HISTORY OF THE

U. S. FOOD AND DRUG ADMINISTRATION

Interview between:

J. William Cook

Retired Director, Division of

Pesticide Chemistry and Toxicology

and

Fred L. Lofsvold, FDA

James Harvey Young, Emory University

Falls Church, Virginia

June 18, 1980

INTRODUCTION

This is a transcription of a taped interview, one of a series conducted by Robert G. Porter and Fred L. Lofsvold, retired employees of the U. S. Food and Drug Administration. The interviews were held with retired F.D.A. employees whose recollections may serve to enrich the written record. It is hoped that these narratives of things past will serve as source material for present and future researchers; that the stories of important accomplishments, interesting events, and distinguished leaders will find a place in training and orientation of new employees, and may be useful to enhance the morale of the organization; and finally, that they will be of value to Dr. James Harvey Young in the writing of the history of the Food and Drug Administration.

The tapes and transcriptions will become a part of the collection of the National Library of Medicine and copies of the transcriptions will be placed in the Library of Emory University.

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION

TAPE INDEX SHEET — CASSETTE NUMBER(S) 1 and 2

GENERAL TOPIC OF INTERVIEW: History of the Food & Drug Administration

DATE: 6/18/80 PLACE: Falls Church, Virginia LENGTH: 100 minutes

INTERVIEWEE	INTERVIEWER *
NAME: J. WILLIAM COOK	NAME: Fred L. Lofsvold
ADDRESS: [redacted]	ADDRESS: U.S. Food & Drug Administration
[redacted]	Denver, Colorado

FDA SERVICE DATES: FROM 1939 TO 1972 RETIRED? Yes

TITLE: Director, Division of Pesticide Chemistry and Toxicology
(If retired, title of last FDA position)

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		6	4	Development of fly bioassay method for organic pesticides.
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		17	6	PCB problem - background and analytical solution.
		23	9	Pesticide methods development through AOAC Collaboration.
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	B	2	14	FDA approval of new pesticides and tolerances for residues.
		8	17	Cook develops spot tests for organo-phosphorus pesticides.
		23	22	Examples of use of spot tests.
2	A	10	28	Discovery of the thermionic detector for gas-liquid chromatograph.
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	B	6	39	Cook receives HEW award.
		9	40	Commissioner Larrick's and Commissioner Edwards' interest in research.
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* Also James Harvey Young, Professor of History, Emory University, Atlanta, GA.

This recording is being made with J. William Cook, a retired scientist from the Food and Drug Administration at his residence in [redacted], [redacted]. The recording is part of the FDA oral history project. The date is June 18, 1980. Also present is James Harvey Young, Professor of History, Emory University. My name is Fred Lofsvold.

FL: Mr. Cook, would you briefly describe your career with the Food and Drug Administration?

JWC: I started with the Food and Drug in September of 1939, reporting to the Seattle District. About two years later, I was transferred to a sublaboratory of Seattle District in Portland, Oregon. During those two periods, I did general laboratory work, including quite a bit of pesticide analysis for lead, arsenic and fluorine. But Mr. Harvey asked me to transfer to San Francisco to do some general research on foods. I moved to San Francisco in 1943 and was there 9 years. Then I was transferred to Washington, D.C. into the Division of Food. I had developed some expertise in the use of enzymes looking for contaminants in foods while in San Francisco. Enzymatic techniques were useful in measuring the organo-phosphorus pesticide chemicals so I began to apply them to FDA's problems and that got me into pesticide work, beginning about 1952. I continued mainly in the field of pesticides until I retired in 1972.

JHY: Would you say just a word about your education before you became employed by FDA?

JWC: OK, I received my Bachelors and Masters from Oregon State with a major in Chemistry and a minor in Nutrition. I was interested in the biological aspects of chemistry. My first job out of Oregon State was on the Agricultural Experiment Station staff of Washington State College, now Washington State University. I was in the School of Agriculture, Biochemistry Department and did cooperative work with the Department of Poultry on poultry nutrition. From there I went to the Food and Drug Administration.

JHY: Can you remember the first moment that you encountered the idea of an organo-pesticide? In contrast with the metallic ones?

JWC: No, I really can't remember when I first knew about the general subject of organo-pesticides. I remember being fascinated by the plant growth hormones, a subject which guided people into herbicides. That was about 1936-37.

I guess it was when I first came to D.C. in 1952 and was attempting to use the enzymes as means of determining food contaminants, that I became fascinated with the organophosphorus compounds. There was some secret work being done in the Division of Food at the same time on the war

nerve gases. The organo-phosphorus pesticides are related to the so-called war nerve gases, the main difference being that the pesticides usually are solids, or liquids rather than gases at room temperature.

JHY: I got you off the track there, but since there was a bridge in history of kinds of things used for pesticides, I was just curious about where you saw them first, the new ones coming?

JWC: For years I had read the current scientific journals and was always attracted by articles that concerned compounds with biological activity. In those days companies that were developing data on new pesticide chemicals brought data on chemistry and toxicology to FDA scientists in order to inform us and to get our reactions to their procedures and data. (This was before such data were required to be submitted by the Miller Amendment to FDA Law in 1954.) When there were meetings concerning organo-phosphorus compounds I was asked to attend. I quickly became interested and intrigued with both the chemistry and the biological effects of these compounds. Organic phosphorus compounds were developed in Germany during World War II. Schrader who worked for Farbenfabriken Bayer at Leverkusen, Germany, was working on them as possible insecticides. The Germans recognized the fantastic toxicity of these compounds and did develop them as war nerve gases. It wasn't until after the war,

when some U.S. people went into Germany that we got acquainted with these compounds and brought some of them to the U.S.

I thought I would give you a little history of the development of the general chemical analysis for pesticides that we in Food and Drug developed through continued intensive research and organization. The first sort of general method was developed by Dr. Ed Laug in the Division of Toxicology. He used the fruitfly as a bioassay organism. He extracted plants, did some clean-up or purification of the extracts, and then exposed groups of flies to the extracts. An estimate of the pesticide in the plant extract was made by comparing the number of flies killed by the extracts, to the number of flies killed by the standards. This simple procedure was useful, but it was soon found to have a number of limitations. Dr. Al Klein in the Division of Food was working closely with Dr. Laug and he found that some samples of food had naturally occurring compounds present which were extracted and had a tendency to either give a false positive or a false negative result. He did considerable work in attempting to clean up the extracts to eliminate the false results and samples for improving the certainty for quantities and identity. Later, Mr. Paul Mills joined the Division of Food and he too began to do work to improve the technique. The fly bioassay was developed primarily for a screening technique, i.e., a technique of looking for any pesticide that may be present in a sample of unknown

history. But, of course, it was useful for those samples in which the pesticide was known and a quantitative result was needed. The pesticides in use at that time were primarily the chloro-organics, DDT, chlordane, benzene hexachloride, etc.

Mills was interested in improving the screening techniques because most samples FDA analyzed were of unknown pesticide spray history and many new chemicals were being developed and put on to the market. He used paper chromatography as a means to clean up or purify the extracts from the plants so that there would be less uncertainty, i.e., fewer false positivies and negatives in the fly bioassay. As he worked with the paper chromatography and was able to detect amd measure the chemicals on the paper, he found that he could estimate better quantitative results on the paper than was possible to achieve with fly bioassay and also was able to identify the chemical which was usually not possible by fly bioassay. He did continue doing both the fly bioassay and the paper chromatography for a while, but finally just stopped using the fly bioassay. Also he found that the paper chromatography did not give a complete clean up. Again, he got some false positives and some false negatives unless the sample was fairly well purified before it was put on to the paper. He started using column chromatography to further purify the extracts. Owen Winkler, in the Division of Food, had been using columns to do clean up for some

arsenic and lead work. Mills got some of his clues from Mr. Winkler. The clean up or purification procedures developed by Mr. Mills to give really good results with the paper chromatography were found to be very useful when gas chromatography was developed.

In 1959, Mr. Mills published an article in which he pulled together the various steps in pesticide analysis identifying the type of detectors for gas chromatography, the clean up or purification procedures, etc. That is the basis for a screening or multi-detection system now utilized by Food and Drug Adminstration and by many other laboratories throughout the world. The procedure is generally known as the Mills procedure; however it has been expanded and developed by a number of other people over the past 20 years to the point where 100, or more pesticides, including chlorinated compounds, carbamates and organo-phosphorus compounds, can be run through the scheme to obtain qualitative and quantitative data with a high degree of certainty. The procedure is useful for a large variety of food products. A number of adaptations have been added so that, if there is some uncertainty, an identifying procedure can be run on certain portions of the extracts to give a second analysis to give a higher degree of certainty of the identity. This publication was in the Journal of the Association of Official Agricultural Chemists, Vol. 42, page 734, 1959. The sequel to the Mills Procedure, that is, the greatly elaborated procedure, is a loose-leaf publication of the Food

and Drug Administration called Volume I Pesticide Analytical Methods.

JHY: This is a complex manual, constantly updated, and, so far as we know, until very recently, still being updated and definitely employed for analyses of pesticide residues by FDA chemists. Now in this complex book of procedures which could be applied, are there incidents in which applications had an important regulatory influence, or in some other way became important to society that you could cite for us?

JWC: Yes, one very striking example is that, by use of this technique, it is possible to separate the PCBs from the pesticide chemicals. The PCBs, (polychlorinated biphenyl compounds), which have become controversies in many places, originally showed up on gas chromatograms as interfering peaks. A separate clean-up procedure or column was devised to separate the PCBs from DDTs, etc., so that more accurate analysis of DDT could be made. But, likewise, even though PCB is not a pesticide, this procedure permitted us to develop a very good procedure for the PCBs, in foods, in packaging materials and in plastic food wraps, etc. That is an outgrowth of this analytical procedure.

JHY: It was sort of a serendipitous side result that initially was kind of a problem to you, but later on proved to be of regulatory benefit.

JWC: Right. This separation of the PCBs from the pesticide chemicals proved to be a real boon to us when the National Cash Register Company's no-carbon copying paper found its way into the manufacturing of food packages and then migrated into food.

JHY: That is to say the scraps of paper were mixed with other paper.

JWC: Right. And relatively large quantities of PCBs were in the cardboard containers.

JHY: Is that because the PCBs were in the mechanism by which the carbon transfer was made in these papers?

JWC: Right. The dyes that produced the color on the second and third copies were dissolved in PCBs and those were encapsulated into minute capsules and were in a layer on the paper. When pressure was put on paper by the pen or typewriter, those capsules broke and allowed the dye to dye the second paper. All the scraps would have heavy layers of these chemicals and those scraps and the waste paper cast out by the industries and government, as scrap, were incorporated into the material from which most cardboard is made.

FL: Then the PCBs would migrate from the packing, the cardboard, to the food that was packed in that kind of a package?

JWC: That's correct. But, besides that, the PCBs got into other elements of the environment, such as the streams, and thereby contaminated fish and other food products. There were many other sources of PCBs, besides the copying paper, which contributed to PCBs in the environment. This analytical procedure was easily adapted to PCB analysis in a variety of food and other products.

JHY: You were more involved personally in developing the methods, rather than in employing the methods with regard to food products, which had been seized as suspect and needed to be tested. This was done somewhat more in the field. So you were on the research side of improving the techniques by which analyses could be made to find the residues.

JWC: I did not do laboratory work on any phase of the Mills Procedure. In 1959 I became Chief of the Pesticide Branch. My job there was to supervise and encourage the continued improvement of the procedure, as well as supervise the petition review for tolerances. In 1963 I became Deputy Director of the Division of which Pesticide Branch was a part. As Deputy I continued to monitor the pesticide work. Our division was involved with improving this technique, but

we also did a good deal of testing to be sure that it would work and we did work with the field districts a good deal in performing these analyses. It so happens that the problem of PCBs in cardboard packages developed at the time when we first produced this clean up procedure for eliminating the PCBs from the DDTs. And so our laboratory did get deeply involved in the analysis. We even helped industry laboratories, had meetings of industry people and field laboratory people to help them proceed with the analysis for themselves.

JHY: Was this also true in connection with pesticide residues, did you meet with industry in an effort to put your joint talents together in such a way as to reduce or eliminate problems in connection with residues getting into the food supply?

JWC: Oh, yes in many ways. For instance, the procedure as it is in the manual now has been the result of a fantastic amount of research work, most of that research work has been oriented toward the theme of the Association of Official Analytical Chemists over the years. And there are many references in the manual to the collaborative work done in the AOAC system. And that system involves state and federal Food and Drug scientists, as well as industry, municipal health and food officials. Many times the samples for collaborative studies were sent to a number of Food and Drug

District laboratories and may have gone to a number of industry state, municipal, and other governmental laboratories, including Canadian Food and Drug, to determine the results that they obtained as against those obtained by the author or developer of the various aspects of the procedure. The results of these collaborative studies were then presented to the Association at their yearly fall meeting, and are heard by all interested parties. Any interested party is welcome to attend the meeting and has opportunity to comment. The results are then reviewed by Association committees and accepted or rejected as official or unofficial methods and ultimately incorporated into the AOAC manual.

JHY: One of the striking things about the manual is that it provides procedures for analyzing samples that may have multiple pesticides in the sample. Now why, from the state of the marketplace, was it necessary to develop such complex procedures? Does this mean that apples or lettuce or some other product in this early age of the new pesticides were being sprinkled with many pesticides so that you had samples that might have 4 or 5 and therefore needed the new complex procedures?

JWC: The procedures in this manual are really relatively simple. Many, many of the pages you see are data which show the chemist the supporting data for each page, for example, some tables show the % recovery of maybe 100 chemicals

through a certain step of the procedure, etc. There are many facets to the answer to that question. Generally speaking, the same lettuce sample, for instance would not necessarily have a variety of chemicals on it, but there were a number of chemicals that were registered for use on lettuce, or apples. Therefore, we wanted a procedure to analyze lettuce that would identify and measure any possible chemical or combination of chemicals that may be present on samples of unknown spray history, without going through a multitude of individual analyses. You indicated that this procedure is complex. Basically, to the chemist, this is much less complex than running the procedures for a number of different chemicals on lettuce. With this multi-residue procedure, it is necessary only to make one extract, one clean-up or purification, one pass through the gas chromatograph, and any of a large number of chlorinated and many organophosphorus pesticides that may be present would be identified and measured. Such a lettuce sample can be analyzed for all these pesticides in a matter of a new hours, in contrast to many days by individual methods.

JHY: Now most samples would only have one or two pesticides on them?

JWC: That's right. So again this is sort of a fortuitous outgrowth of attempting to make a good simple analysis. With

DDT for instance, it used to be a relatively difficult to be certain that you were analyzing for and measuring DDT. In an attempt to make that analysis and adding these new techniques of chromatographies and broad spectrum detectors, when we determined DDT, we automatically determined the others, so we just took advantage of that.

JWC: For instance, when the first petitions for aldrin, dieldrin and endrin came, the method's sensitivity was so poor in relation to the toxicity, that we decided not to set a tolerance for endrin because the method would not determine the amount that we considered to be toxic. Aldrin and dieldrin were marginal methods with respect to toxicity. And those chemical methods were very complex and very non-sensitive. The new procedure that is in this manual is so simple and so accurate, so sensitive to dieldrin and aldrin both that they can be determined if only one thousandth of one millionth of a gram was present, aldrin responds so beautifully in this multiresidue procedure that aldrin was used as a reference standard to be sure that the gas chromatagraph was working properly and that the techniques are working properly. So it was just a fortuitous benefit to all the problems of analysis to work and develop this kind of technique.

JHY: That really makes it much more clear to me. Thank you. Now, let me throw in a judgmental question, you were

watching the new pesticides come along and developing tests to detect them. And also the Food and Drug Administration had the responsibility at this period of deciding whether or not new ones should go on the marketplace. As you, as a scientist deeply involved in this look back upon your experience when these new ones were coming on the market so fast, what is your impression about the part of FDA's responsibility toward admitting them? Do you feel that FDA was properly cautious about admitting them to the marketplace or too cautious or too lenient?

JWC: A number of the pesticide chemicals, primarily the chloro-organics, were in use before the 1954 Miller Amendment to FDC Act. This amendment mandated that FDA set tolerances based on chemical, toxicity and residue data submitted to FDA by the manufacturer. Before the Miller Amendment, Food and Drug had to prove in court that the food, that that particular sample of food was poisonous and did not have responsibility or authority of approving or disapproving of a chemical before it was in use.

JHY: It had to set tolerances after the things were on the market?

JWC: The only official tolerances set before the Miller Amendment was after long drawn out hearings held in 1950. At

those hearings, the proponents did not have to have any toxicity or other data except to show that the chemical had been used. In 6 months of formal hearings, 20,000 pages of testimony were recorded. From this, Food and Drug did set some tolerances based on the use data and FDA's and Public Health's knowledge of toxicity.

JHY: Even though there might be a toxicity question still somewhat open.

JWC: Right, when the Miller Amendment was enacted, it provided that toxicity data had to be provided to the Division of Pharmacology so they could determine a safe level and a method had to be available to adequately analyze for the tolerance level in food before the tolerance could be established. Of course, some of the tolerances established through the Miller Amendment were before we had really good methods. So there was a degree of uncertainty in the methods of analysis for some of these compounds. For instance, DDT--the analysis for it was based on the total chlorine analysis even though there is some chlorine normally present in food products. For example, 0.2 part per million DDT might be calculated from the chlorine present in an untreated sample. And then you might calculate 0.4 parts per million based on a sample that had been treated with DDT. The chemist subtracted the two tenths from the four tenths and said that the DDT present was two tenths. But the

calculation was made on the assumption that the chlorine did in fact come from DDT. However, this was not a certainty. It could have been other problems. Now in the case of the aldrin and dieldrin that I referred to before, again it was basically a total chlorine procedure. With a blank of 0.1 ppm calculated as aldrin and a sample of 0.1 ppm and the proposed tolerance in the range of .1, there was uncertainty. Even though the Miller Amendment mandated method submission to FDA, we felt that we had to have more efficient analytical procedures to do adequate surveys of food samples in commerce. The multi-residue procedure previously described was the answer to many of the methods problems we faced in setting and enforcing the tolerances. And the toxicity -- there is never enough data to be absolutely sure of anything, so judgements had to be made on all data. Sometimes tolerances were not established because of lack of proof of safety. Sometimes we went back and rescinded tolerances or lowered some tolerances because of new data.

JHY: So you did feel a sense of urgency because you knew that things were going on the market and that there was scientific uncertainty. It was part of your task to develop the scientific certainty which would clarify some of these doubtful matters.

JWC: We assumed it to be our task. Certainly the law didn't say that, but we assumed it to be our task to continue to develop methods to make it easier for us to know or easier for us to make surveys to find the end product of having set a tolerance and to pass judgment on extension of the tolerances to other products. When I discuss the organo-phosphorus history, I'll bring in some details on that. As I said previously, when I worked in San Francisco, I was attempting to use enzyme systems to help identify food contaminants. I was transferred to D.C. in 1952 to continue that kind of work. There wasn't anybody else doing similar work. Mr. Vorhes, who was then the Director for the Division of Food, suggested that I look around and find some aspect of the Division work that might be enhanced with the use of the enzyme systems. I went to Nutrition and to Antibiotics and people in the Division of Food, various aspects of foods, freezing and so forth--and the pesticide people and I, of course, was studying the literature a good deal. It seemed to me that one of the best uses of the enzyme work at that time would be the organo-phosphorus compounds, because these compounds are toxic by virtue of the fact that they are inhibitors of the cholinesterase enzymes. The cholinesterase enzymes hydrolyze to a compound called acetylcholine. Acetylcholine is involved in the transmission of nerve impulses. Therefore muscle activity is based on acetylcholine being formed and hydrolyzed quickly. When those enzymes are inhibited, the person

becomes rigid or has tremors. I attended meetings with industry as industry brought in data to tell Food and Drug about the chemicals that they were anticipating putting into use, especially when organo-phosphorus compounds were discussed. It was then that I learned about the earlier work done on the organo-phosphorus compounds. When I started looking into the literature to see how people were doing the research on the esterase systems and in inhibition of esterases, I found that most of them had relatively complicated (for those days) pieces of equipment. For me that was impossible, because Food and Drug was pretty poor then. Our total budget was about five million dollars and that's for the whole nation. There was no laboratory equipment really, even beakers and pipettes available for me. I had to figure out some less expensive way to do the work. I had been fascinated with what is called spot tests and that is the use of qualitative reagents that reveal small amounts of chemicals spread out like ink in a blotter. For instance, in San Franciso, I devised a test for urea by having the enzyme urease put in a piece of paper along with a dye that would change with acid-base. So if you put a spot or a chunk of flour or some wheat grains or something like that that had rat urine on them, they would turn the paper a different color in spots where there was some urine. It's simple, cheap and quite accurate for identification of urea, a component of urine from mammals.

FL: Incidentally, we still use that in our cases that we bring on insanitation.

JWC: Do you really?

FL: Yes.

JWC: I wondered about that the other day. So I thought I might as well start to work with a similar technique for the organo-phosphorus compounds, if it were at all possible. However, I didn't think right at first to use the enzyme spot test. So I started by doing chromatography on the O-P coupounds. I got a suggestion from Joe Levine, a chemist in the Division of Pharmaceutical Chemistry, that the sulfur in most O-P pesticides might be sensitive to bromine. So I used that reaction as a spot test. After I developed the chromatogram, I sprayed the whole paper with a bromine containing compound called N-Brom-succinimide. Then I superimposed that with a dye chemical (fluorescein) spray that was sensitive to bromine. Wherever the sulfur in the organo-phosphorus compound used up the bromine, it was not available then to change the color of the fluorescein. The brominated fluorescein is pink and the non-brominated is yellow. So I'd have yellow spots where there was organo-phosphorus compounds. Most of the organo-phosphorus compounds that were used as pesticides had sulfur to

stabilize them. I found that to be a very useful test and almost immediately I discovered some startling reactions of the organo-phosphorus compounds. First I spotted Systox, chromatographed it, and sprayed my two reagents. Beautiful, just like that. In less than an hour I had a beautiful analysis showing the two isomeric compounds which comprise technical Systox. So I thought well I'd better test some of the other organo-phosphorus compounds to see if they're equally sensitive. So I took an 8" square piece of paper and spotted 10 different O-P pesticides in microgram quantities.

JWC: First, I spotted Systox on the paper. Then I washed the small spotting pipette and then spotted parathion. I repeated the wash and spotting of eight other O-P products. When I chromatographed and sprayed the spot test reagents, I found that Systox wasn't Systox any more. I got different spots than I did when I spotted it alone on the chromatogram. I immediately thought, well gee whiz, the only difference between now and the other time was it took me maybe 15 or 20 minutes to spot the chemicals on the chromatogram, as against previously it was just a matter of seconds. So I took three different pieces of paper, and spotted them with Systox. I left one under the fluorescent lamp that I was working under. I stuck one in the drawer, and I took one over to the window and let the sun shine on it. And the one where even the fluorescent light was on it

changed. The one in the drawer didn't change. And the one in the window changed. So that showed me that spread out in a thin layer on the paper Systox is almost instantaneously changed from the technical product of 2 isomers to two other compounds that are more soluble in water. It probably was the explanation of why Systox is a systemic insecticide. Because, even though it is basically an oil soluble technical product, it immediately changed to more nearly water soluble compounds. And the combination of the water and oil solubility permits it to go into the plant and translocate. There were changes in the other O-P pesticides also.

Knowing that some of these technical products were good in vitro inhibitors of cholinesterase, and some of them were not good in vitro inhibitors of cholinesterase, I thought I would devise another test in which I did superimpose both spot tests, the cholinesterase inhibition and the bromine-fluorescein test. Much to my surprise, I found that the bromination technique that I used for the first spot test converted the non-cholinesterase in vitro inhibitors, tors, to in vitro inhibitors. This technique gave me a tool to determine or visualize some of the general chemical characteristics of these compounds. From this line of work I developed many useful clues to look for when petitions came in for new O-P compounds. I was able then to accept or reject confidently the data that were submitted in

the petitions. I almost felt that at times I knew more about the compounds than the companies that made them.

JHY: This distinction that was new to science that you had discovered, was interesting analytically, but could you hypothesize things from it about relative degrees of toxicity or toxicity in different parts of the body, or anything of that kind? Did it have implications of that sort?

JWC: I gave considerable consideration to the possibility that some degree of projection of toxicity to humans from animal studies could be made. For instance, I postulated that it might be possible to determine the in vitro inhibitory effect of a series of organo-phosphorus compounds on a number of different enzyme systems in experimental animals and some in human, then knowing the toxic effect of these compounds on the experiental animals make a calculated projection of the toxicity to man.

We ran one experiment with this in mind, but did not follow it up. In that experiment we measured the in vitro inhibitory effect of (1) parathion (2) malathion and (3) methyl parathion on 8 different enzyme sources. Those eight were:

1. Rat red blood cells
2. Rat whole blood
3. Rat plasma

4. Rat brain
5. Dog plasma
6. Dog red blood cells
7. Human red blood cells
8. Human plasma

It was interesting to find that "... the inhibitory properties of the three pesticides are most similar in their effect on rat brain; the greatest difference is less than a factor of 3." "...the greatest dissimilarity (is) in their effects on rat plasma; parathion is 10 times more effective than methyl parathion and about 3000 times more effective than malathion".

This work was possible only because of the conversion of the technical product to in vitro inhibitors by the use of the bromine oxidation previously noted. This experiment was only the first step toward the postulated purpose of comparative toxicities to man.[1] Unfortunately if was not pursued.

The techiques were aids in pursuing (to a more complete finish, I might add) some other phenomena that were significant to us, such as, the low toxicity of parathion to cows in contrast to the extreme toxicity of parathion to dogs.[2] Another was some work where we showed the reason

[1] Jane McCaulley and J. W. Cook, JAOAC 42, 197-200, (1959)

[2] J. W. Cook Ag. and Food Chem., 5, 859-863 (1957)

why malathion has such a low toxicity to mammals in contrast to its being a good pesticide[3], and also an exploration of the high degree of potentiation of toxicity when malathian and EPN were fed to animals simultaneously[4]

JHY: But it is a clue, a warning flag, in a sense.

JWC: These spot tests and chromatographic pictures and the above described experiments helped a good deal in the evaluation of petitions, because, by this time, any petition on organo-phosphorus compounds would come to me to evaluate.

FL: These petitions were applications from companies to market a product?

JWC: I say petitions--before the Miller Amendment, the people used to bring in data on methods and data on toxicity and ask us to review it. Not really say yes or no, but they did bring it in to review it because they knew they had to ultimately deal with Food and Drug. In reviewing the petitions and keeping in mind the many experiments from our lab I could help resolve some questions that arose in my mind. But, when the Miller Amendment was passed, then, of course, we had to get full petitions including methods of

[3] J. W. Cook, Jane R. Blake, George Yip and Martin Williams, JAOAC 41, 399-411 (1958)

[4] J. W. Cook, Jane R. Blake and Martin W. Williams, JAOAC 40, 664 (1957) and subsequent papers.

analysis and toxicity data. Again these chromatograms helped me a good deal in judging whether the type of method of analysis, the determinative step of the analysis or sometimes even the extraction procedures were adequate to be able to extract these compounds of different solubilities that were produced by the light effect and oxidations to produce the terminal, effective residue which might not be the same as the product sprayed, such as in the care of Systox. I had a number of experiences in which I had to turn down petitions on the basis that I thought that the method was not really testing what the sponsors thought they were testing. Sometimes they would agree and sometimes they felt I was wrong. I was fortunate in being able to take them out in the laboratory and demonstrate why I thought their data were wrong. And on the basis of those demonstrations, some of them went back and took another look at their chemical. In a couple of instances, they had brought in cholinesterase inhibition methods of analysis for their compound and I knew from my work that the pure compound was not a cholinesterase inhibitor unless it was converted somehow. In some instances, one spectacular one, I questioned that their method of analysis was right on the basis of the color compound they were producing. I showed them in the lab why I felt that their method was wrong. They went back to their lab and did not question me.

They ultimately developed a very good method of analysis. They also found out some things about their compound that they did not know previously. They found that the terminal residue was different than they thought it was and would, in fact, not yield the color compound they had used as the basis of the original method.

Another interesting toxicological problem was fairly easily solved with the aid of the simple spot tests I had developed. Dr. Frawley, in the Division of Toxicology, discovered that there was marked potentiation of the toxicities of the two O-P compounds, EPN and malathion when they were fed simultaneously. Potentiation is a condition in which two or more chemicals administered simultaneously produce more biological effect than the sum of the effects of the individals. Frawley reported that an effective in vivo dose of EPN depressed plasma cholinesterase more than red cell cholinesterase; on the other hand, an effective dose of malathion depressed the same cholinesterases in the reverse order. Simultaneous adminstrationn of EPN and malathion depressed the two enzymes similar to malathion. So it appeared that EPN acted to make malathion more toxic. A simple in vitro experiment using rat liver homogenates showed the liver rapidly converted malathion to another compound whereas if EPN was added before malathion, then the malathion was not changed. This simple in vitro experiment was easily accomplished by use of the simple spot tests.

These and other experiments helped us considerably in evaluating the toxicity data. When the potentiation of these two compounds was first found, we had a concern that there may be some mysterious aspect of potentiation which would lead into the need for extreme amounts of work to be able to evaluate the safety of other O-P compounds.

JHY: How did this help out, then? It gave you a method of checking each possible potentiation.

JMC: Well, as far as malathion and EPN were concerned, potentiation wasn't a mystery any more. For instance, I tested the combination, the in vitro combination of a number of organo-phosphorus compounds with malathion and came to the conclusion that -- the data showed that in vitro, parathion was a very potent potentiator of malathion. In other words, it inhibited the destruction of the malathion as did EPN. On the other hand, parathion (which is itself much more toxic than EPN) would kill the animal before it had any opportunity to inhibit the enzyme that hydrolized malathion. There was very little possibility that there was any greater hazard from the two than from the one alone. I did this with other compounds too and came to a judgment that we could do tests like this that would give us a little better feeling in making interpretation of combinations.

JHY: Did potentiation really raise a nightmare vision that, if enough of these got out, that there might be kind of a wholesale disaster?

JWC: Well, I suppose that could have gone through people's minds. We did, in fact, stop processing petitions right when we first found the potentiation. For some time we asked the petitioners of each of the O-P compounds that they do potentiation work between their compound and other compounds that were already in tolerance. That, of course, amounted to a tremendous amount of work on their part. They did that, but this was finally stopped. That is we didn't ask them to do any more of the combination potentiation. Partly because we felt that the new data had not shown any potentiation of other combinations and nothing refuted the in vitro experiments that we had done.

JHY: Fine.

JWC: The best analytical procedure for the organo-chlorine compounds had been developed quite extensively with gas chromatography as the determinative step. That is it had gotten away almost completely from paper chromatography to gas chromatography. It so happened that a detector called the electron capture detector was extremely sensitive to many chlorinated organic compounds, including many of the chlorinated pesticides. There are different sensitivities

for different compounds, but there is extreme sensitivity for some of the very toxic organo-chlorines and therefore made an ideal combination for analysis. Only one or two of the organo-phosphorus compounds were responsive to the electron capture detector because they had chlorine also. A lady in my laboratory had done gas chromatography at NIH before coming to FDA. Her name is Laura Giuffrida. She said she would like to try doing gas chromatography of organo-phosphorus compounds. I almost attempted to discourage her, because we had no detection system; but we did not have a gas chromatograph available either. So she went to NIH, where she had worked previously, and made use of their gas chromatograph which had a flame ionization detector on it. That is the gases that came off of the column would go through a flame and be ionized and change a current to give a response that could be recorded on a graph. This (flame ionization) is sensitive to any organic compound so it does not discriminate an O-P or O-C compound from sugar. Once she decided to clean that detector. So she took it apart and cleaned it and put it back together. When she did that and put an organo-phosphorus compound through, she got roughly 20 thousand times increase in response over what she had been obtaining. That is, let's say, a compound that had 10 carbons and one phosphorus would be 20,000 times more responsive than one that was 10 carbons with no phosphorus. However, this high response was limited to the first few samples put through the dector.

Since she had worked with a pH meter and found how sensitive that detector was to perspiration and salt off the fingers, she thought maybe the fact that she handled that detector as she put it back in the instrument might be contributing somehow to the high response. So she coated the electrode with a little salt and found that she then got this 20,000 times response which persisted. This detector is used throughout the world and is known as a "thermionic dector". This development opened up a new world of analysis to us, because there had been no good way of measuring O-P compounds. So it now occurred to us that, with this detector available, it could be possible to incorporate it into the Mills procedure. We found that it was relatively simple to modify the solvents and the clean-up procedures to include both the O-P and O-C chemicals, then we added a second detector to the gas chromatograph. That is, the effluent from the column would go through the chlorine detector first, which is non-destructive, and pass on into the thermionic detector and thereby give readings for both groups of compounds in the same analytical procedure. Thus, some of the O-P compounds, but not all of them, were included into the whole analytical scheme. We thought that this was a significant enough discovery to merit an award. We were able to convince the Department to give her an award. I was told that it was the largest financial award ever given by HEW. It was $1500.

JHY: To anybody in FDA, you mean?

JWC: To anybody in the Department. That was my understanding.

Now, going back to the spot test technique it was useful in studying other chemicals relating to petitions. One example is as follows: Parathion is extremely toxic to dogs, for instance, one part per million will produce a depression of blood cholinesterase. On the other hand, large quantities fed to a cow will not affect their cholinesterase or permit any parathion to pass into the milk. So I judged that something had to be happening to the parathion before it got to the bloodstream of the cow because, generally speaking, parathion when fed at toxic levels will go from the bloodstream into other mammals' milk and into the meat. The Department of Agriculture had a cow at Beltsville that had a opening into the rumen, so we fed the cow parathion and removed samples from the rumen of this cow.

JHY: Was that Dr. Beaumont?

JWC: Yes, I think so, that's right. By the time I got the samples into the laboratory there was no longer any parathion present. But again a spot appeared migrating to a different position. Some literature indicated to me the possibility that there might be a chemical reduction of parathion, so I checked and found that the nitro group of

parathion had been reduced to an amino group. That compound is much less toxic and therefore it gave me better assurance in approving of a tolerance for parathion on plants being fed to cows, because it would not be transmitted to the milk.

JHY: The reduction would result from some chemical in the cow that was not in the dog?

JWC: That's right. The cow has a very large rumen which digests the food before the food reaches the true stomach. The rumen is sort of a fermentation factory; it's filled with organisms of various kinds which act upon the food. Basically the cow lives on organisms and the organisms live on the plants that the cow eats. Dogs have a much simpler digestive system.

JHY: And it wasn't necessary for you to figure out what the chemical was that did this?

JWC: It was an enzyme system, a reductive enzyme. I published this research in Agriculture and Food Chemistry, volume 5, number 11, page 859, November, 1957.

In July of '64 through December of '69 the Bureau of Food published or republished the selective publications of the Bureau of Science research, in quite large volumes, each of which runs in the neighborhood of 600 pages.

JHY: And that was for 6 months.

JWC: That's right.

FLL: And these were articles that had been published in scientific journals?

JWC: Right. Only selected ones, not all of them. Reprints from the Journal of Microbiology, for instance, Journal of Nutrition, Journal of Food Science, and so forth.

FLL: And a good percentage of those covered the kind of work we've been talking about and method development and research, and pesticide analysis?

JWC: That's right.

In 1963 I was sent to Rome to attend a meeting of the Food and Agriculture Organization's Pesticide Committee. There I was asked to be on a working committee on pesticide residues. We met for a one or two week period each year, and I continued on that committee for about eight years. It was very very fascinating. There were members from about 10 different countries on the committee. We attempted to set up tolerances that we thought could be considered international tolerances which would help the Food and Agriculture Organization help their underdeveloped countries in

providing themselves with food. In other words, it got into how they could do their agriculture and still make use of pesticides, and keep them within tolerance levels that other nations, at least, considered to be safe.

I was also asked to become a member of the Pesticide Section of the International Union of Pure and Applied Chemistry, which also met someplace in Europe each year. In this section we attempted to either provide methods of analysis to interested organizations, including Food and Agriculture Organizations or World Health Organization or to suggest to people throughout the world methods that should be developed or expanded for the use of those two organizations. We also provided methods of analysis to another group called The Codex Alimentarius. These were to be incorporated into a compendium of monographs on food products in international trade, so that the standards on those monographs would be acceptable to all nations and so that residue levels or contents or composition did not become an inhibitory factor in international trade. A lot of work was involved in both committees because, generally speaking, we in Food and Drug had more data available for both these committee meetings than did some of the other people from other countries for they would have only that which they found in the literature, and maybe not too much of that; whereas we would have petition after petition. So it turned out to be a big challenge, but it was a lot of fun.

FLL: Were you the only United States representative on those committees?

JWC: On the FAO one, yes, for most of the time. On the IUPAC committee I was the only U.S. member; there were some other U.S. associate members. Companies could send people, generally by invitation, who were able to provide data that would be useful to the Committee. For instance, American Cyanamid would send one or two people, or Dow Chemical would send people, depending on the chemicals of theirs that we were going to be considering.

The FAO Committee was giving consideration to all the data that we could find on the amount of residue that might be contributed to food products from various rates of application, time intervals, and type of product, and weather conditions, and t deciding whether the residue was the parent compound or some metabolic product. We used the term terminal residue, which may be a metabolite or some other compound produced by the effect of light on the parent pesticide. We tried to evaluate the methods of analysis by which those data were acquired. We developed monographs to incorporate our studies and recommendations. We also wanted to have a method of analysis that would be useful for the various countries to use in judging the products received by their own countries. Of course, I always attempted to promote the multi-detection methods that we had

developed in FDA, primarily because we and other people had put in so much effort to be relatively certain that each step in the method had been tested to show its value; whereas in many instances the data were developed by an unknown method or impure chemicals or problems which raised questions on the validity of the data.

JHY: You mean another nation?

JWC: I mean other laboratories in the U.S. and in other nations. Well, even in our own country, a lot of the data that you find in the literature are pretty difficult to interpret. Of course, this is exactly the kind of thing that we had to do in evaluating petitions for all of the compounds for which we did set tolerances. A petition would consist sometimes of from one or two experiments, to maybe 100 different experiments in a variety of publications, plus data that were acquired by the company's laboratories in their own experimental work, or work that they had contracted out to some university, which may or may not be published. Then from evaluating each one of those individual experiments, one has to make a decision as to the validity of the results of each experiment. Then an overall evaluation has to be made of all of the experiments. And sometimes when you get dozens of experiments you find many of them say yes and many of them say no. So then you have

to pass some kind of judgment on which ones you can accept as being valid, in relation to establishing a tolerance, and which ones you feel you must reject. If the methods of analysis seem to be adequately supported, you are inclined more to depend on those data than on data from some method that is not so well known or adequately supported. So in the petition work, essentially we were doing a fair amount of "paper" research without leading to some more laboratory work but to a decision on, in the case of the petitions, yes or no whether a tolerance can be established. I used some of the same data to support the FAO and IUPAC recommendations. Generally speaking, I tried to promote the use of our multi-residue methods for adoption and to recommend people for their next work. But it is very difficult to talk people into this because each seemed to want to do it the way they're used to, or they want to take credit for the method that they have developed. No other group has ever put the effort, the combined effort, to study in relatively infinite detail essentially all of the steps and other aspects of a multi-residue analysis, as we have on this Mills procedure.

JHY: It isn't that the results of your complex multiple approach are inaccessible, because this volume here on the table before us, "Pesticide Analytical Manual" Volume I, which we have referred to before, would be readily available to any laboratory in any nation which wished to take it and employ it.

JWC: That's right. Some people have some valid arguments against it though, for instance, the method uses acetonitrile for extraction. There is not a pure form available in some nations so they must buy it from the U.S. So some prefer to use a different solvent. If a different solvent is used, then you may have quite a different procedure to follow to clean up or purify the extract beyond that step. So I can understand why some people would be reluctant to use our multi-residue method. But it's still a good approach.

In Cortina, Italy at a IUPAC meeting I had an interesting and rewarding experience at an informal meeting of friends. Dr. Batora from Czechoslovakia, stated that he wanted to thank me because he became interested in pesticide chemistry from reading my papers on malathion and potentiation. He pointed out that he did the first work on pesticides in his country, and represented Czechoslovakia at this international meeting. Then Dr. Pekka Koivistoinen, from Finland, who had been very active in this field for a long time, said he was pleased and interested that Dr. Batora would say that because he himself became interested in pesticides from the same series of papers of mine.

JHY: Have you cited this article on the tape?

JWC: I didn't give the reference. The reference is the Journal of AOAC, May, 1955, pg. 399. The title is

"Malathionase Activation and Inhibition", and others that follow.

JHY: Well that certainly is a tribute to have stimulated two men of this kind who eventually became your peers in an international venture of this sort. Mr. Cook has just brought a printed award which was given to him which has his photograph at the top and his title, Acting Director - Division of Pesticide, Chemistry, and Toxicology, Bureau of Foods, Pesticides and Product Safety - Food and Drug Administration. This accolade for "sustained high quality of performance and exceptional supervisory ability in chemical research on the nature and measurement of intermediate and terminal residues of pesticides in food." Now, I've just read the citation and will you please explain the award?

JWC: Well, the award consists of a distinguished service certificate from the U.S. Department of Health, Education and Welfare. Also, I was awarded a departmental gold metal about the size of an old silver dollar and is entitled "Medal of Award for Distinguished Service" with my name engraved.

JHY: This is from the Department of HEW?

JWC: Right. It was presented in a ceremony, a relatively large departmental ceremony.

FLL: That is the highest award that the Department issues, I believe. What is the date on the certificate?

JWC: 1970. April 10, 1970.

JHY: And you said that before the Department would accept a candidate for this award, that candidate had to have received an award from the agency?

JWC: Well, yes, the FDA Award of Merit.

FLL: Which is the highest award that the Agency issues. Bill, do you have any stories about any of the Commissioners you served under that would sort of illuminate how they operated?

WJC: With respect to the laboratory operations, yes, I can comment that Mr. Larrick used to have meetings over in the South Agriculture Building where our laboratories were located and is away from his own office. Periodically, he would have these meetings and ask the Division Directors to supply chemists or other scientists to report on their work in the laboratory. This, of course, helped him understand what our problems were and what we were doing, but it also

gave the individuals in the laboratory the feeling that he cared; that he was interested in what was going on in the organization. Some of the other commissioners had no meetings of similar nature. Dr. Edwards did ask that there be presentations, but he was not as interested in the details, but wanted more broad reviews of a subject from the scientists, rather than their specific research. For instance, I was asked to give a report on mercury. We were doing some work on mercury, but most of my report was from literature. This did not give the laboratory people the feeling that Dr. Edwards was interested in the scientists themselves or in their work or accomplishments.

FLL: Were those presented by operating researchers, or presented by branch and division directors?

JWC: In the case of Mr. Larrick's meetings, they were presented by the person doing the research, yes. Also the room was large enough that other scientists could attend these meetings. Not so with Dr. Edwards' meetings.

JHY: And this was in the early '60s when you were at that building?

JWC: Yes, well it could have been even the late '50s and the early '60s, right.

FLL: But the Edwards presentations, were they by the individual researchers?

JWC: Not that I recall. In some instances there may have been. Like in some of the aflatoxins and other mycotoxins, some of the people doing some of the laboratory work probably gave some of the talks, I'm not absolutely sure of that.

(END OF THE RECORDING)

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Pesticide Residues

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METHODOLOGY for residue analysis has advanced rapidly during the current review period, from November 1962 through October 1964. Notable progress has been made in the development and refinement of methods of analysis by which any or all of a large number of pesticide residue chemicals can be detected and measured in one general operation. This is of particular significance because great interest has developed—outside the scientific community as well as within—in the possible presence of pesticide chemicals in all parts of our environment, including man himself. Only by the use of improved methodology will it be possible to accomplish the task of detecting, identifying, and measuring the many possible residual pesticide chemicals. It is only after the presence or absence of these chemicals in any part of our environment has been proved unequivocally that the medical man, the lawyer, the lawmaker, the administrators in government and in industry, and other interested groups can assess the significance of such residues.

There are 300 to 400 chemicals registered for use on food products alone, and a few hundred more are registered for other uses whereby they may become part of our environment. The chemist cannot know which of the hundreds of possible pesticide chemical residues to look for in samples of air, water, soil, plants, human and animal tissues, prepared foods, etc. There is an urgent need for general procedures that can identify and measure a large number of chemicals at one time. They must be highly sensitive and accurate, since it is essential that all monitoring of our environment be at a level considerably below any "tolerance" or otherwise critical level, so that trends can be more readily recognized and assessed for significance. Upward or downward trends in any portion of our environment will be recognizable only when the methodology becomes sufficiently sensitive and accurate so that analyses in the fraction-of-a-part-per-million or even part-per-billion range become routinely dependable (Fischbach, H. Pub. 1082, National Research Council, p. 55, Nov. 29, 1962).

The multiple detection procedure of Mills (*145*) for chlorinated pesticides is still being extended and modified. The determinative steps most useful for these general procedures are still the forms of chromatography—gas liquid, thin layer, and paper. There is growing recognition that, for these determinative steps to be useful and dependable, great care must be exercised to avoid interferences, false responses, and incorrect interpretations of chromatograms.

Lykken (*134*) emphasizes the point that if a sample is not representative of the lot of material from which it is obtained, results of analysis will not be valid or useful. He presents valuable information for anyone involved in residue work and discusses factors which must be considered in designing experiments, such as proper sampling, compositing, quartering, storage, and shipment of samples.

Greater importance has been assigned to efficient extraction of the pesticide residue from the sample. The use of mixtures of solvents such as hexane and isopropanol to achieve a single continuous phase with the aqueous medium of food products has been studied (*14, 101, 141, 147, 176, 192*). Some investigators dehydrated the tissues by using anhydrous sodium sulfate, which improved the extraction of the residue with an organic solvent (*10, 50, 141, 185*).

The use of solvents which dissolve the pesticidal chemicals while maintaining

miscibility with the aqueous medium of the tissues, such as acetone (*93*, *127*, *185*) and acetonitrile (*14*, *54*, *157*), is reported. Such solvents give generally higher values than those obtained by the use of solvents that are immiscible with tissue media, such as hexane alone. Care must be exercised not to pass judgment on the efficiency of extraction merely on the basis of high recoveries of the chemical added during the analytical procedure. Good recoveries of the chemical added may be achieved when the degree of extraction of the actual weathered residue is poor.

Gas chromatography columns currently available are sensitive to small amounts of certain impurities. A number of papers emphasize the necessity of good cleanup before the extracts can be chromatographed to yield unambiguous results (*22*, *27*, *44*, *45*, *63*, *130*, *133*). These columns must be carefully and thoroughly "conditioned" (*44*, *45*, *63*, *177*) before they can be used routinely to obtain good qualitative and quantitative results.

Barry and Hundley (*17*) have edited a "Pesticide Analytical Manual" comprising a compilation of methods and other information useful to a residue analyst. Although developed as a guide for chemists in the laboratories of the Food and Drug Administration, it has been distributed to many others as well. It presents information on sampling, extraction, cleanup, and determinative procedures; techniques for preparing and conditioning columns; and lists of relative retention times and detectable quantities of many pesticides for different chromatographic procedures and detectors.

Although gas chromatography with various detectors is now the most popular technique in residue analysis, other procedures have not been neglected. Thin layer chromatography, with its greater speed of development and increased resolution and sensitivity, has to some extent replaced paper chromatography. Colorimetric, ultraviolet, and infrared procedures also are used.

GAS CHROMATOGRAPHY—GENERAL

The greatest advances during this period have been made in gas chromatography. Because of the extreme sensitivity of the electron capture detector, it has been used by many workers. Dimick and Hartmann (*64*) have published a general description of electron capture gas chromatography as used in pesticide analysis. They discuss the principle of electron capture and the geometry and operating parameters of the detector. Although only one specific instrument is described and some of the steps in the outlined procedure have since been improved, this report does provide a good introduction to those unfamiliar with the subject.

With the search for ever-increasing sensitivity and speed of analysis has come the realization that these desirable goals encourage the production of methods in which unrecognized side effects, minor interferences, slight amounts of contaminants, and any lack of care in the use of equipment or interpretation of responses can produce greatly misleading and inaccurate results.

Lovelock (*133*), in a general discussion of electron absorption detectors, points out that with complex mixtures (such as are usually present in residue analysis) these detectors may give " . . . not only inaccurate but even totally false results." Causes of various false responses, both positive and negative, are discussed and a pulse-sampling technique which minimizes the errors is described. Barney, Stanley, and Cook (*16*), working with Systox, have shown that in a poorly designed detector, electron capture and ionization may take place at the same time and that pulse mode of detector operation will not eliminate interferences from ionization.

Burke and Giuffrida (*44*) point out the need for adequate cleanup before a sample extract is injected into the electron capture gas chromatograph. They show that injection of poorly cleaned extracts may contaminate a column and result in weak or spurious responses. Since solvents used must be "pure," redistillation is frequently required. The use of plastic containers for solvents is discouraged, since extractables in the plastics may cause response of the electron capture detector. In some cases these spurious effects are so strong that responses due to pesticide residues may be completely masked. The need for proper preconditioning of the gas chromatography column is also thoroughly discussed. Unless properly conditioned, the column mayca use degradation of some pesticides. Not only may the degradation result in loss of the pesticide but also the degradation products may cause responses at the retention times of some other common pesticides for which they may be mistaken. Equipment and operating parameters described permit detection of chlorinated pesticides, such as heptachlor epoxide, at levels of 0.01 to 0.001 p.p.m. Relative retention times are listed for 65 pesticides.

Bonelli, Hartmann, and Dimick (*36*) describe two columns used with electron capture gas chromatography. Pesticides which cannot be resolved on one column may be resolved on the other. Operating parameters, sensitivity data, and retention times for a number of pesticides, including chlorinated, organophosphorus, and organosulfurs, are given.

Burke and Holswade (*46*) present similar data for microcoulometric gas chromatography. Retention times relative to aldrin are listed for 87 chlorinated compounds, and those relative to sulfone for 26 thio compounds. A table which lists the amount of each pesticide required to give a half-full scale recorder deflection may be found very useful. Recovery data indicate that responses are linear when the pesticide is present above a definite minimum quantity. The need for proper cleanup of sample extract before injection is emphasized, and conditions and precautions for most effective use are described. The general level of sensitivity obtainable is given as 0.01 p.p.m.

Shuman and Collie (*177*) describe the preparation of a gas chromatography column; they also emphasize the need for proper conditioning. They recommend a 6-foot, 6-mm. i.d. column packed with 10% Dow Corning 200 (12,500 centistokes) silicon fluid on Anakrom ABS. Other workers (*44*, *45*) have also found this type of column superior for pesticide work.

De Faubert-Maunder, Egan, and Roburn (*63*) give details for preparing a column. Good columns, columns which decompose pesticides, construction, linearity of responses and cleaning of detectors, effects of rate of injection, peak measurements, and chromatogram interpretation are discussed. The use of a glass injection liner is recommended.

Beckman and Bevenue (*24*) studied the effect of column tubing composition on recovery of chlorinated hydrocarbons. Working with 6-foot by $^1/_4$-inch columns and a microcoulometric detector, they checked columns made of copper, stainless steel, aluminum, and quartz. Copper tubing gave the poorest recoveries, quartz the best. Aluminum and stainless steel were satisfactory.

It has been apparent for some time that complete reliance on the retention time for identification of a compound may yield erroneous results. Robinson and Richardson (*170*) emphasized the need for caution in interpreting the results of gas chromatography of plant and animal extracts, both as to identity and quantity, when only one column is used. They described four different columns and tabulated the resolution of pairs of pesticides on these various columns.

Goulden, Goodwin, and Davies (*88*, *89*) were also concerned about improving the certainty of identification. They found that a column packed with a 2.5% silicon oil and 0.25% Epikote 1001 on Celite gave good resolution. With column temperature of 163° and gas flow of 100 ml. nitrogen per minute, they obtained complete separation of at least 11 pesticides in 30 minutes. They also proposed the use of simultaneous chromatography using five parallel

columns leading to one electron capture detector. The stationary phases of the columns differ so that 3 to 5 peaks may be obtained for each pesticide. They called this a "spectrochromatogram" and stated that the pattern is characteristic of the specific pesticide. They also described the use of a halogen-sensitive cell of the type used in detecting refrigerator leaks. The response to individual chlorinated pesticides of this cell differs from that of the electron capture cell. By connecting this detector in series with the electron capture detector and recording responses from both detectors, identification of individual compounds is made more reliable.

Programmed temperature gas chromatography is also coming into use as a means of improving resolution and separation, speeding up runs, and chromatographing mixtures containing both very fast and very slowly eluting compounds. Burke (*43*) used programmed temperature with a microcoulometric detector; he tabulated relative retention times for 22 compounds. Other workers have also used this technique (*20, 36, 118*).

The technique of preparation of a derivative of a pesticide before injection into the gas chromatograph has been continued. For some time, it has been the practice to convert 2,4-D and other chlorinated phenoxy acid herbicides to their methyl esters because the free acids will not pass through the common gas chromatographic columns. Derivatives are now being used for other reasons. Klein and Watts (*120*) found that Perthane, *o,p'*-DDT, and *p,p'*-DDD have similar retention times and are difficult to resolve on many gas chromatographic columns; however, olefins of these three compounds prepared by refluxing cleaned-up sample extracts with 2% NaOH in ethanol were separated on a 3-foot gas chromatographic column of Celite 545 with a 2.5% coating of SF-96 and 2,2 - diethyl - 1,3 - propandiolisophthalate polyester (1:1). Klein and Watts obtained recoveries ranging from 84 to 105% from samples of leafy vegetables containing residues added at levels of 1 to 10 p.p.m. One striking benefit from the use of the olefins is that the Perthane olefin gives an electron capture response about ten times greater than Perthane.

Beckman and Berkenhotter (*20*) used derivatives to increase the reliability of identification of pesticide residues. They separated the individual compounds by gas chromatography with a thermal conductivity detector and then dechlorinated the individual fractions with sodium and liquid ammonia. After that they chromatographed the dechlorinated portions again and obtained chromatograms. The results obtained from the two chromatograms can be used to characterize the pesticide.

Gutenmann and Lisk (*97, 99*) prepared brominated derivatives which had strong electron capturing ability and chromatographed these as a means of obtaining increased sensitivity. They worked with diphenyl, Guthion, MCP, and MCPB as pure compounds, and also used this technique to determine residues of CIPC, monuron, diuron, and linuron in fruits and vegetables. Recoveries from crops of 76 to 116% were obtained at levels of 0.05 to 1.2 p.p.m. when only one pesticide was present at a time.

A word of caution may be in order about using this technique in any procedure which does not include extensive cleanup of residue before gas chromatography. Valuable information may be obtained when only one pesticide is added and when the untreated crop is available so that chromatograms of sample and control can be compared. However, if the technique is used on crops of unknown spray history with no control crop available for comparison, chromatograms would probably contain so many unidentified and unidentifiable peaks that accurate interpretation would be impossible.

Bache, Lisk, and Loos (*13*) prepared nitro derivatives of MCP, MCPB, and NAA in order to increase the response of these herbicides on the electron capture gas chromatograph. They used this technique to determine MCP and MCPB in timothy and peas and NAA in apples, and reported finding residues of MCP on snap bean plants treated with MCPB.

The above discussion has been concerned primarily with gas chromatograph detectors for halogenated compounds. One of the most exciting and promising developments of the past year was the appearance of two dissimilar detector systems, each of which is reported to be highly specific for phosphorus - containing compounds. Giuffrida (*86*) modified a conventional flame ionization detector by fusing a sodium salt onto the electrode. The result was a detector 600 times as responsive to a compound containing 10 carbons and 1 phosphorus atom as was the conventional flame detector. Response to compounds containing six chlorine atoms was twenty times as great, while the response to compounds containing neither Cl nor P was the same as that of the conventional flame ionization detector. When the extraction procedure of Mills, Onley, and Gaither (*146*) was used, diazinon, ronnel, parathion, ethion, and Trithion, when added to broccoli at levels of 0.05 and 0.1 p.p.m., were easily detected. There was no interference from crop materials even when the equivalent of 5 grams of original sample was injected. Construction and operating conditions of the detector are described, and retention time lists for 23 organophosphate compounds are given.

Burchfield, Rhoades, and Wheeler (*42*) report the development of a microcoulometric detection system which is specific for phosphorus. The effluent from the usual gas chromatographic column is passed through a quartz tube heated to 950° C., with hydrogen as the carrier gas. Organic compounds are reduced to hydrocarbons, water, PH_3, H_2S, and HCl. The latter three compounds precipitate silver ion and so register on a microcoulometric titration cell. Insertion of a short silica gel column removes HCl; substitution of Al_2O_3 for silica gel removes both HCl and H_2S and permits measurement of PH_3 with absolute specificity. Response of the cell to PH_3, H_2S, and HCl is in the ratio of 2 : 2 : 1. When a model C-100 microcoulometer at maximum sensitivity is used, 0.1 μg. of P gives a peak area of 5 square inches. Cleaned-up extracts from crops examined do not interfere with the reduction or detection steps.

CHLORINATED PESTICIDES—GENERAL PROCEDURES

More attention has been given to the development of multiple detection procedures for the chlorinated pesticides than for any other class of pesticides. This is only natural, since these compounds are widely used and many are so persistent that traces of some compounds, such as DDT, are being found almost everywhere. Moreover, these compounds have been found to be more amenable to this type of analytical method.

Mills, Onley, and Gaither (*146*) have combined and modified previously reported methods to provide a rapid, simple procedure for extracting and cleaning up residues from nonfatty foods. Used with gas chromatography, thin layer chromatography, or paper chromatography, the procedure will determine 21 chlorinated pesticides. Good recoveries were obtained of 5 pesticides added to 11 products at levels from 0.02 to 0.2 p.p.m.

Taylor, Rea, and Kirby (*185*) extracted chlorinated pesticide residues from animal tissue by blending the tissue with acetone and anhydrous Na_2SO_4. The pesticides were transferred to hexane and injected into a gas chromatograph. Recoveries for lindane, endrin, dieldrin, *p,p'*-DDE, and heptachlor epoxide ranged from 75 to 99% at 2.5 to 10 p.p.m. levels.

Several procedures have been reported for extracting chlorinated pesticide residues from water. Kahn and Wayman (*113*) describe a continuous extractor using refluxing petroleum ether.

The water sample was passed through the extractor at a rate of 0.5 to 1.0 liter per hour. Nonpolar compounds were extracted by the petroleum ether, concentrated, and determined by electron capture gas chromatography. Some of the intermediates in the manufacture of aldrin and endrin were determined at levels as low as 0.3 p.p.b. by using a 135-liter sample. Infrared spectra can be run on extracts after cleanup on alumina columns.

Breidenbach *et al.* (*38*) describe equipment and procedures for collecting large volume samples of water by carbon adsorption as well as analysis of discrete bottled samples of water. The procedure used by the Public Health Service–Water Pollution Surveillance System for analysis of carbon-chloroform extract by thin layer chromatography, electron capture, and microcoulometric gas chromatography, and infrared is reported, but no data on the efficiency of such a system are presented.

Schwartz *et al.* (*176*) have used electron capture gas chromatography for determining "Polystream," a mixture of chlorinated benzenes, in clams and oysters.

Minyard and Jackson (*147*) analyzed 101 samples of commercial animal feeds, using electron capture gas chromatography. They extracted the samples with an isopropanol–Skellysolve B mixture (1 + 3) and used a Florisil column cleanup. They state that they were able to detect less than 1 p.p.b. of most of the chlorinated pesticides.

Baetz (*14*) reported using Norit-A for cleaning up sample extracts. The sample was extracted by blending with acetonitrile or mixed solvents and the residues were partitioned into petroleum ether. An aliquot was evaporated to dryness, taken up in benzene, shaken with Norit-A, and filtered. The filtrate was reported to be suitable for injection into the microcoulometric gas chromatograph but it could not be used for electron capture gas chromatography because of excessive interference. The method is reported to have given satisfactory recoveries of 11 chlorinated pesticides from carrots, collards, okra, and peas at 0.1 to 1.0 p.p.m. levels. Recoveries of lindane and BHC, however, were as low as 53%.

Moats (*152*) reported a one-step cleanup procedure using a column of Nuchar C 190–Celite 545 (1 + 2). With paper chromatography as the determinative step, sensitivity was believed to be about 0.03 to 0.1 p.p.m.

McKinley, Coffin, and McCully (*137*) have reviewed cleanup procedures for both chlorinated and organophosphate pesticide residues. They point out the advantages and limitations of the various methods, and list 41 references. Gutenmann and Lisk (*93*) have used electron capture gas chromatography as the determinative step in which the samples were extracted with acetone and the residues partitioned into Skellysolve B, a portion of which was injected into the gas chromatograph without additional cleanup. They used technical grade solvents without purification and reported recoveries ranging from 60 to 112% for 11 pesticides, with sensitivities from 0.04 to 0.001 p.p.m. (The writers believe that it is well to caution readers once more against using such abbreviated procedures unless the spray history of the crop is known and untreated samples of the same product are available for comparison determination.)

The determination of chlorinated pesticide residues in fatty foods has presented a special problem, since the pesticides are fat- and oil-soluble and separation is difficult. The analysis of milk presents an added challenge in that, for many procedures, the fat must first be separated from the milk.

Onley (*161*) has reported a rapid method for milk which combines the usual two steps into one. Instead of first separating the fat from the milk and then extracting the pesticide from the fat, the milk is blended with a mixture of acetonitrile, ethyl ether, dioxane, and acetone (3:1:1:1) and anhydrous sodium sulfate. After filtering, water is added and the residues are transferred to petroleum ether. From this point, a modification of the Mills procedure is followed. By electron capture gas chromatography, satisfactory recoveries were obtained for 19 pesticides at levels ranging from 0.005 to 0.1 p.p.m.

Henderson (*106*) reported a collaborative study involving two samples of milk and 22 laboratories. Advantages and disadvantages of various methods were discussed, and results by paper chromatography and microcoulometric and electron capture gas chromatography were compared.

Several methods have been reported for separating fat from dairy products prior to pesticide analysis. Langlois, Stemp, and Liska (*131*) used the conventional Babcock test procedure and reported that although endrin was apparently destroyed, DDT, DDE, lindane, heptachlor, heptachlor epoxide, and dieldrin were recovered satisfactorily. Lampert (*128*) used a detergent solution and a Babcock cream test bottle for separating the fat from milk.

A number of different ways have been suggested for separation or cleanup of pesticide residues from fats and oils. Eidelman (*67*) used dimethylsulfoxide to extract the residues from acetone and petroleum ether solutions of fat. After water was added and the residues were partitioned into petroleum ether, they were further cleaned up by the Mills Florisil column procedure and then determined by microcoulometric gas chromatography. Fish oil samples required additional treatment of the 15% eluate from the Florisil column to eliminate interferences. Saponification and MgO-Celite column cleanup were used prior to injection into the gas chromatograph.

McCully and McKinley (*135*) used a freezing technique to separate chlorinated pesticide residues from fats and oils. The fat or oil was dissolved in a benzene-acetone mixture (1 + 19) and the fat precipitated by cooling to −70° C. The solution was filtered through a charcoal-wood cellulose column and concentrated for injection into the electron capture gas chromatograph. A special apparatus for use in this procedure was described in a second publication (*136*). Working with organophosphate compounds, Crosby and Laws (*57*) reported that freezing out of waxlike substances from acetone solution removed some impurities but also removed pesticides.

Ott and Gunther (*163*) used forced volatilization to separate pesticide residues from butter fat by use of a newly designed device. The fat was heated to about 190° C. and volatiles were carried to a cooled trap by a stream of nitrogen. Determination was made by microcoulometric gas chromatography; the entire analysis required about one hour. Sensitivity was reported at about 0.5 p.p.m. for some of the more common chlorinated pesticides. However, DDT broke down to form some DDE and DDD.

De Faubert–Maunder, Egan, and Roburn (*62*) compared dimethylformamide and dimethylsulfoxide for extracting residues from hexane solutions of fats, and reported that dimethylformamide gave better recoveries. They described procedures for analysing samples of fat, milk, butter, and eggs. Moats (*151*) used a column containing 100 grams of standardised Florisil to clean up as much as 2 grams of fat. Pesticides were eluted from the column with 20% methylene chloride in petroleum ether in a form adequate for spotting the entire sample for paper chromatography.

Langlois, Stemp, and Liska (*130*) extracted pesticide residues from dairy products by grinding the samples with Florisil that was partially deactivated by the addition of 5% water to the adsorbent. The mixture was added to the top of a Florisil column and the pesticides were eluted with 20% methylene chloride in petroleum ether. The eluate was evaporated and the residue taken up in hexane for injection into the electron capture gas chromatograph. The procedure took 30 to 90 minutes and recoveries were reported as being consistently better than 90%. The sensitivity was stated to be 0.05 p.p.m.

for DDT and endrin and 0.1 p.p.m. for some of the other chlorinated pesticides. The same procedure was also adapted for analyzing egg yolk and poultry tissue (*181, 182*). The fact that in this procedure different volumes of eluting solvent are used for different pesticides presents a difficulty. For example, to remove endrin, 650 ml. of eluting solvent and 90 minutes of eluting time are required, and in screening for all pesticides this most time-consuming version of the procedure would have to be used routinely.

Onley and Mills (*162*) modified the conventional Mills procedure for use on eggs. To eliminate interferences, they passed an acetone solution of the extracted oil through a filter paper pulp column. They obtained recoveries of 73 to 110% for seven pesticides at levels down to 0.02 p.p.m.

Thin layer chromatography has become increasingly important in pesticide residue analysis. Kovacs (*124*) studied the chromatography of 16 pesticides on alumina and silica gel plates. He found that for the silica gel plates, prewashing was desirable and the ultraviolet exposure time was critical. R_f's relative to DDD are listed and results by thin-layer chromatography and microcoulometric gas chromatography are compared, using the extraction and cleanup procedure of Mills, Onley, and Gaither (*146*). Use of good cleanup before thin layer chromatography, allowed determination of many pesticides in the p.p.b. range.

Walker and Beroza (*196*) have made an extensive study of thin layer chromatography. They list the R_f's in 19 solvent systems for each of 62 pesticides, including chlorinated compounds, organophosphates, and carbamates, and they discuss chromogenic sprays, choice of solvent system, and the use of thin layer chromatography as a cleanup procedure.

Kawashiro and Hosogai (*114*) have reported a new spray reagent for detecting chlorinated pesticides on silica gel thin layer chromatography plates. The plates are sprayed with 0.5% *o*-tolidine or *o*-dianisidine and then irradiated with ultraviolet at 2536 A. The pesticides appear as green spots against a white background. Amounts of 0.5 to 1 μg. are detectable for many of the pesticides. This reagent does not appear to be as sensitive as the conventional $AgNO_3$.

Morley and Chiba (*153*) have used thin layer chromatography as a cleanup procedure for gas chromatography. Samples were spotted and developed on each half of a plate. One half of the plate then was covered with aluminum foil and the other half sprayed and exposed to ultraviolet light to locate the spots. Similar areas on the covered half then were scraped off and extracted for gas chromatography. They also report the use of thin layer chromatography on uncleaned plant extracts as a rapid screening method for DDT and DDE in plants.

Paper chromatography continues to receive attention. Mitchell (*148*) reports the minimum detectable quantities of 22 chlorinated pesticides, using the $AgNO_3$-phenoxyethanol chromogenic agent. Data for an aqueous system and a nonaqueous system are given along with R_f values. Krzeminski and Landmann (*126*) describe a spray reagent for paper chromatography which gives no curtain effect and in which impurities in the paper do not interfere. The reagent, an alcoholic water solution of methyl yellow, was tested on 14 chlorinated pesticides and showed detection limits of 2 to 8 μg.

Heinisch and Neubert (*104*) report the use of wedge-shaped strips for the ascending paper chromatography of chlorinated pesticide residues. They state that this procedure gives better separation than other methods.

A matter of concern to all residue chemists is the possible presence of unsuspected degradation or metabolic products of pesticides. Roburn (*171*) studied the effect of sun and ultraviolet light on several chlorinated pesticides. Using gas chromatography, he found that grass treated with dieldrin and exposed to sunlight for several months gave an unknown second peak with response approaching that of dieldrin in magnitude. Fifty 100-μg. quantities of pesticides were then deposited as films on glass and exposed to a germicidal ultraviolet lamp for 2 to 3 hours. With gas chromatography as the examining medium, dieldrin so exposed showed one derivative; endrin showed one main product and several minor ones; aldrin showed dieldrin and a small amount of another derivative; *p,p'*-DDE, three main products and several minor ones; *p,p'*-TDE, a small amount of a dehydrochlorinated product; and *p,p'*-DDT, a small amount of DDE. The α, β, γ, and δ isomers of BHC did not show any reaction products.

CHLORINATED PESTICIDES—SPECIFIC PROCEDURES

Friestad (*77*) reports a spectrophotometric method for aldrin which requires prior cleanup of the sample. The aldrin is reacted with nitrosyl chloride, then heated in acid to form dihydrochlorketo aldrin. This compound reacts in alcoholic solution with *m*-dinitrobenzene and potassium hydroxide to give a red-violet color, which is extracted with chloroform and its absorbance read at 525 mμ. Dieldrin and endrin interfere with the assay. This reaction is sensitive to about 10 μg. aldrin.

Bache (*11*) used thin layer chromatography to determine amiben in tomatoes. The sample was treated with sodium hydroxide; the hydrolyzed amiben was extracted and, after transfer to acetone, spotted on silica gel plates. Bache, Gutenmann, and Lisk (*12*) used electron capture gas chromatography for the same determination, methylating the hydrolyzed amiben prior to injection into the gas chromatograph. They report recoveries of 70 to 123% at levels of 0.05 to 1.25 p.p.m.

Klayder (*119*) has modified the A.O.A.C. method for captan for use on green vegetables. A collaborative study involving eight laboratories showed average recovery of 95% at levels of 50 to 105 p.p.m. He also reports that captan was largely destroyed by a canning process in which the food was heated at 14-pound pressure for 35 minutes to one hour.

Beckman and Bevenue (*22*) determined chlorobenzilate in grapes and cottonseed, using microcoulometric gas chromatography. They report a sensitivity of 0.05 p.p.m. and state that rigorous cleanup is essential; otherwise the gas chromatographic column quickly becomes contaminated.

Schafer, Busch, and Campbell (*173*) have reported a rapid screening method for DDT in milk, using electron capture gas chromatography. The milk was treated with alcoholic potassium hydroxide to saponify the fat and convert DDT to DDE. A hexane extract of the milk was then injected into the gas chromatograph. Recoveries of 95% are reported at levels of 0.04 to 0.12 p.p.m. on a whole milk basis.

Blinn and Gunther (*31*) present the results of a collaborative study of two versions of a colorimetric procedure for DDT in milk and butter fat. They prefer the version which includes an oxidation step in the cleanup.

Klein, Watts, and Damico (*121*) used the conversion of DDT to DDE as confirmation of identity in the analysis of butter and oils for DDT. They used the Mills procedure for extraction and cleanup and determined DDT by electron capture gas chromatography. A second aliquot was converted to DDE by treatment with sodium hydroxide, and again chromatographed.

Hardin and Sarten (*101*) compared five procedures for extracting DDT from field-treated collards. They found that blending first with isopropyl alcohol and then with hexane gave better recoveries than did tumbling or grinding with hexane.

Espadas and Loaeza (*70*) used aniline in place of alcoholic sodium methylate or alcoholic potassium hydroxide in the color development after nitration of

DDT. They state that a more stable color was obtained and the method was simplified.

Beckman and Bevenue (*23*) determined Dilan residues in pears by using microcoulometric gas chromatography after cleanup with Nuchar. DDT, if present, was removed by elution from a Florisil column.

Albert (*6*) has suggested a modification which saves about two hours in the determination of endrin by the Mills procedure. He reports that the 15% eluate can be cleaned up and fat eliminated by passing the eluate through a potassium hydroxide–Celite column. This replaces the lengthy saponification step. By electron capture gas chromatography, the recoveries from carrots, onions, collards, and broccoli at the 0.05 p.p.m. level ranged from 100 to 106%. EDITOR'S NOTE. This procedure may work equally well for dieldrin.

Gordon, Haines, and Martin (*87*) determined Kelthane in milk colorimetrically with a sensitivity of 0.01 p.p.m. based on whole milk or about 0.25 p.p.m. on the fat basis. They extracted the fat from the milk and hydrolyzed the Kelthane with tetramethylammonium hydroxide. The released chloroform was determined by the Fujiwara reaction. The procedure eliminated the need for a special steam system.

Ackermann, Carbone, and Kuchar (*4*) modified an earlier spectrophotometric method for the determination of pentachloronitrobenzene in soil and crops. They used a direct extraction from food products with ethanol and partition into petroleum ether to eliminate pigments. The method was said to be sensitive to about 3 μg.

The A.O.A.C. method for sulfone was studied by Shuman (*176*). He found that hexane as the stripping solvent gave better recovery from field-sprayed peaches, whereas benzene worked better for apples. He checked the remainder of the fruit by blending with an isopropanol-benzene mixture and found that the stripping had removed over 90% of the residues.

Two gas chromatographic columns are described by Carey (*48*) for use in the determination of 2,3,5,6-tetrachloronitroanisole in vegetables and grains by electron capture gas chromatography. By using a cleanup column of activated magnesia and diatomaceous earth, as little as 0.02 p.p.m. can be detected without interference from crops.

Beckman and Bevenue (*21*) describe a method for the determination of tetrachlorothiophene and 1,2-dibromo-3-chloropropane in brussels sprouts and walnut meats, using electron capture gas chromatography. The sample is blended with petroleum ether, and the extract is passed through a Florisil column and injected into the gas chromatograph. The sensitivity of the method is reported as 0.01 p.p.m. Recoveries of 90 to 100% were obtained at levels of 0.05 to 1.0 p.p.m.

Burke and Mills (*46*) used a modification of the Mills procedure to determine Thiodan and Tedion residues. DDT was removed by elution from the Florisil columns with 6% ethyl ether in petroleum ether. Thiodan and Tedion then were eluted with 30% ethyl ether in petroleum ether. Determination was made by microcoulometric gas chromatography. Extracts of broccoli required additional cleanup on a column of sodium sulfate, attapulgus clay, Celite 545, and Nuchar 190 N. Dieldrin and endrin, if present, gave overlapping peaks with the column used in the work.

A colorimetric method for the determination of Thiodan in vegetables and beef fat is described by Maitlen, Walker, and Westlake (*141*). Vegetables were extracted by tumbling with an *n*-hexane–isopropanol mixture (2 + 1). Sugar beet extracts required cleanup by shaking with a charcoal–magnesium oxide mixture. Beef fat was ground with anhydrous sodium sulfate and extracted with *n*-pentane. Cleanup included acetonitrile partitioning and the use of a Florisil column. The actual determination was carried out in a single test tube; an aliquot was evaporated, methanolic sodium hydroxide–pyridine reagent was added, and the developed color was read at 520 mμ. Of 45 pesticides tested, only captan, chlordane, heptachlor, and ovex caused any interference. A somewhat similar procedure had been used earlier by Butler, Maitlen, and Fahey (*47*) to determine Thiodan in strawberries and alfalfa.

A modification of the colorimetric method for toxaphene has been reported by Nikolov and Donev (*160*). They state that the sensitivity of the determination was increased about tenfold by preliminary treatment with nitric acid before development of the color.

HERBICIDES

A number of methods have appeared for the determination of the chlorophenoxy acids and their esters in food products. The free acids are generally converted to their methyl esters for determination by gas chromatography. This is necessary because the free acids will not pass through the common silicon oil gas chromatographic column.

Bevenue, Zweig, and Nash (*27*) describe a method for determining 2,4-D and its esters in potatoes, using microcoulometric gas chromatography. Cleanup on a Florisil column was found necessary to remove unidentified interferences, and diazomethane was used to esterify any free 2,4-D. The method was sensitive to 0.01 p.p.m.

Both paper and thin layer chromatography were used by Abbott *et al.* (*1*) to determine MCPA, MCPB, 2,4-D, 2,4-DB, and 2,4,5-T in soil and water. The thin layer procedure separated dinoseb and DNOC from the above herbicides and detected their presence. Other chromatographic procedures for determining some of these herbicides in various crops are described by Bevenue, Zweig, and Nash (*26*) for 2,4-D in dry crops and walnuts; by Yip (*198*) for 2,4-D in wheat; by Gutenmann and Lisk (*94*) for 2,4-D and 2,4-DB in forage; by (*100*) for MCP in soil; and by (*98*) for silvex in water.

Daoud and Luh (*59*) describe a colorimetric method for determining 2,4,5-T in canned apricots at a level of about 1 p.p.m. After cleanup on activated basic aluminum oxide, the extracted residue was reacted with chromotropic acid and sulfuric acid. The resulting color was read at 565 mμ. The authors state that about 40% of the 2,4,5-T was converted to a combined form not extractable with ether.

Coakley, Campbell, and McFarren (*52*) used a somewhat similar color reaction to determine 2,4-D and its butoxyethanol ester in fish and shellfish at a sensitivity reported to be 0.1 p.p.m. The blended samples were treated with sodium hydroxide to hydrolyze the ester to 2,4-D which, after acidification, was extracted with benzene. A Florisil column was used for cleanup, after which the color was developed with chromotropic acid and read at 570 mμ. Gas chromatography was used to verify the identity of the 2,4-D.

Kirkland and Pease (*118*) used temperature-programmed microcoulometric gas chromatography for the determination of the herbicides Trysben (2,3,6-trichlorobenzoic acid) and Zobar (polychlorinated benzoic acid). The herbicides were extracted from samples of sorghum, wheat, barley, pineapple, and sugar cane by blending with methyl ethyl ketone. The extracted residues were cleaned up and converted to the methyl esters before they were chromatographed. Satisfactory analyses were carried out at 0.04 p.p.m.

Getsendaner (*84*) reports a method for the determination of dalapon in cranberries, bananas, and corn cobs. The determination was made by electron capture gas chromatography using a column consisting of 3.85% diethylene glycol adipate polyester and phosphoric acid on Gas Chrom S or Chromosorb W.A.W. These columns permitted the successful chromatographing of dalapon as the free acid so long as the acidity of the column (H_3PO_4) was maintained. Recoveries at levels of 0.25 to 5 p.p.m. ranged from 80 to 100%.

ORGANOPHOSPHATES—GENERAL PROCEDURES

The development of multiple detection schemes for the organophosphate pesticides has proved more difficult than for the chlorinated. Extraction and cleanup procedures, to be useful, must be capable of handling compounds of widely differing polarities, since the parent pesticides and their metabolites range from oil-soluble to water-soluble.

Coffin and Savary (*54*) report a procedure which includes acetonitrile extraction, elution from polyethylene-coated alumina with slightly acidified 40% acetonitrile, partitioning into chloroform, and final elution of the organophosphates from Magnesol with successive portions of chloroform, acetone, and methanol. The pesticides were separated by paper chromatography and the spots were located by one of several means. The portions of the paper chromatogram containing the spots were cut out and phosphorus was determined after digestion in a Schöniger flask. Recoveries of 80 to 107% were obtained for 41 organophosphate standards and for 25 organophosphates added to lettuce at levels of 0.4 to 1.5 p.p.m. Eight compounds tested were not determined by this procedure.

As mentioned earlier, a big step forward in the methodology for organophosphate residues has been the development of gas chromatographic detectors highly specific and sensitive for phosphorus-containing compounds (*42*, *86*). It is anticipated that as they become more readily available these detectors will find widespread use in the development of complete procedures that will be adequate for the determination of both the parent pesticide chemical and the significant metabolic products. Meanwhile earlier detectors are being used. Nelson (*157*) made use of microcoulometric gas chromatography with the sulfur cell for determining 10 thiophosphate pesticides in fruits and vegetables at levels ranging from 0.15 to 1.5 p.p.m. Samples were extracted by blending with acetonitrile, and the residues were partitioned into petroleum ether. No additional cleanup was used. It was pointed out that the study was made only on parent compounds. Many of these formed toxic metabolites which contained no sulfur or were water-soluble and so would not be detected by this procedure.

Egan, Hammond, and Thomson (*66*), using electron capture gas chromatography, obtained recoveries of 73 to 91% for a number of the parent organophosphates from lettuce, onions, apples, etc. The samples were blended with an ethyl methyl ketone–hexane mixture (3 + 2) and the extract was washed with sodium sulfate solution, passed through anhydrous sodium sulfate and then through an alumina or magnesia column, concentrated, and injected into the gas chromatograph. Two columns are described, and relative retention times and sensitivities for 19 organophosphate compounds are reported.

Anyone using conventional electron capture gas chromatography for determining organophosphate residues should remember that the electron capture detector is much more responsive to halogenated compounds. Chlorinated pesticide residues, if present even in trace amounts, can be mistaken for significant amounts of organophosphates with similar retention times.

Gutenmann and Lisk (*96*) took advantage of this high electron capture response to halogenated compounds in a method they used to determine ethion and malathion in solutions. They reported that organophosphates which contained methoxy or ethoxy groups reacted with HI (Zeisel alkoxyl reaction) to form methyl and ethyl iodides. These were injected into the gas chromatograph. It is pointed out that alcohols, ethers, and esters must be removed, since they also undergo the Zeisel reaction. This procedure, of course, does not identify the compound being measured other than as one containing a methoxy or ethoxy group.

Crosby and Laws (*57*) used gas chromatography as an additional cleanup step in preparing extracts for infrared determination. The entire effluent from the gas chromatograph was caught by passing it through methylene chloride. After evaporation of the methylene chloride, the residue was dissolved in carbon disulfide and the infrared spectrum was determined over the 5- to 15-micron range, using a cavity cell with beam condenser and scale expander. Good spectra were obtained with as little as 1 μg. residue. Recoveries of 50 to 80% were obtained from fruit and vegetables at 0.2 to 4.0 p.p.m. levels, and 40 to 50% at the lower limit of 0.1 p.p.m. Data are reported for 15 organophosphate compounds.

Frehse (*74*) has written a most extensive review on the use of infrared in pesticide residue analysis. Although it covers all classes of pesticides, the greatest emphasis is on organophosphates. Among the subjects discussed are extraction and cleanup procedures (where it is pointed out that thorough cleanup is indispensable), cells, solvents, analysis of solid substances, special equipment, and the infrared characteristics of organophosphate pesticides.

MacRae and McKinley (*139*) used a Solka-Floc and activated charcoal column to clean up residues prior to paper chromatography. Two systems are described which can be used to identify 13 parent organophosphates. However, when added to crop extracts, many of the compounds were not recovered.

Zadrozinska (*200*) determined parathion, methyl parathion, malathion, and diazinon in strawberries, cabbage, spinach, etc., at levels of 0.5 to 2 p.p.m. by a paper chromatographic procedure. After bromination, fluorescein was used to detect the spots on the chromatograms.

Three color reagents (metanil yellow, yellow RFS, and methyl orange) for detecting thiophosphates on paper chromatograms are reported by Dutt and Seow (*65*). Metanil yellow was found to be the best of the three when tested on parathion, malathion, diazinon, and dimethoate. The limit of detection was 1 to 2 μg.

Getz and Friedman (*83*) studied cholinesterase inhibition methods of detecting organophosphates on paper chromatograms. They developed two procedures. In one, a direct method, the developed chromatogram itself was sprayed first with enzyme-indicator solution and then with the substrate. In the other, or indirect, procedure, after a second sheet of paper had been sprayed with the enzyme indicator solution, it was placed in firm contact with the developed chromatogram and incubated for 15 minutes. Then the second sheet was treated with substrate and the spots were developed on it.

McKinley and Johal (*138*) described the use of liver esterase inhibition for detection of organophosphate spots on paper chromatograms. The substrate was 1-naphthyl acetate and the color reagent was azoene fast blue RR. About 30 organophosphate pesticides and metabolites, as well as carbaryl, were studied. Most of the compounds were detectable at levels between 0.01 and 0.50 μg.; some required as much as 3 μg.

Thin layer chromatography has also been used in the determination of organophosphates. Uchiyama and Okui (*190*) list R_f values for 14 compounds chromatographed on silica gel plates using a hexane-acetone (4 + 1) mixture as developing solvent. Bunyan (*41*) adapted both the bromophenol blue–silver nitrate reagent for thiophosphates and the cholinesterase inhibition method for use on thin layer chromatography. The bromophenol blue–silver nitrate reagent was found to be more sensitive on silica gel plates (<0.1 to 0.6 μg.) than on alumina (about 0.5 μg.). The cholinesterase inhibition technique would not work directly on the thin layer plates and required the use of sprayed paper placed in contact with the plate, similar to the method described above (*83*). Again, silica gel plates worked better than alumina.

Several procedures based upon the molybdenum blue method for determination of phosphorus after extraction,

cleanup, and oxidation to inorganic phosphate have been reported. Blinn (*28*) used a Schöniger combustion flask to determine dimethoate in a number of agricultural products, with a sensitivity of 0.1 p.p.m. Brewerton (*39*) used perchloric acid digestion. Isaeva and Enoshevskaya (*109*) used a mixture of nitric and sulfuric acids and potassium permanganate as the digesting and oxidizing agent in determining a number of the organophosphates.

These last three procedures, of course, do not identify the pesticide but simply measure the total phosphorus. However, when combined with paper or thin layer chromatography, the method becomes more specific. The cleaned-up sample extracts may be chromatographed on paper or thin layer and the identity of the residue determined by the R_f of the spot. The spots then may be cut out from the paper chromatogram or scraped off the thin layer plate and total phosphorus determined to obtain a quantitative value. Ruelene in milk (*132*) and phosphamidon in vegetables and fruits (*8*) have been determined in such a manner by paper chromatography. Thin layer chromatography has been used similarly in the determination of dimethoate (*180*).

A rather novel approach for semiquantitative determination of organophosphates was taken by Brusaux, Dormal, and Thomas (*40*). It is based upon the fact that esterases from various bovine organs separate into five to seven zones when extracts of the organs are submitted to agar-gel electrophoresis on microscope slides. Total or partial disappearance of one or more of the zones occurred when organophosphates were added to the extracts prior to electrophoresis. Inhibition patterns for 14 compounds and procedures for the analysis of samples of unknown history are described. With kidney extracts, the sensitivity is reported as 0.05 p.p.m. for some of the pesticides.

ORGANOPHOSPHATES—SPECIFIC PROCEDURES

Van Middelem, Waites, and Wilson (*191*) used both electron capture gas chromatography and the dinitrochlorobenzene colorimetric method to determine dimethoate in snap beans and found results by the two procedures in good agreement. Only the parent compound was actually measured, since the oxygen analog was not recovered by the procedures.

A modification of the colorimetric method for diazinon was used by Enos and Frear (*69*) to determine dimethoate in fruits and forage. The sample was extracted with a solvent (varying with nature of sample) and, after cleanup by solvent extraction, the dimethoate was extracted from hexane with hydrobromic acid. Acid hydrolysis produced hydrogen sulfide, which was swept into a receiver containing zinc acetate and reacted with *N,N*-dimethyl-*p*-phenylenediamine hydrochloride to form methylene blue, the absorbance of which was read at 670 mμ.

Giang and Schechter (*85*) determined dimethoate in milk and various crops by a colorimetric procedure, which measured both the parent compound and its oxygen analog. The compounds were hydrolyzed with alkali to thioglycolic acid, which was reacted with sodium phospho-18-tungstate, and the absorbance was measured at 720 mμ.

Enos and Frear (*68*) used paper chromatography to determine dimethoate in milk. The dimethoate was extracted from milk with an ethyl ether–hexane mixture. After transfer to hexane, the extract was cleaned up on a Florisil column and then spotted for paper chromatography. After development the paper was sprayed with 2,6-dibromo-*N*-chloro-*p*-quinoneimine. Dimethoate showed up as a red spot. Diazinon, Guthion, Systox, Trithion, and malathion did not react.

Cerna (*49*) reports a colorimetric method, based on the Fujiwara reaction, for the determination of Dipterex (trichlorfon) in foods.

Mitsui *et al.* (*149*) describe a colorimetric method for DDVP based on an orange-red complex that is formed between DDVP and acetone in the presence of alcoholic potassium hydroxide. Absorption at 370 mμ follows the Beer-Lambert law. They report that the method is also applicable to Dipterex and Dibrom, and the procedure for Dibrom is described (*150*).

Sun and Johnson (*183*) have developed a fly bioassay procedure which can determine as little as 0.1 p.p.m. DDVP in the presence of many other insecticides.

Archer *et al.* (*9*) report a nonspecific cholinesterase inhibition procedure for the determination of ethion in olives. They used peracetic acid to oxidise the ethion because the olefinic compounds present in olives interfered with the usual bromine treatment. The method, with modified cleanups, worked well on a number of fruits and vegetables.

Graham and Orwoll (*90*) describe a procedure in which the ethion is hydrolyzed with ethanolic sodium hydroxide and the diethyl phosphorodithioic acid formed is determined spectrophotometrically as its complex copper salt absorbing at 418 mμ. To make the procedure specific for ethion, Delnav is eliminated by a mercuric chloride treatment and other phosphate pesticides are eliminated by a dilute sodium hydroxide wash. The method is reported as applicable to a number of fruits and vegetables.

Dawson, Donegan, and Thain (*61*) used electron capture gas chromatography to determine Fenitrothion [dimethyl(3-methyl-4-nitrophenyl phosphorothionate)], parathion, Chlorthion, and paraoxon in cocoa beans.

Cox (*55, 56*) studied the colorimetric procedure for Guthion in which the pesticide is hydrolyzed to anthranilic acid, diazotized, and coupled with *N*-(1-naphthyl)ethylenediamine dihydrochloride. He reports a collaborative study in which Guthion was added to various fruits and vegetables at levels of 0.3 to 1.56 p.p.m. Recoveries ranged from 53 to 137%.

Miles (*144*) describes a new and rapid colorimetric method for Guthion, Ethyl Guthion, and their oxygen analogs. It is based on the direct coupling of the pesticide with *N*-(1-naphthyl)ethylenediamine dihydrochloride in the presence of acetic and hydrochloric acids to produce a purple solution with absorption maximum at 556 mμ. The samples were extracted by blending or tumbling with chloroform and were cleaned up with an Attaclay-Celite mixture. The oxygen analogs were separated from the parent compounds on a Florisil column. Recoveries from fruits and vegetables ranged from 78 to 97%.

Frehse, Niessen, and Tiets (*75, 76*) report an infrared method for fenthion (Lebaycid) in beet leaves, lettuce, cabbage, apples, and cherries as well as olives and olive oil. After extraction and cleanup, the residue was oxidized with potassium permanganate. The sulfone band at 7.55 microns was used for quantitation and the spectrum from 7 to 11 microns for identification. A micro phosphorus determination may also be run on the cleaned-up residue.

Bates and Rowlands (*18, 19*) have studied the conventional colorimetric method for malathion. This procedure involves the alkaline decomposition of malathion to sodium dimethyldithiophosphate, which is extracted and complexed with copper. They found that many stored food products, such as citrus pulp, coconut meal, copra, flour, etc., gave troublesome emulsions unless a preliminary chromatographic cleanup on alumina or silica gel column was included. They also found that recoveries from some rice brans were low (*18*). This they believe to be due to the formation of free fatty acid in the bran during storage. Rowlands (*172*) eliminated interferences in the determination of malathion in pimento by using polyethylene-coated alumina and acid-washed alumina cleanup columns.

Fischer and Uhlich (*72*) report an infrared method for the determination of malathion in kohlrabi, lettuce, and cauliflower. The cleaned-up extracts were dissolved in carbon disulfide and the absorption band at 9.82 microns was used to determine the malathion.

Considerable work has been done on the determination of parathion and

related compounds. Van Middelem, Waites, and Wilson (*192*) studied various extraction and cleanup procedures for parathion in leafy vegetables. They found blending with a mixture of isopropyl alcohol and benzene to be the preferred method of extraction. Straight tumbling with benzene gave very low recoveries. They also describe a chromatographic cleanup column which is superior to shaking the raw extract with a decolorizing mixture.

George (*82*) reports a micro method for the determination of parathion and such similar compounds as methyl parathion, binapacryl, EPN, and Guthion. This method is based on the Averell-Norris colorimetric procedure but is said to be ten times more sensitive. In the determination, Guthion was first hydrolyzed to break the nitrogen ring. Karathane and Chlorthion interfere in the method but unhydrolyzed Guthion gives only a slight color.

Coffin and McKinley (*53*) report both a colorimetric and a paper chromatographic method for parathion, methyl parathion, EPN, and their oxons. To determine the total *p*-nitrophenol, the cleaned-up extract is treated with hydrogen peroxide and potassium hydroxide and the *p*-nitrophenate is measured colorimetrically at 400 mμ. To determine individual compounds, the cleaned-up extracts are chromatographed on paper, the developed chromatogram is treated with bromine and potassium hydroxide, and the individual spots of *p*-nitrophenate are eluted and read at 400 mμ. Recoveries of 84 to 101% are reported from lettuce, strawberries, and apples at levels from 0.4 to 1.3 p.p.m. As little as 1 μg. of each compound was readily detected on the paper chromatogram, and aromatic amines did not interfere.

Kubistova (*127*) described a method for parathion and *p*-nitrophenol in animal tissue. The sample was blended with acetone, the residue transferred to chloroform, and *p*-nitrophenol extracted with a sodium carbonate solution. In the determination of total parathion and *p*-nitrophenol, a second sample was extracted and parathion was hydrolyzed to *p*-nitrophenol. The *p*-nitrophenol was determined as an indophenol blue after reduction with titanium trichloride and reaction with *o*-cresol.

Gajan (*79*) developed a polarographic method for the determination of parathion. It was tested on green beans, apples, tomatoes, broccoli, spinach, and brussels sprouts and was able to detect as little as 0.1 p.p.m. parathion. Methyl anthranilate and *p*-nitrophenol did not interfere.

Several methods have been proposed for the determination of phorate. Waldron *et al.* (*195*) used an improved colorimetric method in which phorate is hydrolyzed to release formaldehyde which is then reacted with chromotropic acid. Reagent and crop blanks are required, and the authors point out possible interference from formaldehyde in the air or from phosgene in the chloroform.

To determine phorate, Blinn (*29*) used thin layer chromatography with infrared or the colorimetric chromotropic acid method. The residue was oxidized by peracetic acid to the oxygen analog sulfone. By using thin layer chromatography the residue was separated from potential interfering pesticides and positively identified. A palladium chloride chromogenic agent did not interfere with the colorimetric or infrared determination of the eluted spots. Excellent infrared spectra were obtained with as little as 7 μg. by using ultramicropotassium bromide pellets and beam condenser. Blinn (*30*) later compared the ability of 12 oxidants to convert phorate to its oxygen analog sulfone. He reports that *m*-chloroperbenzoic acid worked best. He also suggests the use of silica gel thin layer plates buffered at pH 6 to prevent hydrolytic decomposition of the organophosphate esters.

Although Archer *et al.* (*10*) also used peracetic acid to oxidize phorate, the phorate was determined after oxidation by cholinesterase inhibition. Potatoes were anlyzed by extracting with chloroform and anhydrous sodium sulfate without further cleanup. Sugar beet leaf extracts were cleaned up on a sodium carbonate–Celite 545–charcoal column, and cottonseed extracts on a Florisil column.

Cholinesterase inhibition was used by Blumen (*33*) to determine Phosdrin in fruits and vegetables. A modification of the procedure in which unhydrolyzed acetylcholine is converted to hydroxamic acid and reacted with ferric chloride to form a red complex was studied collaboratively. Recoveries from apples, cabbage, and tomatoes ranged from 70 to 117% at levels of 0.164 and 0.328 p.p.m.

Claborn and Ivey (*51*) report a colorimetric method for determining Nemacide (VC-13) and ronnel in animal tissue. After extraction and cleanup, the pesticides are hydrolyzed and the resulting chlorophenols are steam-distilled and reacted with 4-aminoantipyrine. The resulting color is extracted into a nitromethane-pyridine mixture and read at 490 mμ. Sensitivity is estimated to be 0.05 p.p.m. Teasley (*186*) used a different version of the colorimetric procedure to determine Nemacide [*O,O*-diethyl O-(2,4-dichlorophenyl)-phosphorothioate] in fruits and vegetables. The method was subjected to collaborative study, and although three collaborators obtained fair results, two others were unable to do so. Magat (*140*) describes a modified method for ronnel in meat in which the ronnel is also hydrolyzed and the trichlorophenol steam-distilled, but, in place of using a colorimetric determination, the trichlorophenol is determined directly by ultraviolet spectroscopy at 315 mμ. This method, however, is not applicable to samples containing less than 1 p.p.m.

Adams, Anderson, and McDougall (*5*) report a paper chromatographic method for determining Systox (demeton) and its toxic metabolites. An ethanol solution of the extracts is cleaned up on a column of acid-washed alumina and then is chromatographed on silicone-treated paper. The paper is sprayed with potassium permanganate and treated with potassium hydroxide. Systox and its most important metabolites form *O,O*-diethylphosphorothioic acid which is then detected by spraying with 2,6-dibromo-*N*-chloro-*p*-quinoneimine. This procedure is said to have a sensitivity of 0.3 p.p.m. and to distinguish residues of Systox and its metabolites in the presence of other organophosphate pesticides.

Trotsenko (*189*) describes a method for detecting Systox in air. The method is based on the ability of the thiol isomer to extinguish the fluorescence of eosin. Geldmacher-Mallinckrodt and Weigel (*81*) studied the reaction of the hydrolysis products of Systox and Meta-Systox with heavy metals. They suggest the use of copper and cobalt solutions as spray reagents after separation of the compounds by thin layer chromatography.

A spectrophotofluorometric method for Zinophos and its oxygen analog is described by Kiigemagi and Terriere (*115*). After extraction and cleanup, the residue is hydrolyzed and washed with strong alkali. It is then activated at 315 mμ and the fluorescence measured at 375 mμ. The method was tried on a number of fruits and vegetables and is said to have a sensitivity of 0.05 p.p.m.

CARBAMATES

A general infrared method for the determination of *N*-methyl carbamates in plants has been described by Niessen and Frehse (*158*). Samples were extracted by blending with acetone. Interfering plant material was precipitated with an ammonium chloride–phosphoric acid coagulating solution and, after additional cleanup on alumina, the infrared spectrum from 2.95 to 2.83 microns was recorded. The absorption at 2.88 microns due to the N—H stretching vibration was used for quantitation. As little as 0.2 p.p.m. of the pesticides could be determined.

After the infrared determination, the carbon disulfide solution was used for thin layer chromatography on alumina

G, which served to determine the identity of the pesticide. R_f values are listed for seven compounds.

An infrared method was used by Ferguson *et al.* (*71*) to determine CIPC in white potatoes. After extraction and cleanup the residue was dissolved in carbon disulfide and the infrared spectra were obtained of the solution in 0.5-mm. cells. Peaks at 1110 and 1210 $cm.^{-1}$ were used for calculation. Monuron and diuron did not interfere and the three compounds could be distinguished by their infrared spectra. The method was used on samples which contained between 2 and 15 p.p.m. CIPC. To determine CIPC in milk and urine, Gard and Ferguson (*80*) used modifications of other methods. The CIPC was hydrolyzed; the 3-chloroaniline was distilled, diazotized, and coupled with *N*-(1-naphthyl)-ethylene diamine dihydrochloride. In order to obtain consistent low blanks, it was necessary to add formalin to the urine and to age it for 48 hours prior to analysis.

Hardon, Brunink, and Van der Pol (*102*) made use of similar diazotization and coupling to determine dichloran (2,6-dichloro-4-nitroaniline), a fungicide. Although not a carbamate, it is listed here since it can, if present, interfere in the determination of some of the carbamates.

Johnson (*110*) conducted a collaborative study of the colorimetric method for carbaryl. After minor modifications were made to improve the method, an additional collaborative study was run (*111*) on samples of apples and lettuce. Recoveries averaged 87.8%.

Chiba and Morley (*50*) introduced a rapid thin layer chromatographic screening procedure for carbaryl without any prior cleanup. The sample was extracted by blending with methylene chloride and anhydrous sodium sulfate, evaporated, dissolved in petroleum ether, and spotted on silica gel plates. After development, the plates were sprayed with methanolic sodium hydroxide and the hydrolyzed 1-naphthol was coupled by spraying with a solution of *p*-nitrobenzene diazonium fluoborate. The authors note that with suitable cleanup much lower amounts of carbaryl can be detected.

Bracha (*37*) used a different diazonium salt in the determination of *O* - isopropoxyphenyl - *N* - methylcarbamate. For the determination of residues on various surfaces, he coupled the hydrolyzed insecticide with diazotized 3-nitroaniline-4-sulfonic acid and measured the absorbance at 490 mμ. The developed color was very stable in water. This method has been adapted for the determination of carbaryl, Isolan, Pyrolan, Dimetilan, and Hercules AC-5727 (*m*-isopropylphenyl-*N*-methylcarbamate).

Marquardt and Luce (*142*) report the use of a new color reagent in determining Zectran in peaches and cottonseed. The Zectran was extracted from the sample and hydrolyzed to yield 4-dimethylamino-3,5-xylenol, which then was reacted with luteoarsenotungstic acid. Absorbance was measured at 700 mμ. The luteoarsenotungstic acid is said to be highly specific for 4-dimethylamino-3,5-xylenol.

Cullen (*58*) modified the standard procedure for dithiocarbamates. The residue was decomposed directly on the crop and the evolved carbon disulfide was collected and reacted with a solution of cupric acetate and diethanolamine in ethanol. Absorbance was measured at 435 mμ. Cullen points out that the dithiocarbamates decompose very quickly when in a slurry of a crop or in contact with slightly polar solvents. Samples should either be analyzed immediately after harvest or frozen for storage. The method was tested on ferbam, ziram, maneb, zineb, thiram, and metiram.

DINITRO COMPOUNDS

From a study of methods for determining the dinitro compounds, Boggs (*34*) concludes that the paper chromatographic procedure is still the best general method. He lists R_f values for six compounds for both the aqueous and nonaqueous systems.

Potter (*168*) determined Dinoseb in potatoes by measuring its absorbance in ethyl methyl ketone at 379 mμ after extraction and cleanup.

Abbott and Thomson (*2, 3*) used a wedge-layer type of plate chromatography as cleanup in the determination of Dinoseb in a number of fruits and vegetables. The plates were coated with a layer of silica gel-kieselguhr, which varied in thickness from 2 mm. at one edge to 0.1 mm. on the opposite edge. The sample extract was applied as a streak near the thick edge and the plate was developed. The yellow Dinoseb band was then scraped off; the pesticide was eluted with a solvent and determined by infrared or gas chromatography or colorimetrically by the method of Potter (*168*) described above.

Kilgore and Cheng (*116*) note that Karathane dissolved in *N,N*-dimethylformamide gives a strong yellow color without the addition of alkali. They used this phenomenon as a basis for the determination of Karathane in fruit. A hexane extract of the sample was cleaned up, either on a Florisil column or by washing with concentrated sulfuric acid, and evaporated. The residue was dissolved in *N,N*-dimethylformamide and absorbance read at 444 mμ. A sensitivity of about 0.05 p.p.m. was attained.

Heinisch and Panser (*105*) report a method for dinitro-*o*-cresol in plants used for fodder. A dilute sodium hydroxide extract of the sample was acidified and extracted with petroleum ether. The solvent was evaporated and the residue was dissolved in 5 ml. of ethanol and treated with 0.5 ml. of propanol and 2 drops of 10% aqueous potassium cyanide to produce an orange color, which was measured. The method is said to be good for residues as low as 0.1 p.p.m.

FUNGICIDES

Gunther, Blinn, and Barkley (*92*) describe a procedure for determining biphenyl and *o*-phenylphenol in citrus fruit. The sample was blended with water and the residues were isolated by steam distillation into cyclohexane. After separation, the *o*-phenylphenol was coupled with *p*-nitrobenzenediazonium fluoborate and determined colorimetrically at 540 mμ. Biphenyl was determined directly by measurement of its absorbance at 248 mμ.

Souci and Maier-Haarlaender (*179*) used a similar procedure for biphenyl but modified the steam distillation apparatus. Rajzman (*169*) reports a method for biphenyl in citrus fruit based upon the blue color given by biphenyl with sulfuric acid and traces of formaldehyde and ferric iron. The absorbance was measured at 610 mμ. There is no interference from *o*-phenylphenol; it does give a pink color but this disappears during treatment with sulfuric acid.

Vogel and Deshusses (*194*) used steam distillation to separate *o*-phenylphenol from citrus fruit. The *o*-phenylphenol was then reacted with 2,6-dibromoquinone-chloroimide and absorbance was measured at 619 mμ.

To determine diphenylamine in apples, Gutenmann and Lisk (*95*) used electron capture gas chromatography. The residue was extracted and brominated to form what was believed to be a hexabromo derivative of diphenylamine, which was then injected into the gas chromatograph. Solvents were redistilled and contact with rubber, which might contain diphenylamine, was avoided.

Anderson and Adams (*7*) report a colorimetric method for the determination of Dexon (*p*-dimethylaminobenzenediazo sodium sulfonate) in corn, cottonseed, and several other crops. The sample was blended with 1% sodium sulfite and the Dexon was isolated by dialysis. The Dexon was then reacted with resorcinol and sodium hydroxide and irradiated with light from two projection spotlights to produce a yellow color read at 450 mμ.

Pasarela (*164*) conducted a collaborative study of the colorimetric method [Steller *et al.*, *J. Agr. Food Chem.* **8**, 460 (1960)] for dodine in fruit at levels

of 0.1 to 10.4 p.p.m. Recoveries ranged from 64 to 119%, with most values falling between 80 and 110%.

Kleinman (*122*) conducted a collaborative study of the colorimetric method for glyodin in pears and peaches. Recoveries averaged about 88% for pears and 85% for peaches. Two collaborators, however, reported difficulties with peaches.

Niessen, Frehse, and Tietz (*159*) developed a quantitative procedure for Fungilon (Bayer 32394) residues on apples, using a microtitration in a two-phase chloroform–water system. Apples were stripped with chloroform, waxes were removed by precipitation from cold methanol solution, and the extract was cleaned up on an alumina column. The Fungilon then was titrated with Aerosol OT (dioctyl-sodium-sulfosuccinate), with methylene blue as indicator. At the end point, color intensity was equal in the two layers. Glyodin and dodine are reported to interfere.

MISCELLANEOUS PESTICIDES

Analytical methods have been reported for a number of herbicides and other growth regulators in addition to those discussed above.

HEH (β-hydroxyethylhydrazine), also known as "Omaflora," is used to induce flowering in pineapples. Thomas and Ackermann (*187*) have developed a colorimetric method for its determination. After extraction with water and removal of interfering color pigments with ion exchange resins, the HEH is reacted with cinnamaldehyde to produce a yellow color which is read at 420 mμ.

Fletcher and Zalik (*73*) developed a method for 3-indoleacetic acid in which a methanolic extract of the plant material was chromatographed on paper and part of the chromatogram was sprayed with a chromogenic reagent to locate the indoleacetic acid. The corresponding R_f region from the unsprayed area was eluted with methanol and the ultraviolet spectrum was determined. Absorbance at 280 mμ was used for quantitative determination.

Lane (*129*) conducted a collaborative study of the colorimetric method for maleic hydrazide in potatoes. Recoveries were satisfactory.

Zweig *et al.* (*201*) developed a method for the determination of naphthaleneacetic acid in olives, using gas chromatography as part of the cleanup procedure. The olives were blended with chloroform and hydrochloric acid and the extract was passed through alumina and silica gel columns. The residue then was methylated with diazomethane and injected into the gas chromatograph. Fractions were collected. The eluate was nitrated and naphthaleneacetic acid was determined from the absorbance at 360 mμ.

Young, Shimabukuro, and Aono (*199*) determined naphthaleneacetic acid in pineapples by its ultraviolet absorbance after eliminating interferences by oxidation with potassium permanganate.

Petunova and Martinson (*165*) based their method for simazine in plant tissue on the ultraviolet absorbance of hydroxysimazine. After extraction and cleanup, the simazine was treated with sulfuric acid and hydrolyzed to hydroxysimazine. The absorbance then was measured at 225, 240, and 255 mμ.

Benfield and Chilwell (*25*) have proposed a method for determining the s-triazines in soil and in crops by gas chromatography of the cleaned-up extract. They used a 4-foot column packed with 0.1% ethylene glycol adipate polyester on glass beads. An unusual feature of their method was the addition of a second related triazine to the sample as an internal standard before extraction. Final determination involved only the ratio between the amounts of the two components present.

Blinn and Gunther (*32*) developed a procedure for distinguishing between residues of Aramite and OW-9 in foodstuffs. The two acaricides have similar structures, and OW-9 responds to the usual colorimetric procedure for Aramite. To distinguish between them, Blinn and Gunther used gas and thin layer chromatography as well as gas chromatography of their parent carbinols after hydrolysis.

Tietz *et al.* (*188*) made use of a red nickel chelate complex formed with ammonia to determine Eradex (2,3-quinoxalinedithiol cyclic trithiocarbonate) in fruit. Absorbance was measured at 530 mμ. Havens, Adams, and Anderson (*103*) used a similar reaction to determine Morestan (6-methyl-2,3-quinoxalinedithiol cyclic carbonate) in apples and pears. They read the absorbance at 540 mμ.

Sinclair, Lindgren, and Forbes (*178*) determined ethylene chlorobromide, using the procedure of Sinclair *et al.* for ethylene dibromide [*J. Econ. Entmol.* **55**, 236 (1962)]. This procedure consisted of steam distillation, alkaline hydrolysis, and determination of the bromide. It was pointed out that since ethylene chlorobromide undergoes degradation in products, inorganic bromides should also be determined.

Kimura and Miller (*117*) modified the colorimetric method to determine metaldehyde in plant material. Emulsification problems were resolved by passing the extract through a Florex column. Objectionable interfering colors were eliminated by evaporating the chloroform extract to dryness.

After minor modifications, the ultraviolet method for nicotine in fruits and vegetables was studied collaboratively by Martin and Schwartzman (*143*). Recoveries from apples, cabbage, spinach, and mustard greens at 1.4 to 2.4 p.p.m. levels ranged from 84 to 120%.

Munday (*155*) conducted a collaborative study in which the A.O.A.C. method for piperonyl butoxide was tested on a number of processed grain products. It was found that plant extractives gave an abnormal brown color and interfered in the determination.

Hoffman and Gordon (*107*, *108*) studied the A.O.A.C. colorimetric methods for arsenic and found that the arsine–molybdenum blue method gave slightly better reproducibility than did the silver diethyldithiocarbamate procedure, although both were suitable for determining arsenic in foods. They report that antimony does not interfere with the arsine–molybdenum blue method and that its interference with the silver dithiocarbamate method can be prevented by adding more stannous chloride to the generating mixture.

Methods for cyanide have been reviewed by Bark and Higson (*15*) who compare and evaluate the various procedures. Jones and Schwartzman (*112*) report a rapid method for determining mercury in wheat containing treated seed kernels. The treated kernels were picked out visually under ultraviolet light and burned in a Schöniger combustion flask. Mercury was determined with dithizone. Good agreement with the official A.O.A.C. method is claimed. An analysis can be completed in about two hours.

Pickard and Martin (*167*) describe a method for determining mercury in soil. The sample is digested with sulfuric and nitric acids and selenium, and the mercury is distilled from boiling sulfuric acid with hydrogen chloride gas. After treatment with EDTA and sodium thiosulfate, the mercury is determined with dithizone.

Phillips, Bowman, and Schultheis (*166*) have developed a screening procedure, the main purpose of which is to single out samples that may contain overtolerance residues. Bioassay, organic chlorine, and acetylcholinesterase inhibition determinations were run on the same extract. Comparison of various ratios provided the basis for characterization and estimation of most insecticides that inhibited cholinesterase or that contained chlorine.

Polarography has come into wider use in residue analysis work. Davidek and Janicek (*60*) and Kosmatyi and Shlyapak (*123*) used polarography to determine DDT, and Gajan (*79*) made use of the technique to determine parathion. Nangniot and Dardene (*156*) describe three polarographic methods for determining captan and folpet (Phaltan) on plants.

Veksler and Tsukervanik (*193*) list a number of defoliants which can be determined quantitatively by polarography. They state that there is a relationship between the polarographic behavior of the compounds and their activities as defoliants.

Morris and Haenni (*154*) determined the infrared spectra (2 to 35 microns) of 24 pesticides, using potassium bromide disks. They discuss the relation of absorption band to structure and note the maxima of analytical significance.

Guillemin (*91*) separated the isomers of BHC by gas chromatography, using a 3 m. × 6 mm. stainless steel column packed with 40- to 60-mesh glass beads coated with 0.25% polypropylene glycol Niax 1025.

In spite of advances in instrumentation, bioassay methods continue to have their uses. Sum *et al.* (*184*) discuss factors that may affect results and suggest precautions to be taken in bioassays. Weinmann (*197*) describes methods of purifying the extracts and evaluating results. Funderburk and Lawrence (*78*) determined Diquat and Paraquat by measuring their bleaching effect on duckweed (*Lemna* minor L.). as little as 0.0005 p.p.m. Diquat or 0.00075 p.p.m. Paraquat can be detected.

Two methods make use of newer techniques to accomplish the familiar determination of organic chlorides in fat. Krzeminski and Landmann (*125*) used sodium in liquid ammonia to release the chloride from pesticide residues and then determined the chloride potentiometrically. Schmitt and Zweig (*174*) used neutron activation to determine total organic chloride in butter fat. The sensitivity is reported to be 10 p.p.b. total organic chloride and the time of analysis to be less than 1 hour per sample, which can be reduced considerably if many specimens are processed simultaneously.

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Pesticide Residues

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RESEARCH IN THE FIELD of methodology for residue analysis has received much attention during the two-year review period from November 1964 through October 1966. In preparing this review, no attempt has been made to include all articles dealing with residue analyses but, rather, the authors have tried to select those which, in their opinion, will be most useful to the worker in this field.

Much of the literature refers to the "multidetection" methods in which many chemicals can be determined during one analysis. The possible inclusion of so many compounds in one analysis is a tremendous step forward but it does increase the problem of interpreting the responses properly so that one has adequate assurance of the identity of the compounds reported. This problem is basically no different from interpreting the response of the older methods such as a color reaction of the "wet chemical" or "specific" methods; for example the "specific" colorimetric method for parathion also responds to paraoxon, methyl parathion, EPN, Chlorthion, etc. The GLC methods generally give separate response for each of these; thus the possible misinterpretation is more obvious. This problem has been given a great deal of study and considerable progress has been made as indicated in numerous places throughout the review.

Closely associated with the field of methodology, particularly with the multidetection methods, is that of the "nature of the terminal residues." A pesticide chemical may be altered by its environment after application to yield terminal residues sufficiently close to the parent chemical to appear as a response to the detection system of a multidetection method. Developments in this field should be closely watched and taken into consideration as aids in the interpretation of responses in many of the methods now available.

There is no single uniform system of nomenclature used in the literature for pesticide chemicals, so for many pesticides there is no single generally recognized name. Both the coined patent names and the common names vary with the country and worker; thus there may be a number of names to describe the same compound. On the other hand, some names have recognition by all workers in the field. Because of these problems and because the authors believe that the use of chemical nomenclature exclusively would make the review difficult to follow, we have arbitrarily adopted a mixed system. In Frear's "Pesticide Index" (*111*), probably the most comprehensive listing of pesticides, common names, trade and chemical names are listed and cross-indexed. We have tried to use the name which we believe will be most meaningful to the reader and, where possible, to use a name which may be found in Frear's Index. Common names which appear in the United States Food and Drug Administration tolerance regulations have been used where possible. Common names of other compounds have been used whenever they exist and are considered to be well known. For those compounds best known by a single trade name we have used the trade name in capitalized form. In those instances where the common or trade name may be confusing or is not well known, we have tried to include the chemical name as well. Thus the reader should, with the help of Frear's Index, be able to

identify almost every compound referred to in this paper.

The literature on pesticide residues has maintained its rapid growth in the two years since the previous biannual review (*75*). Gunther (*133*) has continued his excellent series of "Residue Reviews" and 15 volumes have now been published. These volumes contain articles on many aspects of pesticide residues written by experts in the specific fields. Volume 1 of the Food and Drug Administration's "Pesticide Analytical Manual" (*19*), probably the most widely used manual of methods for pesticide residue analysis, has undergone continuous revision and updating. The methods in this volume have been studied in FDA laboratories and are known to be useful combinations of extraction, cleanup, and determinative steps which yield quite satisfactory qualitative and quantitative results for many compounds.

The U. S. Public Health Service has published a 2-volume "Guide to the Analysis of Pesticide Residues" (*55*) which includes methods for the analysis of water and soil as well as foods. However, this is primarily a compilation of methods recommended by a variety of laboratories and which have not necessarily been used or tested by the U. S. Public Health Service.

Several new periodicals of interest have appeared in the period covered by this review. The "Bulletin of Environmental Contamination and Toxicology" (*53*) is a bimonthly journal designed to provide a rapid publication in fields including pesticide residue methodology. The first issue was dated January-February 1966.

The U. S. Department of Agriculture began the publication of "Pesticides Documentation Bulletin" (*243*) on March 19, 1965. This biweekly is a "... computer produced permuted title index in three parts: Keyword Index, Bibliography, and Author Index." This is excellent as a general reference for those interested in many aspects of pesticides but is of little value to the residue analytical chemist as a specific reference because only those words which appear in the title of an article will appear as entries. There is no grouping of subjects such as "methods of analysis" or "chemical methods of analysis" unless those words appear in the titles of the original article.

More recent and potentially most valuable to the pesticide residue chemist is the "Health Aspects of Pesticides—Literature Bulletin" (*146*). This is an experimental monthly publication of the Office of Pesticides of the U. S. Public Health Service. The first issue, dated September 1966, contained 117 abstracts including 26 of recent articles dealing with analysis. In addition to author and subject indexes, the contents are also divided into sections dealing with pollution, toxicity and toxicological factors, analysis, etc.

This two-year period has also seen the beginnings of attempts to automate pesticide residue analysis. Gunther *et al.* (*136*) designed a system for the determination of total chlorine. They combined an automatic combustion apparatus with a continuous flow chloride ion detector. It was capable of handling as much as 2-gram equivalents of plant extractives and had a useful range of 0.01 to 500 ppm. The burning cycle of 7 minutes was automatic and the chloride ion measured and recorded. Ott and Gunther (*233*) reported an automated colorimetric phosphorus determination which had a sensitivity well below 0.1 μg phosphorus per milliliter of final solution. Samples were cleaned up and burned in a Schöniger flask and the solution was transferred to an *Auto*Analyzer which carried out the analysis and recorded the result. Several procedures were reported for the automatic determination of cholinesterase inhibition using the *Auto*Analyzer. Voss (*283*) described a procedure in which acetylthiocholine was used as the substrate. The liberated thiocholine acted on 5,5-dithiobis-2-nitrobenzoic acid producing a color which was measured at 420 mμ. Ott and Gunther (*231*, *232*) used acetylcholine as the substrate and measured the transmittance at 555 mμ as affected by change in color of phenol red.

All of the above procedures required prior extraction and cleanup before the sample solution could be placed in the *Auto*Analyzer. The first fully automated analysis was reported by Gunther and Ott (*137*) for the determination of biphenyl in citrus fruit rind. The sample was automatically homogenized in water and the biphenyl steam distilled. Oils and waxes were removed with H_2SO_4 and a cyclohexane solution of the biphenyl was passed through the cell of a recording UV spectrophotometer with readings taken at 246 mμ and recorded. The useful range was said to be from 1 to 150 ppm on a whole fruit basis with a reproducibility of about 3%.

GAS CHROMATOGRAPHY

Another area which received considerable attention was detectors for gas chromatography. As the need for detectors capable of selective specificity for compounds containing halogens, phosphorus, sulfur, and nitrogen became greater, several new approaches were taken. McCormack, Tong, and Cooke (*204*) developed a detector based on selective monitoring of the emission spectra of the eluted organic compound. Using argon as the carrier gas, the spectra are excited in the plasma of a 2450-Mc electrodeless discharge. By measuring the intensity of selected atomic lines and molecular bands, the system can be made quantitative and highly specific for the halogens, phosphorus, and sulfur. Bache and Lisk (*13*) used this emission spectrometer detector to analyze a number of foods for such organophosphorus pesticides as diazinon, dimethoate, disulfoton (Di-Syston), ethion, parathion, and ronnel by measuring the intensity of the 2535.65 Å. line. Recoveries of 72–115% were obtained at levels ranging from 0.03 to 0.60 ppm.

Brody and Chaney (*50*) developed a flame photometric detector for determining sulfur- and phosphorus-containing compounds. The flame emission spectra were generated in a hydrogen–air flame and narrow bandpass interference filters used for isolation of the phosphorus and sulfur emission at 526 mμ and 394 mμ, respectively. The sensitivity was said to be 0.25 ng for malathion or parathion and in the submicrogram range for sulfur-containing compounds.

Coulson (*77*) introduced an electrolytic conductivity detector for gas chromatography. The effluent from the GLC column was passed through a combustion tube where the compounds were oxidized to CO_2, SO_2–SO_3 and HCl. The gases were passed into a stream of deionized water and into an electrolytic conductivity cell where the conductivity was measured by a simple Wheatstone bridge. The detector was 10,000 times as sensitive for halogen or sulfur compounds as for carbon or nitrogen.

Coulson (*78*) later modified this system to determine nitrogen-containing compounds. The effluent from the GLC, instead of being oxidized, was reduced with hydrogen in the presence of a catalyst to change any nitrogen to NH_3. Any acids formed were removed with $Sr(OH)_2$ and the NH_3 was passed through to the electrolytic conductivity detector. The apparatus was used for the determination of such nitrogen-containing compounds as simazine, parathion, amitrole, etc.

Martin (*198*) reported a method for the determination of nitrogen-containing compounds which also reduced the effluent from the GLC column with hydrogen to produce NH_3. The NH_3 was then passed into a titration cell where it was automatically titrated with coulometrically generated hydrogen ion. However, with some compounds, some of the nitrogen atoms were converted to elemental nitrogen which would not be converted to NH_3. Guthion, for example, gave only 39% recovery. Although the method was developed for petroleum industry use, it was said to be applicable to pesticide analysis.

A number of papers were published

on the thermionic detector, all of which gave strong support to the validity of the detector. Beckman and Gauer (*26*) reviewed the literature and development of the sodium thermionic detector. They described the construction and operation of a detector based on Giuffrida's design. Hartmann (*145*) reported a thermionic detector for phosphorus in which cesium bromide was used as the alkali metal source. The cesium bromide plus a suitable filler was pressed under high pressure to form a ceramic-like pellet which was then shaped to serve as the tip of the burner. Coahran (*71*) described a modified detector in which a ceramic tube filled with granular anhydrous Na_2SO_4 was placed around the jet.

Giuffrida and Ives (*123*) used dual detectors in an investigation of cleanup procedures for organophosphorus pesticide residues. The effluent from the GLC column was passed through a stream splitter and into two detectors. The response of a regular flame ionization detector was indicative of the amount of plant extractives present and thus of the cleanup efficiency. The response of the thermionic detector showed the amount of pesticides recovered.

Giuffrida, Ives, and Bostwick (*124*) described and explained the operating parameters for electron capture and the thermionic detectors. Specific details were given on how to adjust each detector and GLC system for most suitable operation for residue analyses. This paper should be required reading for residue analysts using gas chromatography.

Karmen (*173*) described a stacked flame ionization detector for phosphorus and chlorine. He indicated that the detector worked because phosphorus and halides increased the vapor pressure of the alkali metal and thus made more of it available for ionization. Abel, Lanneau, and Stevens (*5*) reported a modified stacked flame detector, claiming a controlled specificity for phosphates and halides in the order of 100,000–200,000 to 1 over other organic species.

Burchfield *et al.* (*54*) discussed various types of GLC detectors, pointing out the advantages of each. Burchfield and Wheeler (*57*) described the use of the microcoulometric detector in both the oxidative and reductive modes. Burchfield *et al.* (*56*) also reported the use of the microcoulometric detector for the determination of phosphorus, sulfur, and chlorine. The effluent from the GLC was carried through a reducing oven with H_2, forming PH_3, H_2S, and HCl. All three products were measured by a microcoulometric titration cell with silver electrodes. By inserting subtraction columns before the cell, HCl (by silica gel) or both H_2S and HCl (by Al_2O_3) could be removed, thus giving the system a high specificity.

The electron capture detector continues in wide use but only a few papers suggested modifications. Yauger *et al.* (*287*) reported the use of Ni^{63} as the radioactivity source, the big advantage being that such a detector can be operated at temperatures up to 300° C. Abbott and de Faubert Maunder (*3*) described a simple electron capture detector that could be constructed from a standard 75-ohm co-axial cable plug and a strip of tritiated copper foil at a total cost of material of less than $10.

Gas chromatography has become so accepted in pesticide residue analysis that its use in procedures is now taken for granted much like the analytical balance or a spectrophotometer. However, several papers have appeared which treat gas chromatography as a general topic. Gudzinowicz (*125*) compiled a vast amount of data on the use of electron capture gas chromatography in pesticide residue analysis. He listed the R_f and sensitivities for a large number of the pesticides on a variety of columns. Burke and Holswade (*58*) tested 17 liquid phases in the search for a GLC column which would elute the common pesticides in a different order than the widely used DC-200 column. They recommended a column prepared by mixing equal portions of Gas Chrom Q coated with 15% QF-1 and Gas Chrom Q coated with 10% DC-200. They listed retention times and response data on the column for over 85 pesticide chemicals using both the electron capture and the microcoulometric detector.

Berck (*29*) listed retention times, both absolute and relative to *n*-pentane, for 34 fumigants on a column packed with 10% SE-30 on Diatoport-S. Kanazawa (*171*) evaluated and compared columns with two liquid phases, 5% Dow Silicone 11 and 2% polyethylene glycol, for the separation of chlorinated and phosphorus pesticides and herbicides. Linear ranges, sensitivities, and separation efficiencies are reported. Gaul (*114*) compared five methods of measuring GLC peaks and discussed the problems with toxaphene, chlordane, and BHC. She suggested ways of measuring the peak areas when the pesticides were separate and in mixture. It was also pointed out that in determining BHC the analyst should bear in mind that the electron capture response to the β-isomer is about 50% of the response to the other isomers.

Giuffrida (*121*) described a GLC system for the collection of fractions for infrared analysis. The fractions were collected individually directly on KBr and then pressed into micro disks. About 10 mg of KBr was used and good spectra were obtained with as little as 3 μg of pesticide.

CHLORINATED PESTICIDES

General Procedures. Chlorinated pesticides continue to be the most widely used group and it is natural that methodology for these compounds received a great deal of attention. Beynon and Elgar (*36*) prepared an excellent review of work published up to May 1965. They list 324 references and cover all aspects of residue analysis from the collection and storage of samples through extraction and cleanup to the numerous means of quantitation and identification.

Mumma and coworkers (*218*) investigated the effectiveness of a commonly used extraction procedure in removing pesticide residues which had been picked up by growing crops. Using crops grown in soil containing dieldrin, they found that the widely used hexane–isopropanol (2:1) extraction procedure removed only about 64% of the dieldrin present. When this was followed by a 12-hourextraction with a 1:1 mixture of chloroform and methanol complete extraction of the dieldrin was obtained.

In the past two years, several collaborative studies were made of widely used analytical procedures. Johnson (*167*) reported on a study of the Mills' procedure involving the determination of heptachlor epoxide and dieldrin in evaporated milk and in butterfat. The results for 20 laboratories showed a standard deviation of ± 0.039 ppm for heptachlor epoxide at the 0.29-ppm level and a standard deviation of ± 0.052 ppm for dieldrin at the 0.26-ppm level. Several collaborative studies of the Mills, Onley, Gaither procedure were also reported. Krause (*187*) studied the recovery of aldrin, DDE, and methoxychlor from potatoes. Gaul (*115*) investigated the recovery of lindane, heptachlor, and TDE from endive and cauliflower, and Davidson (*87*) reported on the determination of BHC, *p,p'*-DDT and endrin in apricots and strawberries. Each study demonstrated the validity of the procedure.

A large number of articles describe general procedures for the determination of chlorinated pesticide residues. Many of these are modifications of previously reported methods. Gunther and Barkley (*134*) modified a microcoulometeric gas chromatograph so that, when desired, the GLC column could be bypassed with the sample going directly to the combustion furnace. This permitted easy determination of "total chlorides." Advantages of the arrangement include a more accurate measurement of toxaphene since the entire residue registered as one peak.

Robertson and Tyo (*246*) determined chlorinated pesticides in oysters using a continuous perforated basket centrifuge for extraction of sample with aceto-

nitrile. After partitioning of the residues into petroleum ether, the determination was made by electron capture GLC. Recoveries for heptachlor, heptachlor epoxide, DDE, and DDT ranged from 97 to 115% at the 0.16-ppm level. Kadis and Jonasson (*170*) used a modification of the method of Langlois *et al.* [*Milk and Food Technol.* **27**, 202 (1964)] to determine chlorinated pesticides in blood. The sample was ground with Florisil, transferred to a Florisil column, and eluted with 30% methylene chloride in petroleum ether. After evaporation and solution in hexane, analysis was by electron capture GLC. Jain and coworkers (*161*) used a simplified procedure to determine 23 pesticides including chlorinated, organophosphorus, and a nitro compound in blood. The sample was extracted with an acetone-ether mixture (1:1), evaporated, taken up in hexane, and injected into an electron capture GLC. There was no interference from the blood but the sensitivity was limited by the size of sample that could be chromatographed (equivalent to 1 mg blood).

Radomski and Fiserova-Bergerova (*245*) described the determination of chlorinated pesticides in tissues using electron capture GLC. They blended the sample with petroleum ether, added anhydrous Na_2SO_4, made to volume with petroleum ether, and injected an aliquot into the GLC without any cleanup. Sensitivities were reported in the range from 0.001 to 0.06 ppm. Hamence and coworkers (*142*) analyzed animal tissue by extracting with acetone, partitioning the residues into petroleum ether, extracting with acetonitrile, and again partitioning into petroleum ether. Final cleanup was on an alumina column. Determination was by electron capture GLC. To confirm identity and separate compounds with similar retention times, aliquots were reacted with HBr, alcoholic KOH, and chlorine, and gas chromatography was repeated. Data are given for 12 compounds.

Stanley and LeFavoure (*263*) used a perchloric–acetic acid mixture to digest samples of animal tissues. The fat and pesticides were extracted with *n*-hexane and cleaned up on a sulfuric acid–Celite column before determination by electron capture GLC. Aldrin, dieldrin, and endrin are destroyed by the procedure. Parker *et al.* (*238*) combined portions of previously reported methods for the determination of chlorinated pesticide residues in animal and human tissues. Frozen samples were blended with Dry Ice to a powder and extracted with hexane. Acetonitrile extraction and a column containing Florisil, Celite, attapulgus clay, and charcoal were used for cleanup before determination by electron capture GLC. Onley and Bertuzzi (*229*) reported a rapid procedure for the analysis of fish, meat, and fat by electron capture GLC. The method combined the use of a mixture of acetone, methyl Cellosolve, and formamide to extract the pesticide residues with the use of calcium stearate to coagulate and hold fatty constituents. Recoveries ranged from 76–108% at levels of 0.002–1.0 ppm. Kotula and Moats (*183*) used TLC to analyze eggs or poultry fat samples in less than 2 hours. Extraction was with ethyl ether with cleanup on a carbon–Celite 545 column. As an alternative, fat could be dissolved in petroleum ether and cleaned up on a Florisil column. In each case, suction was used to speed up the elution from the column. Eight chlorinated compounds were determined with a sensitivity of about 0.1 ppm. Sawyer (*254*) used acetone to extract chlorinated pesticides from eggs. After partitioning into petroleum ether, the residues were cleaned up on a Florisil column for determination by microcoulometric or electron capture GLC. In addition to being fast, it was claimed that this procedure eliminated interferences sometimes found in other procedures. Cummings and coworkers (*83*) combined features of the method of Stemp *et al.* [*Poultry Sci.* **43**, 273 (1964)] with those of Mills *et al.* [*JAOAC* **46**, 186 (1963)] for the analysis of eggs. The sample was ground with Florisil and anhydrous Na_2SO_4 and the mixture transferred to the top of a Florisil column. The pesticides were eluted in two fractions and concentrated for analysis by electron capture GLC. The sensitivity was reported as 0.001 ppm, and recoveries for lindane, heptachlor epoxide, DDT, dieldrin, and endrin ranged from 78 to 109%.

Moats (*214*) used TLC to determine chlorinated pesticides in dairy products with a sensitivity of about 0.125 ppm on a fat basis. Stemp and Liska (*265*) reported a simplified and shortened procedure for the analysis of milk. A 10-ml sample of milk was mixed with deactivated Florisil, slurried with 20% CH_2Cl_2 in petroleum ether, and decanted through a column of deactivated Florisil. The eluate was evaporated; the residue was taken up in hexane and injected into an electron capture GLC. Recoveries were over 90% at levels of 0.1 to 10 ppm whole milk basis. It was stated that 40–50 samples could be cleaned up by one technician in a day.

Giuffrida *et al.* (*122*) described a procedure for milk, fats, and oils. Milk was extracted with acetone and the residues were partitioned into petroleum ether. Fats and oils were dissolved in petroleum ether. The samples were transferred to a column of deactivated Florisil, and after removal of solvent pesticides were eluted with acetonitrile containing 10% H_2O. After partitioning into petroleum ether, the extracts were further cleaned up on an activated Florisil column and determined by electron capture GLC. Tolbert (*276*) used a column of sand, magnesium oxide, and Celite 545 to replace the Florisil column in the analysis of oils by electron capture GLC. Saha (*249*) determined aldrin, heptachlor, endrin, and dieldrin in wheat using electron capture GLC. The ground samples were extracted with acetonitrile in a Soxhlet; residues were partitioned into petroleum ether and cleaned up on a magnesia–Celite column.

Several procedures have been reported for the determination of chlorinated pesticide residues in water. Lamar and coworkers (*190*) extracted large (up to 4 liters) samples of water with hexane and used electron capture GLC for the determinative step. Smith and Eichelberger (*260*) described a thin layer chromatographic cleanup of the carbon chloroform extract (of water) resulting in a solution suitable for electron capture GLC. Lerenard and Simon (*194*) used an automatic liquid–liquid extractor which they found capable of extracting 80–90% of lindane and dieldrin from water at concentrations of 1 ppb. Sanderson and Ceresia (*253*) reported on a continuous liquid–liquid extraction apparatus. With a sample flow rate of 1 liter/hour, recoveries of about 90% at the 1-ppb level were obtained. Teasley and Cox (*271*) compared extraction procedures for removing endrin and DDT from soils. They reported that the Immerex extraction method was the best. The procedure involved a 16-hour extraction with *n*-hexane–acetone (9 + 1) in an Immerex tester, an apparatus designed for the analysis of bituminous paving mixture which uses an extraction basket for the sample container.

Samuel (*252*) reported a screening procedure for chlorinated and thiophosphorus pesticides in dairy products, fruits, vegetables, and animal tissue. After sample extraction, a combination of 1 or more of 3 cleanup procedures prepared the sample for final analysis by electron capture or microcoulometric GLC. Recoveries of 75–100% were reported at levels of 0.05–2 ppm. Water soluble organothiophosphorus compounds do not come through the procedure.

Considerable use has been made of thin layer chromatography in the analysis for chlorinated pesticides. Matherne and Bathalter (*200*) described a cleanup procedure making use of 8- × 8-inch plates with channels 10 mm wide by 2 mm deep which were filled with Al_2O_3 G coating. Sample extracts were spotted on individual channels and the plates developed twice with two different solvents. This separated the

pesticide residues from the plant extracts and after elution from the scraped off adsorbent, the residues were in suitable form for electron capture GLC.

Kovacs (*186*) used $3^1/_2 \times$ 4-inch microplates for TLC and reported that as many as 26 chlorinated pesticides could be resolved in 5–10 minutes and identified. The lower limit of detection for many of the commonly used chlorinated pesticides was 0.005 μg. Crabtree (*79*) used microscope slides coated with Al_2O_3 and developed in hexane in $3^1/_2$ minutes for rapid confirmation of identity. Beckman and Winterlin (*27*) described what they called "thin-strip thin layer chromatography." They used a tool to scrape coated 8 × 8-inch plates in such a manner that individual TLC strips or channels 4 mm wide were formed. As many as 20 channels could be used on one plate. The advantages claimed were that, since the spots could not spread, sensitivity was increased and that it was easier to remove separated spots for GLC and IR. Engst *et al.* (*99*) used silica acid gel plates and reported the detection of 6 chlorinated pesticides with a sensitivity of 0.05 μg. Abbott and coworkers (*4*) studied the effect of temperature on R_f values. R_f values for 16 chlorinated pesticides in 16 solvent/adsorbent systems were given. Ballschmiter and Toelg (*16*) investigated fluorescence indicators for TLC. Twenty-four substances were studied. Fluorescence or quenching of spots at levels of 0.02–5 μg were noted with six reagents. Adamovic (*7*), investigating spray reagents for TLC, reported that the chlorinated pesticides under ultraviolet light reacted with aromatic amines to form characteristically colored spots even without zinc chloride or iodine. A total of 18 aromatic amines were tested with the 6 most promising showing sensitivities down to 0.5 μg.

There have been several papers on chlorinated pesticides which are of general interest to the residue chemist. Gunther, Hylin, and Spenger (*135*) investigated the nature of the organic chlorine interferences in the total halogen methods for organic chlorine pesticides. Using Cl^{36} tracer, they have tentatively identified the interferences as quaternary chloride salts of lecithins. Burke *et al.* (*59*) studied the losses of pesticides in various methods of concentrating solutions down to volumes of 0.5 ml or less. They found that large losses occurred when the solutions were evaporated by a stream of air. Losses increased as the residual volume approached dryness and the percentage losses were greater when smaller amounts of pesticides were present. They found that by using a micro Snyder column, solutions could be rapidly concentrated to 0.1–0.3 ml on the steam bath without loss of pesticide. Moats and Kotula (*215*) speeded up the elution from cleanup columns by using suction. They reported that elution rates of 250 ml/min from Florisil columns and 100 ml/min from carbon–Celite columns gave good recoveries without adversely affecting the cleanup.

Mumma and Kantner (*217*) made use of the mass spectrometer for more positive identification of pesticides. They determined the mass spectra of several chlorinated pesticides and found that each gave easily recognizable molecular ion peaks and characteristic ion fragments. Their procedure was to collect the GLC peaks in medicine droppers containing GLC column packing material. The pesticide was washed out, concentrated, and injected into the mass spectrometer. The procedure has been run on dieldrin, DDT, and DDE from wheat and alfalfa. The sensitivity was 0.1 ppm, and 0.1 μg has given a good mass spectrum. Payne and Cox (*239*) used infrared for the identification of chlorinated pesticide residues in sludge, soils, industrial effluents, and fish and other aquatic fauna. Column and thin layer chromatography were used for the cleanup and separation of the individual pesticides. Minyard and Jackson (*213*) attempted to make identification by electron capture GLC more certain through the use of flash heater inserts packed with various salts to modify the pesticides. A number of salts were investigated, and the authors suggested the possible use of several modifiers in parallel ahead of a single column and detector. Sequential injection of an extract into the various modifiers would produce normal and modified chromatograms which were characteristic of the pesticide. Lee and coworkers (*198*) encountered a contaminant which had the same R_f on a silicone elastomer/Celite GLC column as aldrin, and on a column of Apiezon L had the same R_f as β-BHC. By means of infrared they identified the contaminant as dibutyl phthalate which they thought came from plastic containers and plastic-based paints used in the laboratory.

Specific Procedures. Shuman and Cieri (*257*) reported a method for determining residues of chlorbenside including its sulfoxide and sulfone oxidation products. Samples were extracted according to the Mills, Onley, Gaither procedure and all forms of chlorbenside residue converted to the sulfone by oxidation with chromic–acetic acid solution. After cleanup on an Al_2O_3 column, determination was made by electron capture GLC. Thruston (*275*) compared the electron capture gas chromatogram of a chlordane standard with that of a weathered chlordane residue found on squash. He noted that in the weathered residue, the first 4 major peaks of chlordane were small or had disappeared, while the last 3 peaks were not changed significantly. Gajan and Link (*113*) used oscilloscopic polarography for the determination of DDT. They reported that with an electrolyte of 0.1*M* tetramethyl ammonium bromide in 50% aqueous acetone–ethanol, only DDT and those analogs such as methoxychlor containing the trichloroethane group gave responses in the −0.3 to −1.7-volt range. The regular wave showed a peak indicating the total *o,p'*- and *p,p'*-DDT whereas the derivative showed two peaks whose ratios was equal to the ratio of the two isomers.

Several papers dealt with the determination of DDT. Dingle (*90*) modified Davidow's sulfuric acid cleanup for fat [*JAOAC* **33**, 130 (1950)] and obtained solutions suitable for PC, GLC, IR, or colorimetric determination. Recoveries were said to be better than 98%. Stempkovskaya and Vekshtein (*266*) reported a modification of the Schecter-Haller technique using KNO_3 or NH_4NO_3 with H_2SO_4 in place of fuming HNO_3. They also described a stable artificial color standard consisting of a solution of $CuCl_2$, crystal violet, and $K_2Cr_2O_7$ which was said to correspond to the color produced by 100 μg of DDT. Crosby and Archer (*80*) determined the DDT group in milk, blood, and tissue as their dehydrohalogenated compounds after treatment with KOH. Extraction was with pentane and determination by electron capture GLC. The first foot of the GLC column was packed with calcium carbide to remove traces of water and ethanol. Beckman and coworkers (*24*), after showing that the pesticides were present only in the yolk of eggs, determined DDT and DDE by extracting the yolks with acetone. The extract was evaporated; the residue was taken up in hexane and cleaned up on a Florisil column before determination by electron capture GLC. Recoveries at 0.05–1.0-ppm levels averaged 94%. The time required for analysis was less than 1 hour per sample. Schuntner and Schnitzerling (*256*) used gradient elution from a cooled, water-jacketed, silicic acid column to separate DDT and its metabolites into individual components. Compounds separated included *o,p'*- and *p,p'*-DDT, DDE, *p,p'*-DDD, Kelthane, *p,p'*-dichlorobenzophenone, and *p,p'*-DDA.

Hansen (*143*) reported that the colorimetric method of Jones and Riddick [ANAL. CHEM. **23**, 349 (1951)] for the determination of Dilan could be made about 10 times more sensitive by decreasing the total volume of the final solution while keeping the same ratios of reagents.

Harrison (*144*) was able to determine endrin in wildlife in the presence of

large amounts (100–500-fold) of DDE and dieldrin by making a preliminary separation on TLC before using GLC. Although recoveries were slightly better on silica gel plates, alumina gave better separation of endrin from dieldrin. Engel *et al.* (*97*) determined heptachlor and heptachlor epoxide in alfalfa hay by blending the sample with water and ethanol and then extracting with hexane. They used the procedures of Samuel (*252*) for cleanup. Ott *et al.* (*235*) used thin layer chromatography and oscillopolarography to determine *p,p'*-Kelthane and *p,p'*-dichlorobenzophenone. They suggested that in analyzing crop extracts, TLC be used for cleanup before polarographing the sample. Mestres and Chave (*209*) determined lindane in flour by extracting with acetonitrile, cleaning the extract on a Na_2SO_4 and Florisil column, and then using electron capture GLC. Recovery of 0.25 ng of lindane was 95 ± 3%. To determine toxaphene in milk, fat, blood, and hay, Archer and Crosby (*12*) used KOH to dehydrohalogenate the toxaphene before injection into the electron capture GLC. Advantages claimed for the procedure were very rapid and effective cleanup, a higher and more compact peak which eluted before the DDT group, and a two-fold increase in sensitivity. Recoveries ranged from 74–95% at 0.1 and 0.5-ppm levels. Faucheux (*103*) investigated the use of diphenylamine–$ZnCl_2$ as a chromogenic reagent for toxaphene, DDT, and chlordane on alumina TLC plates. Characteristic colors were obtained from these pesticides. Five μg each of toxaphene and chlordane could be detected in mixture. DDT and TDE could be estimated semiquantitatively when all forms were present. Color reactions of 34 pesticides at the 20-μg level were reported.

ORGANOPHOSPHORUS PESTICIDES

General Procedures. Storherr and coworkers (*268*) reported a procedure they used for the determination of five organophosphorus pesticides (malathion, parathion, methyl parathion, diazinon, and carbophenothion) in a number of vegetables and fruits. The sample was blended with acetonitrile and filtered. The extract was concentrated under vacuum to remove the acetonitrile and was then extracted with ethyl acetate. After cleanup on a column of carbon and Celite, the pesticides were determined by GLC using the thermionic detector. Other aliquots were examined by the colorimetric *p*-nitrobenzyl pyridine method (*118*), a total phosphorus procedure (*117*), and by paper chromatography. Watts and Storherr (*285*) described a rapid extraction procedure in which the crop sample was extracted by blending with ethyl acetate. Storherr and Watts (*269*) developed a rapid cleanup procedure for organophosphate pesticides which also shows promise of being useful as a general cleanup method for many other types of compounds. The procedure, called sweep codistillation, makes use of a heated short glass column packed with glass wool. An ethyl acetate extract of the sample was injected into the tube, and pesticides and solvent were swept through into a receiver by a stream of N_2. Repeated injections of small amounts of ethyl acetate resulted in nearly complete recovery of the pesticides while crop material remained in the tube. The effluent was clean enough for gas chromatography using the thermionic detector. Recoveries from five crops fortified with a mixture of carbophenothion (Trithion), diazinon, malathion, parathion, and methyl parathion ranged from 89 to 101%, and only 20 minutes was required to clean up each sample.

Getz and Watts (*118*) reported a colorimetric procedure which had a sensitivity of 2 μg of organophosphorus compound. A cleaned up residue was heated with 4-(*p*-nitrobenzyl) pyridine and cyclohexylamine for 2 minutes, diluted, and the absorbance was read at 520 mμ. The procedure worked for all 24 organophosphorus pesticides tested. Getz (*117*) described a procedure in which the organophosphorus compounds were quantitatively converted to orthophosphates by ammonium persulfate and then measured as molybdenum blue with an absorbance at 660 mμ. Residues were separated on paper chromatograms, the spots were cut out, and the determination was run directly with the piece of paper. Sensitivity was 0.1 μg P, equivalent to about 1 μg pesticide. Irudayasamy and Natarajan (*159*) used paper chromatography for the determination of thiophosphorus pesticides. After development, the dried chromatogram was exposed to bromine vapors, air dried, and sprayed with a solution of Congo red. The pesticides appeared as blue spots on a red background and were stable for 10 days if protected from light. The test was sensitive to 0.5 μg of pesticide. Zadrozinska (*290*) used paper chromatography in the determination of organophosphorus pesticides in various food crops. He extracted the sample with carbon tetrachloride and after separation by paper chromatography used enzymatic and fluorescein methods for making the spots visible. Bates (*20*) also used paper chromatography but extracted the food samples with acetone, cooled the extracts to −80°C. and filtered them to remove fats and waxes and used a MgO column for further cleanup. The pesticides were separated and identified by 2-dimensional paper chromatography. For quantitiation, the spots were cut out and phosphorus was determined by the molybdenum blue reaction after wet digestion or Schöniger flask combustion. R_f's are listed for 20 compounds.

Thin layer chromatography was used by a number of workers. Kovacs (*185*) separated thiophosphorus compounds on Al_2O_3–G plates and located the pesticides with a tetrabromophenolphthalein–$AgNO_3$ spray. R_f's were given for 19 compounds and 11 of these were detectable at the 0.05-μg level. Barney (*18*) investigated previously reported chromogenic reagents for organophosphorus compounds and developed 2 new tests. The developed plates were sprayed with HI solution, heated, sprayed with ammonium persulfate solution, heated again, and sprayed with ammonium molybdate followed by buffered benzidine solution. The procedure determined all compounds tested except for one phosphonium compound. Omitting the ammonium persulfate resulted in a test which detected organophosphates but only some of the organophosphonic acids. The lower limit of detection was less than 1 μg. Watts (*284*) adapted the *p*-nitrobenzyl pyridine reagent (*118*) for use as a chromogenic spray in paper and thin layer chromatography. Twenty organophosphorus pesticides tested gave distinct and persistent spots. The test was sensitive to about 0.5 μg for both the thio and nonthio organophosphorus pesticides. Klisenko (*180*) used Al_2O_3 plates and 3 chromogenic sprays for detecting organophosphorus residues. Zadrozinska (*291*) used silica gel and talc adsorbents with 16 mobile phases. El-Refai and Hopkins (*96*) described the use of plates coated with cellulose powder containing 10% $CaSO_4$ as binder, for the separation of organophosphorus pesticides and their oxons. Two solvent systems were used, each including an immobile solvent phase. Four spray reagents were used including one based on cholinesterase inhibition directly on the plate which was sensitive to 0.001–2 ng of the various pesticides. The authors discussed the choice of systems for specific separations and listed R_f values. Melchiorri *et al.* (*206*) used Silica Gel GF 254 plates for the separation and identification of 13 organophosphorus pesticides in vegetable oils. Various solvent systems are described. Salamé (*250*) studied the chromatography of 10 organophosphorus compounds on silica gel using 16 solvent systems and reported R_f values. He used two chromogenic reagents, one (Br_2, $FeCl_3$ and sulfosalicylic acid) had a sensitivity of about 5 μg and the other (palladium chloride) a sensitivity of about 2 μg. Stanley (*262*) used 3 × 1-inch microscope slides coated with silica gel-G. He listed R_f values for 31 organophosphorus compounds in 6 solvent systems and described 7 spray reagents.

A number of workers have used GLC for the separation and identification of organophosphorus pesticides. Horiguchi *et al.* (*155*) separated 9 compounds on three different GLC columns using an electron capture detector. Kanazawa and coworkers (*172*) were able to separate any combination of 19 organophosphorus pesticides by the use of three GLC columns, although no one column gave complete separation of all 19 compounds. Hrivnak and Pastorek (*157*) reported the successful separation of 11 *O,O*-dialkyl-*O*-(4-nitrophenyl)thiophosphates describing the columns and operating conditions used. Nelson (*223*) used microcoulometric GLC for the determination of 16 thiophosphates in 25 crops at levels as low as 0.1 ppm. Samples were extracted by the Mills, Onley, Gaither procedure [*JAOAC* **46**, 181 (1963)] and the residues partitioned into petroleum ether before the gas chromatography. Recoveries of over 70% were obtained for all compounds except Guthion (16%), demeton (46%), and dimethoate (0%). Later modifications (*224*) increased the recovery of dimethoate and Guthion to 70–98%. Cook and coworkers (*74*) studied the electron capture response of 7 organophosphorus pesticides in an attempt to correlate structure to response. They found that in general the electron affinity changed in the manner:

$$\begin{matrix} O \\ \| \\ P\text{—}O \end{matrix} < \begin{matrix} S \\ \| \\ P\text{—}O \end{matrix} < \begin{matrix} O \\ \| \\ P\text{—}S \end{matrix} < \begin{matrix} S \\ \| \\ P\text{—}S \end{matrix}$$

and that the methoxy group bonded to the central phosphorus atom resulted in lower electron affinity than did the ethoxy group.

McCaulley (*203*) used a combination of GLC with infrared for the determination of organophosphorus residues from fruits and vegetables. The sample was extracted by blending with acetonitrile, water salted out and the acetonitrile extract evaporated. The residue was subjected to distillation under a vacuum of about 0.5 micron with the pesticide residues being collected on a cold finger cooled with liquid nitrogen. The residues were rinsed off, adjusted to volume, and injected into a GLC. For identification, the peaks of interest were collected and their infrared spectra obtained. Hermann (*151*) used frustrated multiple internal reflection (FMIR) infrared for the identification of trace amounts of organophosphorus pesticides eluted from column, paper, and thin layer chromatograms.

Nangniot (*221*) determined 22 phosphoric acid ester pesticides by oscillographic polarography. Operating conditions for each are listed.

Damico (*85*) determined the mass spectra of 23 organophosphorus pesticides. In addition to giving the spectra, rearrangement and fragmentation patterns are discussed.

Specific Procedures. Blinn and Pasarela (*41*) used a colorimetric procedure to determine Abate, (*O,O,O′,O′* - tetramethyl - *O,O′* - thiodi - *p* - phenylene phosphorothioate) in water, mud, and oysters. After extraction and cleanup, the Abate was hydrolyzed to 4,4′-thiodiphenol and reacted with 4-aminoantipyrine under oxidizing conditions. The color formed was extracted into butanol and read at 510 mμ. Recoveries from water at 0.025–0.045 ppm levels ranged from 79 to 88% and from mud and oysters at 0.1–0.66 ppm levels, 60 to 82%.

Katague and Anderson (*174*) used electron capture GLC for the determination of Bay 37289 (*O*-ethyl-*O*-2,4,5 - trichlorophenyl - ethylphosphonothioate), its oxygen analog, and 2,4,5-trichlorophenol, in a number of crops including alfalfa, beans, carrots, and potatoes. After extraction of the sample with acetone/benzene, the 2,4,5-trichlorophenol was removed with 0.1*N* NaOH for separate determination. Bay 37289 and its oxygen analog were then hydrolyzed to 2,4,5-trichlorophenol, acetylated, and injected into the GLC. The sensitivity of the method was about 0.1 ppm with recoveries ranging from 75 to 104%.

Several procedures have been reported for the determination of Bidrin (dimethyl phosphate of 3-hydroxy-*N,N*-dimethyl-*cis*-crotonamide). Sun *et al.* (*270*) described a fly bioassay said to be specific for Bidrin in the presence of 1 or more of 46 insecticides. It was sensitive to 0.05 ppm. Stevens and Van Middelem (*267*) used electron capture GLC to determine Bidrin in cabbage with a sensitivity of 0.01 ppm. After extraction and cleanup, the Bidrin was reacted with NaOH and iodine–KI solution to form iodoform which was extracted and injected into the GLC. The method, which is specific for methyl vinyl phosphates will thus also detect Phosdrin and phosphamidon. Murphey *et al.* (*219*) described a procedure in which Bidrin was hydrolyzed with NaOH and the resulting dimethylamine distilled and determined colorimetrically as dimethyl dithiocarbamate following addition of Cu^{+2} and CS_2. Recoveries from alfalfa, lettuce, orange peel, string beans, etc. at levels of 0.2–10 ppm ranged from 80 to 108%. The color reaction is specific for dialkylamines. Thus, *N,N*-dimethylcarbamates such as Dimetilan isolan, and Pyrolan would interfere and could be determined by this reaction. Lau (*192*) used cholinesterase inhibition for the determination of both Bidrin and the closely related Azodrin (dimethyl phosphate of 3-hydroxy-*N*-methyl-*cis*-crotonamide) in crops with a sensitivity of 0.1 ppm. The two compounds could be separated from each other and from other insecticides through procedures that are described.

The insecticide, diethyl-1-(2,4-dichlorophenyl)-2-chlorovinyl phosphate, or compound 4072, is known in England as chlorfenvinphos or by the trade name Birlane. Claborn and Ivey (*70*) described a procedure for its determination in milk and tissue in which compound 4072 is hydrolyzed to 2,2′,4′-trichloroacetophenone and determined as such by electron capture GLC. Beynon and coworkers (*34*) reported the analysis for compound 4072 in soil and crops. After extraction of the sample and cleanup on a Florisil column, the insecticide was determined either by cholinesterase inhibition or by electron capture GLC. Compound 4072 consists of 6% cis isomer and 90% trans. When these isomers were gas chromatographed as the intact compounds, they had different retention times. Sensitivity of the two determinative procedures was about equal, 0.01 ppm. Robinson *et al.* (*247*) determined compound 4072 in sheep fat, liver, and other tissues. The parent compound and its metabolite, trichloracetophenone, were separated from chlorinated pesticides and from each other on a column of unactivated Florisil. Each was then determined by electron capture GLC. The sensitivity for compound 4072 was 0.003 ppm and for the trichloroacetophenone 0.001 ppm. Bazzi and Fabbrini (*22*) determined Cidial (ethyl mercaptophenylacetate, *O,O*-dimethyl phosphorodithioate) in oil by extracting a hexane solution of the oil with acetonitrile and after cleanup determining phosphorus as molybdenum blue.

Irudayasamy and Natarajan (*160*) reported a colorimetric method for the determination of carbophenothion (Trithion). The pesticide was hydrolyzed with alkali to *p*-chlorothiophenol which was then reacted with diazotized *o*-dianisidine to give a yellow color with maximum absorbance at 375 mμ.

Boone (*43*) used microcoulometric GLC to determine DDVP and naled (Dibrom) in apples, cabbages, and carrots. A silicic acid column was used for cleanup. Buechler *et al.* (*51*) modified and improved the resorcinol method for the determination of DDVP.

El-Refai and Giuffrida (*95*) used GLC with the thermionic detector to determine DDVP and trichlorfon (Dipterex) in water and in formulations. They also studied the hydrolysis rates of each pesticide and the rate of conversion of trichlorfon to DDVP. Anderson and coworkers (*10*) reported a method for the determination of trichlorfon and its metabolites, chloral hydrate, and trichloroethanol, in plant and animal tissue using electron capture GLC. After extraction and cleanup,

trichlorfon and chloral hydrate were injected into the GLC with trichlorfon breaking down and both compounds registering as chloral. To determine the trichlorethanol, a separate aliquot was acetylated to form trichloroethylacetate and then chromatographed. Mustafa *et al.* (*220*) used a colorimetric procedure for determining trichlorfon. The sample was spotted on a filter paper impregnated with 3,5-dintrobenzoic acid and heated at 70°C. for 2 minutes. Trichlorfon gave a blue spot, which was measured in a densitometer with a 550 mμ filter. The reaction, based on the cleavage of the P — C bond and reaction of the phosphite esters with the 3,5-dinitrobenzoic acid, distinguished between trichlorfon and DDVP, which did not react. Szyszko (*298*) reported an oscillopolarographic method for trichlorfon in which lindane and DDT did not interfere. However, maneb and zineb did interfere. Szyszko (*297*) also reported an oscillopolarographic method for demeton-*S*-methyl in foods where maneb and zineb did not interfere. Giang and Schechter (*119*) described a method for the determination of demeton and its metabolites in fruits and vegetables. After extraction and cleanup, the parent compounds and metabolites were all converted to the sulfones by oxidation with *m*-chloroperbenzoic acid. After additional cleanup on a cellulose column, the residue was dissolved in CS_2 and read in a 5-mm infrared cavity cell using 5× scale expansion. The absorption at 7.55 microns was used for calculation. Recoveries at 0.6 ppm levels ranged from 76 to 102%.

Gilmore and Cortes (*120*) used dual band TLC as cleanup for the determination of diazinon. By means of a divider in the applicator, the plates were coated with a mixture of Darco G60 and Solka-Floc on the lower 4 cm and with silica gel H on the remaining 16 cm. The crude extract along with a standard were applied to the charcoal–cellulose band, and after development, the sample spots, located by comparison with the standard R_f's, were removed for analysis. Fifty grams of spinach was purified by the above procedure and recoveries as followed by S^{35} labeled diazinon, averaged 95%. Abbott and coworkers (*2*) reported the use of multiband chromatoplates for the TLC determination of dimethoate. They prepared plates having 3 bands of different adsorbents and spotted cleaned up sample extracts. Development separated the dimethoate from the remaining plant materials and most other organophosphorus compounds. The dimethoate spots were made visible by spraying with Brilliant green and exposure to bromine vapor. The square root of the spot areas plotted against the log. of amount of dimethoate gave a straight line. Recoveries from fruits and vegetables at 0.1–0.5 ppm levels ranged from 80 to 108%. George *et al.* (*116*) described a colorimetric method for dimethoate in plants and milk. After extraction and cleanup, the residue was treated with methanolic NaOH and 1-chloro-2,4-dinitrobenzene to form a color which was read at 505 mμ. Although the oxygen analog would react, it did not come through the cleanup. The authors tested 33 insecticides, 3 herbicides, and 1 fungicide and found that they did not interfere with the analysis. Smart (*259*) compared three colorimetric procedures for the determination of dimethoate in fruits and vegetables, and reported that the procedure of Chilwell and Beecham worked best. Bache and Lisk (*15*) reported the use of GLC with the emission spectra detector (*204*) for the determination of dimethoate and phorate in soil.

Blinn and Boyd (*40*) reported a colorimetric as well as a thin layer procedure for the determination of the dithiolane insecticides, 2-diethoxyphosphinothioylimino-1,3-dithiolane, and its oxygen analog. After extraction and cleanup, the insecticides could be determined on TLC plates made with equal parts of silica gel-G and silica gel-HF. Under ultraviolet light, the compounds appeared as dark areas on a fluorescent background. In the colorimetric procedure, the cleaned up residue was treated first with acid and then with alkali to form thiocyanate which was converted to cyanogen bromide and reacted with benzidine in pyridine to form an intense red solution with an absorption maximum at 530 mμ.

Adams and Anderson (*8*) reported a spectrophotofluorometric procedure for the determination of Guthion [*O,O*-dimethyl - *S* - 4 - oxo - 1,2,3 - benzotriazin-3(4*H*)-ylmethyl phosphorodithioate] in milk and meat. After extraction and cleanup by liquid–liquid partitioning and the use of an alumina column, the pesticide was hydrolyzed to anthranilic acid, and divided into 2 equal aliquots; standard hydrolyzed Guthion was added to one. The fluorescence of both solutions was measured at 400 mμ using an activation wavelength of 340 mμ. The oxygen analog was measured as well as the parent compound. Sensitivity of the procedure was reported as 0.005 ppm in milk, 0.02 ppm in tissue, and 0.03 ppm in fat.

Anderson and coworkers (*11*) used a somewhat similar procedure for the determination of the anthelmintic, *N*-hydroxynaphthalimide diethyl phosphate. Based on the procedure of P. A. Giang [*J. Agr. Food Chem.* **9**, 42 (1961)] for the sulfur analog, Bayer 22,408, the fluorescence was measured at 480 mμ using an activation wavelength of 372 mμ.

Szyszko (*296*) used oscillographic polarography for the determination of Guthion. A most characteristic curve was obtained using a pH 4.0 acetate buffer as electrolyte.

Bowman and Beroza (*44*) reported two procedures for the determination of Imidan [*O,O*-dimethyl-*S*-phthalimidomethyl phosphorodithioate] in milk and corn plants. After extraction and cleanup, electron capture GLC was used for the determination step using a column which was preconditioned by injection of Imidan just prior to use. A colorimetric method, based on the hydrolytic cleavage to liberate formaldehyde which was then reacted with chromotropic acid, was also described although it was not so good as the GLC procedure.

Gutenmann and coworkers (*138*) also used electron capture GLC for the determination of Imidan. They reported that Imidan, its oxygen analog, folpet, and phthalic acid all had the same retention time. It was therefore believed that all broke down to phthalic anhydride on GLC.

Gudzinowicz (*126*) described some of the GLC properties of fenthion (*O,O*-dimethyl - *O* - [4 - (methylthio)-*m*-tolyl]-phosphorothioate) also known as Lebaycid. He used both electron capture and flame ionization detectors and reported that as little as 22 ng was easily detected.

Koivistoinen *et al.* (*182*) studied procedures for the extraction of malathion from fruits using a colorimetric procedure for the determinative step. They reported that for samples analyzed 2–3 days after pesticide application, tumbling the unmacerated fruit with benzene gave the highest values. However, for samples with longer periods between application and analysis, procedures which called for blending of sample with polar or mixed solvents gave higher values. Mestres and Chave (*210*) described a procedure for the determination of malathion in flour which involved extraction with acetonitrile and petroleum ether and Florisil column cleanup. Determination was by GLC using paired thermionic and flame ionization detectors. Sensitivity was reported as 0.1 ppm.

A number of workers reported procedures for the determination of parathion. Lodi (*195*) used electron capture GLC for its determination in wine and biological materials. With wine, a preliminary cleanup by paper or thin layer chromatography was needed. Ott *et al.* (*234*) described a rapid thin layer procedure having a sensitivity of 0.5 ppm by which they were able to obtain qualitative and semiquantitative results on canned peaches in one hour. Szyszko (*295*) used oscillopolarography in which zineb did not interfere but maneb did. Beckman and coworkers (*25*) analyzed for parathion in cole crops,

using a Florisil column to remove crop interferences and chlorinated pesticides before the final determination by either electron capture GLC or the Averill-Norris colorimetric method.

Moye and Winefordner (*216*) reported a rapid method for the determination of *p*-nitrophenol in urine, using phosphorimetry. The method could determine as little as 0.01 μg in 5 ml of urine in 40 minutes with an average recovery of 88%. Skuric (*258*) described a fluorometric method for the determination of methyl paraoxon based on the oxidation of indole in the presence of methyl paraoxon.

Winnett and Katz (*286*) described a colorimetric procedure for phorate (Thimet) in vegetables in which the cleaned up phorate residue was hydrolyzed with HBr and the released H_2S determined as methylene blue. Claborn and Ivey (*69*) determined ronnel in milk and in animal tissues, using electron capture GLC after cleanup on a Florisil column. As little as 0.001 ppm could be determined in milk and 0.005 ppm in tissues. Sulfotepp was measured by oscillopolarography by Szyszko (*299*).

Sumithion [*O,O* - dimethyl - *O* - (3 - methyl - 4 - nitrophenyl)phosphorothioate] has been determined colorimetrically by alkaline hydrolysis to sodium 3-methyl-4-nitrophenolate with the absorbance at 400 mμ being measured for quantitation. Kovac and Sohler (*184*) used this procedure following extraction and cleanup on Al_2O_3 thin layer plates to determine Sumithion in fruits and vegetables at levels as low as 0.1 ppm, while Franz and Kovac (*110*) reported a similar determination in milk. Oi and Umeda (*227*) used infrared for the simultaneous determination of Sumithion and methyl parathion in spinach and lettuce at about 1 ppm. The absorption peak at 10.3 microns was used to measure the Sumithion and the peak at 10.8 microns for methyl parathion. Coahran (*72*) reported the use of GLC with the thermionic detector for the determination of Zinophos (*O,O*-diethyl-*O*-2-pyrazinyl phosphorothioate) in soil following overnight extraction in a Soxhlet.

CARBAMATES

General Procedure. Eberle and Gunther (*94*) conducted an extensive investigation of 5 carbamates—carbaryl, Dimetilan, isolan, Pyrolan and Zectran. They studied the effect of natural sunlight and ultraviolet light on these compounds and presented useful basic information concerning their analytical behavior with GLC, TLC, oscillographic polarography, and fluorescence spectrometry. Henkel (*149*) described the TLC behavior of herbicidal carbamates and presented methods for their determination in soil, water, and potato extract. Adsorbents, solvent systems, and spray reagents were discussed. In a later publication, Henkel (*150*) reported on 3 thin layer chromatographic systems and 5 chromogenic color development systems for a number of *N*-methyl and *N,N*-dimethyl carbamates. Limits of detections for these carbamates ranged from 0.05 to 0.15 μg. Hylin (*158*) used thin layer chromatography to determine the dithiocarbamates on leaves. R_f values were given for ziram, thiram, zineb, maneb, and others. Sensitivity was approximately 2.5 μg.

Zielinski and Fishbein (*293*) presented data on the GLC behavior of *N*-substituted carbamates on 3 different columns while Fishbein and Zielinski (*107*) described the GLC behavior of the trimethylsilyl derivatives of a number of carbamates and ureas. Damico and Benson (*86*) developed and tabulated the mass spectra of 14 carbamate pesticides. The significant fragmentation ions were noted and ions were postulated for 8 *N*-methylcarbamate rearrangements. Chen and Benson (*63*) reported the infrared spectra of 32 carbamate pesticides and model compounds. The characteristic absorption frequencies and associated structures were tabulated in a summary and presented in a correlation chart. Broderick *et al.* (*49*) reported that methyl anthranilate, because of its absorption bands at 2.86 and 2.95 microns, interfered in the infrared determination of methylcarbamates. They described a method for removing methyl anthranilate in the analysis of Concord grapes for carbamate residues.

Specific Procedures. Johnson and Stansbury (*162*) reviewed the various methods for determination of carbaryl in a variety of products and in water, and described extraction and cleanup procedures.

Gutenmann and Lisk (*139*) used electron capture GLC for the determination of carbaryl in various crops. After extraction and cleanup, the carbaryl was hydrolyzed to α-naphthol, which was then brominated with I and Br_2 in glacial acetic acid. The brominated residue was taken up in benzene and injected into the GLC, which actually determined brominated 1-naphthyl acetate. Van Middelem and coworkers (*278*) reported a somewhat similar technique in which the α-naphthol formed by hydrolysis was brominated with bromine and the electron capture GLC determination made of brominated α-naphthol. Results were reported for levels as low as 0.1 ppm. Benson and Finocchiaro (*28*) modified the official AOAC colorimetric method for carbaryl [Johnson, D. P., *JAOAC* **47**, 283 (1964)] to eliminate the need for special equipment and to shorten the time of analysis. Johnson and Stansbury (*164*) modified the official AOAC method to determine carbaryl in bees, using a Florisil column for additional cleanup.

Gajan *et al.* (*112*) reported an oscillographic polarographic procedure whereby carbaryl could be determined in the presence of α-naphthol. Using a modified cleanup, recoveries of carbaryl from fortified crops averaged 95% at levels from 0.2 to 10.0 ppm. Among a number of pesticides tested, only *o*-phenylphenol interfered. Engst and coworkers (*100*) formed nitro derivatives of carbaryl by treatment with HNO_3. These derivatives were then determined quantitatively by both d.c. and pulsed polarography with a sensitivity of 0.5 and 0.005 ppm of carbaryl, respectively.

Finocchiaro and Benson (*105*) used thin layer chromatography for the determination of carbaryl. After the samples were spotted and the plates developed, the carbaryl was hydrolyzed by spraying with KOH and then coupled with *p*-nitrobenzenediazonium fluoborate to produce blue spots. The procedure was sensitive to about 0.05 ppm and distinguished carbaryl from α-naphthol, which had a lower R_f. Dingle (*91*) determined carbaryl in cattle dipping solutions by simple dilution with ethanol and measuring the absorbance at 280 mμ. Correction for α-naphthol was based on its absorbance at 324 mμ.

Johnson and Stansbury reported similar colorimetric procedures for the determination of Temik, 2-methyl-2(methylthio) propionaldehyde *O*-(methylcarbamoyl) oxime (*165*) and for Tranid, 3-exo-chloro-6-endocyano-2 - norbornanone - *O* - (methylcarbamoyl)-oxime (*166*). The oxime carbamates were hydrolyzed with NaOH to form the oxime which was then hydrolyzed with HCl to release hydroxylamine. The hydroxylamine was oxidized with iodine to nitrous acid which diazotized sulfanilic acid. The latter was coupled with 1-naphthylamine to form a color which was read at 530 mμ. The sensitivity of these methods was reported to be about 0.03 ppm. Niessen and Frehse (*225*) described a colorimetric procedure for the determination of Bayer 39,007 (Baygon, Unden) (*o*-isopropoxyphenyl methylcarbamate) in leafy vegetables. After extraction and cleanup, the pesticide was saponified, neutralized, and treated with triethanolamine, aminoantipyrine, and $K_3Fe(CN)_6$ to form a color read at 490 mμ.

CHOLINESTERASE INHIBITION

Enzyme inhibition continues to be a useful tool in pesticide residue work. Its lack of specificity, objectionable as it may be in many uses, actually enhances its value as a screening tool,

since dangerous amounts of inhibitor may be detected no matter of what nature. Beynon and Stoydin (*37*) reported such a rapid screening test for cholinesterase inhibition making use of agar-agar plates. As little as 0.001 μg of DDVP and other inhibitors could be detected.

Ortloff and Franz (*230*) conducted the test for detection of organophosphorus pesticides on TLC plates, using either 2-azobenzene-1-naphthylacetate (yielding white spots on a red background) or indoxyl acetate (white spots on blue) as substrate. Ackermann (*6*) used silica gel TLC plates for the semiquantitative estimation of organophosphorus and carbamate pesticides. Beam and Hankinson (*23*) reported a procedure for the determination of organophosphorus compounds and carbaryl in milk based on cholinesterase inhibition.

Several workers described the automation of cholinesterase inhibition determinations using the Technicon *Auto*Analyzer. Among these are Voss (*283*) and Ott and Gunther (*231*) whose procedures required prior extraction and cleanup. In a later publication, Ott and Gunther (*232*) used the spots scraped off a TLC plate as input sample for the *Auto*Analyzer.

Guilbault and Kramer (*128*) reported 2 new fluorogenic substrates, resorufin butyrate and indoxyl acetate. Both are nonfluorescent compounds which are hydrolyzed by cholinesterase to highly fluorescent materials. As little as 0.0003 units/ml of horse serum cholinesterase could be determined. However, in addition to cholinesterase, such enzymes as acylase, acid phosphatase, and chymotrypsin also hydrolyzed the substrates to varying degrees. Bauman *et al.* (*21*) reported an immobilized enzyme system which could be used for continuous monitoring of substrate concentration and thus for the detection of cholinesterase inhibitors. A urethane foam pad was impregnated with starch-immobilized cholinesterase and a solution of the substrate, butyrylthiocholine, passed through it. Any inhibition acting on the enzyme reduced the hydrolysis to easily oxidized thiocholine iodide. This caused a change in current flowing through 2 platinum electrodes placed on opposite sides of the pad and thus signaled the presence of an inhibitor. Guilbault and Kramer (*131*) used a similar immobilized enzyme pad in a continuous fluorometric system for determining anticholinesterase compounds in air and water. The substrates, the acetyl and butyl esters of 1- and 2-naphthol, which do not fluoresce, were continuously passed through the pad and were hydrolyzed to the fluorescent phenols. Upon inhibition, the fluorescence dropped.

HERBICIDES

Faust and Hunter (*104*) have reviewed the chemical methods for the detection of aquatic herbicides including diquat, paraquat, and the phenoxy alkyl acids. They discussed various cleanup and determinative procedures. Henkel (*148*) reported on the TLC behavior of the phenoxyalkyl acid herbicides. Adsorbents and pretreatment, liquid phases, R_f's and reagents for detection were discussed. Hosogai and Kawashiro (*156*) separated 16 herbicides in mixtures by TLC, using various nonpolar and polar solvents. Johnson (*168*) described a colorimetric method for the determination of *N*-1-naphthylphthalamic acid in cabbage, asparagus, and alfalfa meal. The sample was heated with zinc and NaOH and the released 1-naphthylamine steam distilled. After cleanup, the 1-naphthylamine was coupled with diazotized sulfanilic acid and the absorbance read at 535 mμ.

Olney and coworkers (*228*) used a modified procedure for the electron capture GLC determination of amiben in vegetables. The sample was digested with alkali to release amiben from any complexes. After extraction and cleanup, it was methylated and further cleaned up on a Florisil column before being injected into the GLC. Sensitivity of 0.01 ppm was reported.

Hilton and Uyehara (*152*) modified the colorimetric procedure of Storherr and Burke [*JAOAC* **44**, 196 (1961)] to determine amitrole in sugar cane. Recoveries ranged from 71 to 125% at levels of 0.0025 to 0.5 ppm. Pease (*240*) used temperature programmed, microcoulometric GLC for the determination of bromacil in tissue, plants, and soil. Recoveries averaged 98% at levels of 0.04 to 5.6 ppm.

A number of methods have been reported for the determination of 2,4-D and other chlorophenoxy alkyl acid herbicides. Hagin and Linscott (*141*) described a procedure for the determination of 2,4-D and 2,4-DB in forage plants which made use of quick freezing and blanching of the plant material before extraction. Determination was by electron capture GLC after esterification with diazomethane.

Meagher (*205*) reported a procedure for 2,4-D and 2,4,5-T in citrus. The peel was extracted by blending with hot acetone, and the bound, the free acid, and the ester forms were separated, and each was hydrolyzed to the free acid. The free acids were esterified with 2-butoxyethanol saturated with HCl gas and cleaned up on a Florisil column before determination by electron capture GLC. Chromatographing the compounds as their butoxyethyl esters had the advantage that the long retention times separated the peaks from interferences present near the solvent front. Recoveries ranged from 89 to 93% at 0.0002–0.4 ppm levels. Crosby and Bowers (*81*) reported a method for the determination of 2,4-D in milk and other high protein samples where the 2,4-D may be bound to the sample. They refluxed the sample with NaOH and methanol to release the 2,4-D which was methylated for electron capture GLC determination. Yip (*288*) used programmed temperature microcoulometric GLC to determining a number of the chlorinated herbicides in oils. Recoveries ranged from 87 to 113% at 0.02–0.08 ppm levels. Yip and Ney (*289*) determined free 2,4-D and its esters in milk and forage. After extraction, cleanup, and methylation, determination was made by both microcoulometric GLC and paper chromatography.

Flanagan *et al.* (*108*) reported a paper chromatographic procedure for dalapon, using $AgNO_3$ and phenoxyethanol as chromogenic reagent. Smith and coworkers (*261*) described a method for dicamba in milk and a number of crops, using electron capture GLC after methylation. Meulemans and Upton (*211*) determined dichlobenil and its metabolite 2,6-dichlorobenzoic acid in fruits, soil, water, and fish. The two were separated and determined by electron capture GLC after cleanup. The dichlobenil was chromatographed as such but the metabolite was first methylated. Beynon and coworkers (*35*) reported an electron capture GLC method for the determination of dichlobenil and Chlorthiamid (2,6-dichlorothiobenzamide) in crops, soils, and water. Several extraction and cleanup procedures and 3 GLC columns are described. Recoveries ranged from 80 to 100% at levels of 0.03–5.0 ppm. Boyack *et al.* (*48*) used GLC with a flame ionization detector to determine diphenamid in vegetables and peanuts, with a sensitivity of 0.05 ppm.

Engelhardt and McKinley (*98*) studied the polarographic behavior of diquat. Using previously published extraction and cleanup procedures, they were able to determine diquat polarographically at levels as low as 0.01 ppm with recoveries of 84–97%.

Calderbank and Yuen (*61*) described an improved ultraviolet method for diquat in potatoes. After extraction and cleanup on a cation exchange column, the diquat was reduced to a free radical with sodium dithionite and its absorbance read at 379 mμ. Earlier, they had reported a similar method for paraquat (*60*). Katz (*175*) reported both colorimetric and TLC procedures for five substituted urea herbicides in water. After extraction with chloroform, diuron, monuron, linuron, neburon, and fenuron were hydrolyzed, diazotized, and coupled with *N*-(1-naphthyl)ethylenediamine dihydrochloride to form magenta dyes which were extracted with *n*-butanol and read at

555 mμ. TLC with ninhydrin spray reagent was used for identification of the specific herbicide.

Gutenmann and Lisk (*140*) used electron capture GLC to determine DNOC, DNOSBP, ioxynil, and bromoxynil in milk, apples, and grains. They noted that reacting the phenolic pesticides with diazomethane to form the methyl ethers eliminated trailing on the GLC. Boggs (*42*) also reported the superior chromatographic behavior of the methyl ethers of the dinitrophenols. Bache and Lisk (*14*) reported a similar GLC procedure for ioxynil but used the emission spectrometric detector of McCormack, Tong, and Cooke (*204*) to measure the atomic iodine line at 2062 Å. Ford and coworkers (*109*) described a colorimetric procedure for the determination of norea (Herban) in vegetables, grains, and oil seeds with a sensitivity of 0.1 ppm. The herbicide was hydrolyzed by caustic to dimethyl amine and the primary bicyclic amine which were both steam distilled. After reaction with 1-fluoro-2,4-dinitrobenzene, the complex with the bicyclic amine was separated out on an alumina column and the absorbance in alkaline dimethylformamide read at 443 mμ. Koivistoinen and Karinpää (*181*) reported a modified procedure for IPC and CIPC on fruits and vegetables. Samples were extracted by tumbling with benzene and the herbicides hydrolyzed. The amines were steam distilled, diazotized, and coupled with *N*-(1-naphthyl)ethylenediamine; the absorbance was read at 555 mμ. Recoveries from spinach, cabbage, tomatoes, and strawberries ranged from 86 to 113% at 0.5–200 ppm levels.

Pease (*242*) described a gas chromatographic method for the determination of the herbicide 3-cyclohexyl-5,6-trimethyleneuracil in sugar beets and soil. Using the flame ionization detector, crop blanks ran as high as 0.04 ppm. Merkle *et al.* (*207*) used electron capture GLC after methylation to determine picloram (4-amino-3,5,6-trichloropicolinic acid) in soil. They noted that the acidity of the extracting solvent (acidified acetone) was very important. It had to be acid enough to convert the picloram to the free acid but not so acid as to convert the amino group to a quaternary salt.

Kerr and Olney (*176*) determined trifluralin in vegetables by electron capture GLC and prometryne by hydrolysis to hydroxypropazine which was measured spectrophotometrically. Drescher (*92*) described 2 methods for determining pyrazon. In one procedure which can be used for detection on paper or thin layer chromatograms, the pyrazon was diazotized, losing its chlorine atom, and was then coupled with 2-naphthol to form a dye. In the second procedure, pyrazon was treated with NaOH-methanol to split off aniline which was steam distilled, diazotized and coupled with *N*-(1-naphthyl)ethylenediamine The absorbance, measured at 530 mμ, permitted detection as low as 0.05 ppm.

Several workers have reported methods for the determination of the *s*-triazines. Mattson *et al.* (*201*) described a procedure for the determination of both chloro and methylmercaptyl *s*-triazines, using microcoulometric GLC with the chlorine and sulfur cells, respectively. A sensitivity of 0.05 ppm was attainable for most crops and recoveries ranged from 75 to 107%. Abbott and coworkers (*1*) used thin layer chromatography to determine 8 *s*-triazines in soil and water. Using silica gel G as the adsorbent the developed chromatograms were sprayed with an 0.5% solution of Brilliant green and exposed to Br_2 fumes. The triazines appeared as deep green spots on an off white background and were immediately marked in outline. For quantitation, the square root of the area of the spots plotted against the log of weight triazine gave a straight line. Manner (*197*) also used TLC to detect 8 *s*-triazines on silica gel GF254. Ninety-one mobile solvent systems and R_f's for each are listed. Plates were examined under ultraviolet light (254 mμ) with the *s*-triazines appearing as dark brown spots on a yellowish green, fluorescing background. The spots could be eluted for additional determinations. Radke *et al.* (*244*) evaluated the pyridine–alkali colorimetric method for the determination of atrazine. They showed that the color intensity increased with acidity of the system and that 20° ± 2° C was a suitable temperature for color development. Chiba and Morley (*66*) reported a microcoulometric GLC method for trichloroacetic acid in wheat sensitive to 0.1 ppm. Compounds such as Kelthane, which could break down to give $CHCl_3$, interfered.

FUNGICIDES

Gunther and Ott (*137*) described a fully automated procedure for the determination of biphenyl in citrus fruit rind. The sample was automatically homogenized and steam distilled; waxes and oils were removed from distillate and the biphenyl in cyclohexane was fed through a cell of a recording ultraviolet spectrophotometer. Chioffi (*67*) used TLC on silica gel to determine biphenyl and *o*-phenylphenol in lemons. Norman and coworkers (*226*) used TLC for cleanup in the determination of biphenyl in citrus fruit and wrappers. Sample extracts were spotted on Eastman silica gel chromagrams and, after development, the spots were located under ultraviolet light. The spots were then cut out, extracted with ethanol, and the absorbance of the biphenyl was measured at 248 mμ. Sensitivity was reported as 5 ppm in citrus fruit and 5 mg/wrapper. McCarthy and Winefordner (*202*) combined a TLC cleanup with phosphorimetric determination for biphenyl in oranges. For the phosphorimetry they used an excitation wavelength of 275 mμ and emission of 470 mμ. Vogel and Deshusses (*281*) reported a GLC procedure for biphenyl in citrus fruit and wrappers. The biphenyl was distilled and absorbed in cyclohexane, which was injected into a GLC with an ionization detector. Sensitivity was reported as 0.5 ppm. Viel (*279*) reported a colorimetric method for the determination of captan and folpet in grapes and strawberries. After extraction and cleanup, the dried residue was treated with pyridine and then with KOH and the absorbance read at 435 mμ. Fishbein *et al.* (*106*) used thin layer chromatography on silica gel for the determination of captan and Captax (2-mercaptobenzothiazole). As chromogenic reagents, they used resorcinol in glacial acetic acid for captan and cupric chloride–hydroxylamine for Captex. Cheng and Kilgore (*64*) described an electron capture GLC method for the determination of Botran (2,6-dichloro-4-nitroaniline) in stored fruits. A sensitivity of 0.01 ppm was attained by tumbling the macerated sample with benzene, drying the benzene with Na_2SO_4, filtering, and injecting into the GLC. Vogel and Deshusses (*280*) reported a polarographic procedure for 2,6-dichloro-4-nitroaniline which had an accuracy of ±3% at levels of 2–7 ppm.

Hoffman and coworkers (*154*) reported both a colorimetric and thin layer method for the determination of dichlone in tobacco. For the colorimetric determination, the residues were extracted by blending with benzene, cleaned up on a Florisil column, evaporated, dissolved in absolute alcohol, triethylamine added, and absorbance read at 640 mμ. In the TLC method, the developed plates were sprayed with diethylamine and the spots compared with standards. Miller (*212*) investigated 4 colorimetric methods for dichlone and combined parts of 2 for collaborative study. The sample was stripped with benzene and cleaned up on a Florisil column, and color was developed with dimethylamine for reading at 495 mμ. Ten collaborators analyzed samples containing 0.5–4.0 ppm dichlone and obtained recoveries with an overall range of 78–112%. Sensitivity of the method was 0.25 ppm.

Thornton and Anderson (*273*) used electron capture GLC for the determination of Chemagro 2635, a mixture of 1,2,4-trichloro-3,5-dinitrobenzene and 1,2,3-trichloro-4,6-dinitrobenzene. The sensitivity of the method was 0.1 ppm and recoveries from cucumbers, potatoes, spinach, cottonseed, etc. were over 85%. Lyalikov and Solonar (*196*) described the polarographic determination

of hexachlorobutadiene and stated that other chlororganic compounds did not interfere.

Cullen and Stanovick (*82*) used electron capture GLC for the determination of korax, 1-chloro-2-nitropropane, in vegetables. The sample was blended with benzene and methanol and after washing and drying the benzene solution was injected into the GLC. Recoveries averaged 80–102% at 0.005–0.1 ppm levels. Voloshchenko and Klisenko (*282*) described a colorimetric method for the determination of Mylone, (3,5-dimethyl - 1,3,5,2*H* - tetrahydrothiadiazine-2-thione). The compound was hydrolyzed with acid to release CS_2 which was reacted with diethylamine and cupric acetate to form copper dithiocarbamate. The sensitivity of the method was reported as 0.02 ppm and recoveries from vegetables ranged from 93 to 120%. Cotta-Ramusino and Stacchini (*76*) reported a spectrofluorometric method for the determination of o-phenylphenol on citrus fruit. The extract was diluted with 0.1*N* NaOH and the fluorescence measured at 425 mμ, using an excitation wavelength of 325 mμ.

MISCELLANEOUS PESTICIDES

Kroeller (*188*) used the colorimetric method for arsenic in food based on the reaction of AsH_3 with silver diethyldithiocarbamate after wet digestion and preliminary separation by distillation from HCl. Kirchmann and Roderbourg (*179*) used radioactivation for the determination of arsenic in plant matter. After irradiation the arsenic was separated by wet ashing and precipitation as As_2S_5 before measurement of As^{76}. The limit of detection was 2×10^{-8} gram. Banderis (*17*) reported a colorimetric method for the determination of chlorates in plants and soil. It was based on the reaction of chlorates with HCl to release chlorine. The chlorine was reacted with o-tolidine to form a yellow color which was read at 448 mμ.

Several methods have been reported for the determination of cyanide. Kroeller (*189*) used a specially designed still to distill cyanide from foods under nitrogen. The distilled HCN was converted to cyanobromide which reacted with pyridine–benzidine to form a red color which was measured. Guilbault and Kramer (*129*) reported a fluorometric method in which the cyanide was reacted with quinone monoxime benzene sulfonate ester in dimethylsulfoxide to give a green fluorescence. With an excitation wavelength of 440 mμ and emission of 500 mμ as little as 0.5 μg of cyanide was easily detected and no other ions were found to interfere. These authors (*130*) investigated this reaction and those of various other quinone derivatives with cyanide, studying the effect of substituents, solvents, pH, and interferences. They found that the fluorescence produced with p-benzoquinone was proportional to the cyanide concentration over the range of 0.2–50 μg. They later reported (*132*) an ultra-sensitive specific qualitative test for cyanide, using p-nitrobenzaldehyde and o-dinitrobenzene to form a highly colored blue complex by which as little as 3 nanograms total cyanide could be detected.

Steller *et al.* (*264*) described a colorimetric method for cyanimide on cottonseeds. The seeds were extracted by tumbling with water followed by cleanup with activated charcoal. The cyanimide was then reacted with a solution of trisodium pentacyanoammine ferroate to give a red color which was read at 530 mμ. The sensitivity of the method was 0.03 ppm and recoveries at levels of 0.03–0.20 ppm averaged about 85%.

Cottonseed has been analyzed for DEF (*S,S,S*-tributyl phosphorotrithioate), using gas chromatography after Florisil column cleanup. Thomas and Harris (*272*) used the microcoulometric detector while Thornton and Anderson (*274*) used electron capture detection in their procedure. Bielorai and Alumot (*38*) reported a procedure for the determination of ethylene dibromide in foods and feeds, using GLC with a flame ionization detector. Benzene was added to the sample and distilled. The distilled benzene was dried and then injected into the GLC. Results by this method were in good agreement with the chemical titrimetric method at 15–1500 ppm levels. Kimura and Miller (*178*) reported a thin layer chromatographic procedure for the determination of gibberellic acid in rhubarb having a sensitivity of 3 ppb. The gibberellic acid spots were located on the acidified silica gel plate by their fluorescence under ultraviolet light. Zielinski and Fishbein (*292*) reported that they could gas chromatograph maleic hydrazide after reacting it in pyridine with hexamethyldisilazane in the presence of trimethylchlorosilane. Hoffman *et al.* (*153*) discussed possible interferences in the colorimetric method for maleic hydrazide and described a Norit-A cleanup to eliminate interferences. Lane (*191*) conducted a collaborative study of the colorimetric method for maleic hydrazide [J. R. Lane, *JAOAC* **46**, 211 (1963)]. Five collaborators obtained average recoveries of 70–92% from samples of cranberries, potatoes, onions, etc. fortified at 1.3- to 85-ppm levels.

A cold vapor atomic absorption apparatus was designed by Schachter (*255*) to measure submicrogram quantities of mercury in the vapor phase at room temperature. Using this apparatus, Pappas and Rosenberg developed procedures for the determination of mercury in wheat (*236*) and in fish and eggs (*237*) at levels as low as 0.01 ppm. Epps (*101*) used the colorimetric dithizone method for determining mercury in rice following digestion with nitric and perchloric acids. An excellent and thorough study of the dithizone method for mercury in foods was recently reported (*169*). Each step in the procedure was evaluated and the resulting method studied collaboratively. Recoveries at 0.1 ppm were excellent and the sensitivity was thought to be 0.05 ppm (dried sample). Neutron activation has also been used for the determination of mercury. Kim and Silverman (*177*) used it for the analysis of wheat and tobacco, making a chemical separation after irradiation before measuring activity of ^{197}Hg. Tomizawa and coworkers (*277*) used neutron activation to determine mercury in rice. Again, a chemical separation was made after irradiation but these workers measured ^{203}Hg.

Hearth *et al.* (*147*) reported an oscillopolarographic method for the determination of Morestan (6-methyl-2,3-quinoxalinedithiol cyclic carbonate) in orange rind. The hexane stripping solution was concentrated and cleaned up on silica gel TLC plates. The spots were located by their fluorescence under ultraviolet light, scraped off, and eluted with ethanol for the polarographic determination. Martin and Schwartzman (*199*) reported that the ultraviolet spectrophotometric method for nicotine, at times, could not distinguish between crop interference from mustard greens and nicotine; they described a TLC procedure which did make the distinction.

Narahu and coworkers (*222*) used the gas chromatograph with a thermal conductivity detector to determine pentachlorophenol in soy sauce. They chromatographed the PCP as the phenol, using dehydroacetic acid as an internal standard. Cheng and Kilgore (*65*) in determining pentachlorophenol and its sodium salt in fruits, first methylated these compounds with diazomethane before using electron capture GLC for the determinative step. Akisada (*9*) described a colorimetric method for pentachlorophenol and tetrachlorophenol in urine and in air. The phenols were distilled off from the acidified urine while the air was passed through an absorbing solution containing a borate buffer at pH 7.13. They were then reacted with 4-aminoantipyrine and $K_3Fe(CN)_6$ and the colors extracted into xylene. The absorbance was measured at 470 mμ for tetrachlorophenol and at 570 mμ for pentachlorophenol. Zielinski and Fishbein (*294*) gas chromatographed piperonyl butoxide and a number of 3,4-methylenedioxyphenyl derivatives, both as the compounds themselves and as the methyl and trimethylsilyl derivatives of these compounds. Mestres and Barrios (*208*) used gas chromatography to determine propylene oxide and propylene glycol in fruit. By means of a system in which 1–20 mg samples were

introduced directly into the injection chamber, they demonstrated that propylene oxide was rapidly absorbed by prunes in which it was hydrolyzed to propylene glycol.

Delfel (*88*) described the use of HI as a color reagent for the detection of rotenone on paper chromatograms. Rotenone gave a characteristic blue color with the reagent while elliptone gave a pink or violet color. None of the other materials present in crude extracts of *Derris elliptica* or *Tephrosia vogelii* gave any color with the reagent. Delfel (*89*) also studied the TLC behavior of rotenone and related compounds and described a number of solvent systems and chromogenic agents to give desired separations. Johnson and Stansbury (*163*) reported a colorimetric method for the defoliant, sodium cis-3-chloroacrylate (Prep), in cottonseeds. The sample was acidified and the free acid extracted by blending with 1-butanol. After cleanup, it was reacted with pyridine and NaOH to produce a colored solution which was passed through an alumina column and then read at 395 mμ. Toxaphene, chlordane, DDT, and TDE did not interfere. Christian and coworkers (*68*) described a polarographic method for selenium in biological materials while Cummings *et al.* (*84*) used a colorimetric procedure measuring the absorbance of a complex of selenium with 3,3′-diaminobenzidine. Pease (*241*) determined sulfamates in apples and pears by removing all the sulfates and then reducing the sulfamates to H_2S which was reacted with *p*-dimethylaminoaniline to form methylene blue. Bowman and Beroza (*47*) reported a gas chromatographic procedure for the determination of tepa, apholate, hempa, and several other chemosterilants. Using the flame photometric detector of Brody and Chaney (*50*) they could detect as little as 0.1 ng of the sterilants. Bullard (*52*) used GLC with flame ionization detector to determine tetramine (tetramethylenedisulfotetramine), a systemic toxicant used to keep animals from feeding on seed and young seedlings. Recoveries from a variety of foliages consistently averaged above 80%. Billy and coworkers (*39*) reported a spectrophotometric procedure for the determination of the lampricide, 3-trifluoromethyl-4-nitrophenol (TFM), in water and in fish tissue. After cleanup by liquid–liquid partitioning and ion exchange column the determination was made by measuring the absorbance at 395 mμ.

MISCELLANY

Duggan (*93*) described the procedures used by the Food and Drug Administration to validate multiple residue methods on the varieties of foods. To illustrate the magnitude of the problem, he pointed out that with the 12 major food classes and the 50 chemicals most commonly found, there were 1.35×10^{16} possible different combinations that the residue chemist could encounter.

In exploring methods for the determination of organo-metallic fungicides on crops, Gudzinowicz and Luciano (*127*) showed that atomic absorption could be used to determine zinc, manganese, and iron. However, the amounts of these metals found in untreated plants were so high that their measurement did not seem a promising means of detecting fungicide residues.

Beroza and Bowman (*30*) introduced the concept of *p*-values—based on the distribution ratios between 2 immiscible solvents—as the basis for identification of pesticide residues and other compounds. In its simplest form, a solution of the residue was analyzed by GLC and then after shaking with an equal volume of immiscible solvent it was again analyzed by GLC. The ratio of the 2nd result to the first is the *p*-value. The authors refined the procedure by the use of a 5-plate Craig counter current distribution apparatus (*31*); they listed *p*-values for 131 pesticides in 6 binary systems (*45*); they designed an apparatus for rapid extraction (*32*) and a device as well as an equation for obtaining *p*-values using nonequilibrated solvents (*46*). They (*33*) also studied the extraction of added pesticides from milk with hexane–diethyl ether with and without prior mixing of sample with ethanol. They found that without ethanol, the extraction efficiencies paralleled the polarities as judged by *p*-values.

Farrow *et al.* (*102*) reported a cleanup procedure for both chlorinated and organophosphate pesticides based on vacuum sublimation. The dried sample extract was subjected to vacuum sublimation at 85°C. for 15 minutes and the pesticide residues were collected on a cold finger cooled with Dry Ice–acetone. The residues were rinsed from the cold finger, made to volume, and injected into the electron capture GLC. The procedure was tested on 35 pesticides in spinach and recoveries for 25 of these exceeded 80%. Most of the others were recovered in the 60–80% range except for a few low values from waxy plant extractives.

Rybakov (*248*) reviewed the use of polarography and discussed methods for the analysis of pesticides containing sulfur, phosphorus, chlorine, and nitrogen.

Coffin (*73*) reviewed the use of paper chromatography in pesticide residue analysis, discussing its advantages and disadvantages as well as various detection systems.

Salo and Salminen (*251*) tabulated TLC data for 29 common pesticides under a number of solvent systems.

Chen (*62*) described a micro technique for infrared by which good spectra could be obtained from as little as 1 μg of pesticide. The procedure, which has been used to identify pesticides separated by GLC, consisted of incorporating the sample into 4 mg of KBr in a micropellet formed in a 2-mm diameter hole in folded aluminum foil. It was pointed out that it was essential to compare sample spectra with standard spectra obtained in the same manner since weak bands are missing at microgram levels.

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