j**) *Heat-inactivated HFBS.*—Heat FBS (**b**) 2 h at 55 ± 1°. Store at 4–8°.

(**k**) *HFBS-BHI-BS medium.*—Mix 20 mL heat-inactivated FBS (**j**), 10 mL BHI (**h**), 10 mL bile salts No. 3 (**i**), and 60 mL Earle's salts (**f**). Store at 4–8°.

(**l**) *Veal infusion agar slant.*—For maintenance of cultures. Dissolve 500 g veal (infusion), 10 g proteose peptone No. 3, 5 g NaCl, and 15 g agar in 1 L H_2O with gentle heating. Dispense 7 mL aliquots to 16 × 150 mm screw-cap tubes. Autoclave 15 min at 121°. Final pH should be 7.3 ± 0.2.

(**m**) *Dulbecco's phosphate-buffered saline (PBS).*—Dissolve 8.0 g NaCl, 200 mg KCl, 1.15 g Na_2HPO_4, 200 mg KH_2PO_4, 100 mg $CaCl_2$, and 100 mg $MgCl_2.6H_2O$ in 1 L H_2O. Sterilize by filtration. Final pH 7.2 ± 0.2.

(**n**) *Calcium- and magnesium-free Dulbecco's PBS.*—Dissolve 8.0 g NaCl, 200 mg KCl, 1.15 g Na_2HPO_4, and 200 mg KH_2PO_4 in 1 L H_2O. Sterilize by filtration. Final pH 7.2 ± 0.2.

(**o**) *Calcium, magnesium, phenol red-free Hank's PBS.*—Dissolve 8.0 g NaCl, 400 mg KCl, 90 mg $Na_2HPO_4.7H_2O$, 60 mg KH_2PO_4, 1.0 g glucose, and 350 mg $NaHCO_3$ in 1 L H_2O. Sterilize by filtration. Final pH 7.2 ± 0.2.

(**p**) *Trypsin stock soln.*—2.5%. Suspend 2.5 g 1:250 trypsin (Difco Laboratories) in 100 mL Ca- and Mg-free Hanks' PBS (**o**) and let particles settle. Sterilize by filtration. Dil. 10 mL stock soln with 90 mL sterile Ca- and Mg-free Dulbecco's PBS (**n**) to prep. 0.25% trypsin. Store at −10°.

(**q**) *Gentamicin stock soln.*—Dissolve 50 mg gentamicin (Schering Corp., Kenilworth, NJ 07033) in 100 mL Dulbecco's PBS (**m**) to give soln contg 500 μg/mL. Dil. 1 + 9 with Dulbecco's PBS to soln contg 50 μg/mL. Store at 4–8°.

(**r**) *Lysozyme soln.*—Weigh 0.3 g lysozyme, 3× crystalline, salt-free, ca 12 000 Shugar units/mg (Calbiochem-Behring, San Diego, CA 92112), into 100 mL Dulbecco's PBS and stir to dissolve. Store at 4–8° not >2 weeks.

(**s**) *Intracellular growth phase medium.*—Mix 80 mL MEM-FBS medium (**e**), 10 mL gentamicin soln (50 μg/mL) (**q**), and 10 mL lysozyme soln (**r**). Prep. immediately before use.

46.C03 *Diagnostic Reagents*

(**a**) *May-Grunwald stain*—Weigh 2.5 g stain (Matheson, Coleman & Bell) into 50 mL absolute MeOH, dissolve by grinding, and dil. to 1 L with MeOH. Stir 16 h at 37°. Hold stain 1 month at 22° (room temp.). Filter for use.

(**b**) *Giemsa stain.*—Dissolve 1 g stain (Matheson, Coleman and Bell) in 66 mL glycerol by heating 1.5–2.0 h at 55–60°. Add 66 mL absolute MeOH. Store stain 2 weeks in tightly stoppered bottle at 22°. Dil. stock soln (1 + 9) before use.

(**c**) *Decolorizing and dehydrating reagents.*—Acetone; acetone-xylene (50 + 50) and (33 + 67); xylene.

(**d**) *Mounting medium.*—Dil. mounting medium with xylene to give easily dispensed colloidal suspension; 20 mL Permount™ (Fisher Scientific Co.) dild with 5 mL xylene is satisfactory.

(**e**) *Human cervical epithelial cell culture.*—ATCC HeLa culture. Other cultures, including Henle 407 human intestine and human laryngeal carcinoma gave comparable data; however, HeLa cell culture was more suitable with regard to culture characteristics.

46.C04 *Apparatus*

(**a**) *Water baths.*—Maintained at 35 ± 1° and 55 ± 1°.

(**b**) *Microscopes.*—Standard 900× magnification; inverted stage, 100× magnification (Preiser Scientific, Charleston, WV 25322), or equiv.; microscope illuminator.

(**c**) *Carbon dioxide incubator.*—95% air–5% CO_2-moisture-satd atmosphere, maintained at 36 ± 1° (Lab-Line Instruments, Inc., Melrose Park, IL 60160, or equiv.).

(**d**) *Tissue culture chamber slides.*—Clean microscope slides mounted with partitions on plastic gasket to facilitate multiple testing. Lab-Tek units contg 4 chambers are satisfactory (Miles Laboratories, Inc., Naperville, IL 60540), or equiv.

(**e**) *Culture containers.*—Sterile 3 fluid oz (85 mL) glass prescription bottles or plastic tissue culture flasks (Costar, Cambridge, MA 02139, or equiv.).

(**f**) *Glass cover slips.*—1 × 2 in. (2.5 × 5.1 cm).

(**g**) *Cell-counting chamber.*—Spencer Bright Line, Fuchs-Rosenthal (Preiser Scientific), or equiv.

(**h**) *Refrigerated centrifuge with adapter.*—To accommodate 13 × 100 mm tubes and covered centrf. cups to prevent aerosolization of pathogens.

(**i**) *Membrane filters.*—0.45 μm pore diam. (Millipore Corp., Bedford, MA 01230, or equiv.).

46.C05 *Preparation of HeLa Cell Culture*

Using std cell culture technics, grow HeLa strain on inner surface of 3 oz glass or plastic container, using 5 mL MEM-FBS-AC medium, (**d**), for 7 days at 36° in CO_2 incubator. Replace with fresh culture medium on fourth day to prevent accumulation of toxic metabolites. In prepg cells in monolayer for transfer to chamber slides, wash once with 5 mL Dulbecco's PBS (**m**) prewarmed at 36°. Add 5 mL prewarmed (36°) 0.25% trypsin and hold at room temp. 2 min. Aseptically remove ca 4.5 mL trypsin. Incubate flask at 36° with occasional agitation. After monolayer has detached and cells are fairly uniformly distributed in residual trypsin, add 25 mL prewarmed (36°) MEM-FBS medium, (**e**). Est. cell density, using counting chamber. Add MEM-FBS medium, if necessary, to dil. suspension to density of 1 × 10^5 cells/mL. With occasional agitation, rapidly transfer 1 mL aliquots to chambers of slide. Incubate 20–24 h at 36° in CO_2 incubator. Aseptically remove spent medium before infection. Wash each monolayer once with 1 mL prewarmed (36°) Earle's salts, (**f**), and 1 mL prewarmed (36°) uninoculated infection medium, (**k**) (see below).

46.C06 *Preparation of Bacteria*

Inoculate, with needle, 5 mL veal infusion broth, (**g**), using growth from veal infusion agar slant (**l**) incubated at 22°. Incubate presumptive

E. coli broth cultures 18–24 h at 36°. Centrf. suspension 20 min at 1200× g at 18°. Resuspend cells in equal vol. of Earle's salts, (**f**). Recentrifuge 20 min at 1200× g. Resuspend cells in 5 mL Earle's salts. Dil. latter suspension with prewarmed (36°) HFBS–BHI–BS medium, (**k**), to final density of 5×10^7 cells/mL. Add 0.2 mL of each suspension to prepd chamber (above). Use 0.2 mL HFBS–BHI–BS for uninoculated neg. control.

46.C07 ***Infection Stage***

Incubate chambers 2.5 h at 36° in CO_2 incubator. Time factor is critical; shorter period results in min. number of infected host cells and longer period may result in cytotoxic effect arising from medium and possibly bacterial metabolites.

46.C08 ***Intracellular Growth Stage***

Remove infection medium from chamber with Pasteur pipet. To prevent contamination, use sep. pipet for each chamber. Wash each chamber twice with 1 mL aliquots of prewarmed (36°) Earle's salts. Subsequently wash with 1 mL aliquot of prewarmed intracellular growth phase medium (**s**) prepd immediately before use. Add 0.8 mL prewarmed intracellular growth phase medium to each chamber. Incubate 5 h at 36° in CO_2 incubator. Control of extracellular growth is critical at this stage; sensitivity of culture to gentamicin and other antibiotics should be examined by std procedures before pathogenicity testing. Problem is critical in meats and dairy products where antibiotics may have been used in therapy or in feeds.

46.C09 ***Staining***

Remove fluid contents of chambers. Wash monolayer 3 times with 1 mL Dulbecco's PBS (**n**). Add 1 mL absolute MeOH fixative per chamber. Hold at room temp. 5 min. Remove MeOH and side walls of chamber slide. Insert single-edge razor blade between gasket and slide, and gently pry gasket from slide. If necessary, cautiously remove remnants of gasket from slide with razor blade. Do *not* let specimen dry while slide is prepd for staining. Immerse slides in May–Grunwald stain (**a**) 10 min. Withdraw slides, remove excess stain, and immerse in Giemsa stain (**b**) 20 min. Withdraw slides, remove excess stain, and immerse in H_2O 10–20 s. Briefly rinse twice in acetone. Briefly immerse slides in following sequence of solvs: acetone–xylene (50 + 50), acetone–xylene (33 + 67), and xylene. Evenly distribute 4 drops of mounting medium, (**d**) to slide. Place large cover slip on prepn. Remove excess mounting medium and xylene by gently blotting. Gently apply pressure to remove air bubbles from prepn.

46.C10 ***Detection and Criteria of Invasiveness***

Examine specimens with 900× magnification. Criterion for intracellular location of bacteria is parfocality of cytoplasmic ground substance and bacteria. If invasive, *E. coli.* occur within cytoplasm. Frequently, they may be located along nuclear membrane. In addition, they may be elongated. Finally, bacteria may occur within a membrane (phagolysosome) individually or in groups, indicative of intracellular growth. Examine, at random, 10 fields contg 15–25 HeLa cells. Count bacteria in each cell. Criterion for infection is ≥5 bacteria per cell. Criterion for invasiveness of bacterial culture is ≥1.0% infected HeLa cells.

HeLa cells results with *E. coli* strains must be confirmed by Sereny keratoconjunctivitis test (1–3).

SELECTED REFERENCES

(1) *Acta Microbiol. Acad. Sci. Hung.* **2**, 292 (1955).
(2) *Acta Microbiol. Acad. Sci. Hung.* **4**, 367 (1957).
(3) *J. Hyg. Epidemiol. Microbiol. Immunol.* **3**, 292 (1959).

47. MICROCHEMICAL METHODS

No additions, deletions, or changes.

48. RADIOACTIVITY

The official final action method for the determination of cesium-137 in milk by gamma-ray spectroscopy, using simultaneous equations, **48.025–48.029,** was extended to include barium-140 and iodine-131. This extension, which had been adopted interim official first action, was adopted official first action.

Iodine-131, Barium-140, and Cesium-137 in Milk by Gamma-Ray Spectroscopy, Using Simultaneous Equations

Official First Action

48.025 ***Principle***

Applicable to ^{131}I, ^{140}Ba, and ^{137}Cs in fluid milk preserved with HCHO. Known vol. is placed in counting vessel positioned over and around right cylinder scintillation crystal detector, NaI(Tl), of multichannel gamma spectrometer. Gamma radiation is counted for given time. Accumulated pulses from selected photon energy range

are sepd from other gamma-emitting radionuclides and background radiation by simultaneous equations. ^{40}K is always present as natural contaminant and may contribute counts in 1 or more of photopeak ranges. Mutual interferences among these 4 photopeaks are eliminated by applying matrix technic to sep. activities of the 4 nuclides. Measurement of one std source of each nuclide provides the matrix coefficients.

In special cases, newly formed fission products may be present, e.g., ^{133}I and ^{135}I, which may interfere either thru direct overlapping of photopeaks or by contributing Compton-continuum counts. Such interference may be minimized by waiting for decay of short-lived radionuclides, by addnl counting following decay, or by chem. sepn.

Milk contg known increments of ^{131}I, ^{137}Cs, and ^{140}Ba, detd in triplicate by 25 laboratories, and 2nd milk contg known increment of ^{131}I, detd in triplicate by 40 laboratories, showed following results (av. of triplicates):

Amt Nuclide Present, pCi/L	Std Dev. (CV, %)		Bias ± 95% Uncertainty	
	Within Labs	Between Labs	pCi/L	%
^{131}I				
98	6.1 (6.2)	8.2 (8.3)	+0.9 ± 3.7	+0.9 ± 3.8
633	29.0 (4.6)	30.1 (4.8)	+2.3 ± 14.3	+0.4 ± 2.3
^{140}Ba				
72	6.5 (9.1)	11.2 (15.6)	+4.0 ± 4.8	+5.5 ± 6.7
515	19.5 (3.8)	35.8 (7.0)	+7.9 ± 15.8	+1.5 ± 3.1
^{137}Cs				
52	4.7 (9.1)	4.1 (8.0)	+1.3 ± 2.0	+2.4 ± 3.8
305	11.4 (3.7)	13.5 (4.4)	−9.8 ± 6.1	−3.2 ± 2.0
^{131}I				
82	5.6 (6.8)	6.8 (8.3)	−0.4 ± 2.4	−0.5 ± 2.9

48.026 ***Apparatus***

(**a**) *Alignment sources.*—Gamma ray energies, at least 1 near ^{137}Cs spectrum, with well known energies and abundance of gamma rays in photopeaks, for alignment. Solid sources, ca 0.1 μCi, are preferred over liq. sources. ^{207}Ba is satisfactory single source with several photopeaks; ^{137}Cs and ^{60}Co are good pair.

(**b**) *Counter.*—Low level gamma spectrometer consisting of shielded Tl-activated NaI scintillation detector, 4 × 4 in., coupled to multichannel pulse-ht analyzer and readout system.

(**c**) *Counting vessel (Marinelli beaker).*—Use 3.5 L beaker, Figure **48:01,** for 4 × 4 in. detector. Beaker and lid available from plastic laboratory-ware suppliers such as Bel-Art Products, Pequannock, NJ 07440, No. F26862 for beaker and No. 26872 for lid.

48.027 ***Reagents***

(*Caution: See* **51.075.**)

(**a**) *Carrier solns.*—10 mg/mL. Prep. solns of CsCl (1.267 g/100 mL), NaI (1.181 g/100 mL), and $BaCl_2.2H_2O$ (1.779 g/100 mL). Store in polyethylene or glass bottles.

(**b**) *Stock std solns.*—10 000 pCi/mL. Dil. calibrated solns of ^{131}I, ^{140}Ba, and ^{137}Cs to approx. indicated strength.

(**c**) *Potassium-40 stock std soln.*—1.89 dpm (disintegrations/min) ^{40}K/mg K. Dissolve 240 g KCl (equiv. to 126 g K) in 3 L H_2O in Marinelli beaker and dil. to 3.5 L.

(**c**) *Calibrating solns.*—For Cs and Ba, add 3–5 mL carrier soln, (**a**), to 3 L H_2O in Marinelli beaker, mix, add convenient amt of stock std soln, (**b**), sufficient to reduce counting error to ca 1% when counted within 10–100 min, mix, adjust pH to 3.5–4.5, and dil. to 3.5 L. Prep. I soln similarly, but adjust pH to 8.5.

48.028 ***Determination***

Using alignment sources centered on detector, adjust spectrometer to cover range at least between 0 and 2 meV, in intervals (channels) of 10 or 20 keV. Adjust voltage or gain control so that the 2 gamma photopeaks of std fall in their appropriate channels. Check and adjust alignment daily.

Place Marinelli beaker contg 3.5 L calibrating soln, (**d**), over detector, and count std for time

(10–100 min) sufficient to reduce counting error to ca 1%. Repeat with each calibrating soln and with H_2O. Recalibrate spectrometer yearly or more frequently if gamma ray resolution changes.

Transfer 3.5 L well mixed milk sample at room temp. into Marinelli beaker, place over detector, and count 100 min or time sufficient to give desired counting statistics.

48.029 ***Calculations***

(a) *Counter efficiency.*—Total individual counts observed in channels of photopeak range for each calibrating soln. Subtract total background count for same photopeak range. Divide net count by counting time in min and amt of radionuclide in pCi, and record cpm/pCi for each.

(b) *Interference coefficients.*—When counting std soln of each radionuclide, ^{131}I, ^{137}Cs, ^{140}Ba, ^{40}K, e.g., ^{131}I, ratio of net counting rate in energy range of each of the other radionnuclides to net counting rate in its own photon energy range gives its fractional interfering coefficient for each of the other energy ranges, e.g., ^{131}I ratio of net counting rate in ^{137}Cs energy range to net counting rate in ^{131}I photon energy range gives its fractional interfering coefficient for ^{137}Cs energy range.

Designate counting rate for ^{131}I, ^{140}Ba, ^{137}Cs, and ^{40}K with symbols I, B, C, and K, resp. Designate net counting rates (observed—background) in their resp. photon energy ranges as N_i, N_b, N_c, and N_k, resp. Then, f, fractional coefficients or contributions of nuclide in particular range, is designated by 2 lower case subscripts; first one indicates nuclide contributing counts to energy range (column) and second, nuclide photon energy range (row). The following 4 equations:

$$N_i = I + f_{bi}\,B + f_{ci}\,C + f_{ki}\,K \quad (1)$$

$$N_b = f_{ib}\,I + B + f_{cb}\,C + f_{kb}\,K \quad (2)$$

$$N_c = f_{ic}\,I + f_{bc}\,B + C + f_{kc}\,K \quad (3)$$

$$N_k = f_{ik}\,I + f_{bk}\,B + f_{ck}\,C + K \quad (4)$$

can be solved simultaneously by matrix algebra, using inversions to provide numerical consts W, X, Y, and Z in equations 5, 6, 7, and 8. These consts are used to solve for concn of each of these 4 nuclides in sample. Net counting rate for each nuclide is:

$$^{131}I = I = W_1N_i + W_2N_b + W_3N_c + W_4N_k \quad (5)$$

$$^{140}Ba = B = X_1N_i + X_2N_b + X_3N_c + X_4N_k \quad (6)$$

$$^{137}Cs = C = Y_1N_i + Y_2N_b + Y_3N_c + Y_4N_k \quad (7)$$

$$^{40}K = K = Z_1N_i + Z_2N_b + Z_3N_c + Z_4N_k \quad (8)$$

Calibration to derive values for consts in equations 5, 6, 7, and 8 is applicable as long as instrument alignment and mode of operation remain const and gamma-emitting nuclides are limited to the 4 elements in matrix. Long-hand inversion of **4 × 4** matrix is tedious and subject to mistakes. Use of computer is recommended to provide numerical consts for equations 5–8. Thereafter, desk calcns can det. concns of ^{131}I, ^{140}Ba, ^{137}Cs, and ^{40}K in samples in absence of computer by summing counts in each photopeak, subtracting background, and applying equations 5–8.

(c) *Iodine-131, barium-140, cesium-137, potassium-40 activities.*—From spectral gamma counts of sample, substitute net value from equations 5 thru 8 and convert net counts/min for each nuclide to pCi/L milk at time of counting:

$$^{131}I(pCi/L) = (\text{net cpm})_i/(E_i \times V)$$

$$^{140}Ba(pCi/L) = (\text{net cpm})_b/(E_b \times V)$$

$$^{137}Cs(pCi/L) = (\text{net cpm})_c/(E_c \times V)$$

$$^{40}K(pCi/L) = (\text{net cpm})_k/(E_k \times V),$$

where E_i, E_b, E_c, E_k = counting efficiency/pCi from std solns for ^{131}I, ^{140}Ba, ^{137}Cs, ^{40}K, resp., and V = sample vol., L.

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, 1982

The following changes should be made in the 13th edition:

Section	*Page*	
7.066	134	Change method title to read: ***Fritted Glass Crucible Method (20)*** ***Official Final Action***
16.085	249	Line 5: Change to read ". . . for series of ≥8 preanalyzed (**16.056** or **16.057**) . . ."
16.125(f)	254	Change to read: (**f**) *Phenol std solns.*—(*1*) *Stock soln.*—Accurately weigh 1.000 g pure phenol, transfer to 1 L vol. flask, dil. to vol. with 0.1N HCl and mix (1 mL = 1 mg phenol. Soln is stable several months in refrigerator). (2) Dil. 5 mL stock soln to 500 mL with H_2O and mix (1 mL = 10 μg phenol). (3) Dil. 10 mL soln (2) to 100 mL with H_2O and mix (1 mL = 1 μg phenol). Working stds.—Dil. 0.0, 0.5, 1.0, 2.5, and 5.0 mL to 5.0 mL with H_2O to prep. std solns contg 0.0, 1.0, 2.0, 5.0, and 10.0 μg phenol equiv./sample, resp. Prep. fresh daily.
16.125(g)	254	Change to read: (**g**) *Color std solns.*—Prep. working std solns in series of tubes, add 0.5 mL buffer (**a**), then add 0.1 mL CQC soln (**c**) and 2 drops of catalyst (**d**), or use 0.1 mL Indo-Phax soln (c). Mix well, and incubate 5 min at 40°. Remove from bath, and ext with 3 mL BuOH as in **16.126.**
18.075(a)	297	Line 1. Change to read ". . . 0.2M each Na_2CO_3 . . ."

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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Edited by
William Horwitz
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Washington, DC

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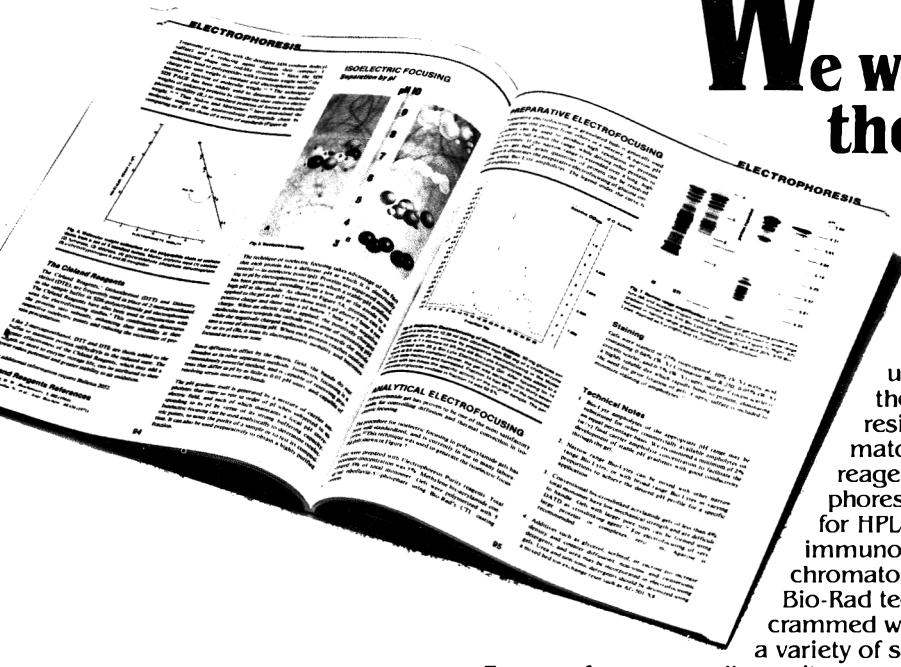

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