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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 (35) 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 (45) 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 hydrog,en as the carrier gas. Organic compounds are reduced to hydrocarbons, watere PH₃, H₂S, and HCl. The latter thre com- pounds precipitate silver ion nd so register on a microcoulometric t tration cell. Insertion of a short si ica gel column removes HCl; substitution of Al₂O₃ for silica gel removes both HCl and H₂S and permits measurement of PH₃ with absolute spec'ficity. Response of the cell to PH₃, H₂S, and HCl is in the ratio of 2 ᐧᐧ 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 Na₂SO₄. 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. (175) 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 analyzing samples of fat, milk, butter, and eggs. Moats (151) used a column containing 100 grams of standardized 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 (139) 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 micro-coulometric 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 AgNO₃.

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 AgNO₂-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.

Getzendaner (*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%.

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 semi-quantitative determination of organophosphates was taken by Bruaux, 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 oxidize 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 chroma-

tography 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 Tietz (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-nitrobenzene-diazonium 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-dimethylamino-benzenediazo 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 naphthalene-acetic 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 naphthalene-

acetic 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-quinox-alinedithiol 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-quinox-alinedithiol 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 Schultheiz (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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