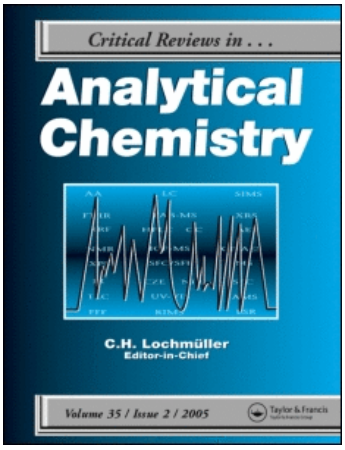

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CHROMATOGRAPHIC ANALYSIS OF PESTICIDE RESIDUES

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I. INTRODUCTION

The purpose of this article is to review methods involving gas, thin-layer, paper, and liquid-column chromatography for the analysis of multiresidues and the determination of some individual residues of pesticides in various types of samples. At present, gas chromatography is by far the most widely used method for the qualitative and quantitative analysis of pesticide residues. Paper and thin-layer chromatography are used mainly for confirming identifications of substances tentatively determined by gas chromatography but may also provide cleanup prior to gas chromatography and semiquantitative estimation in cases where gas chromatography is unavailable or not applicable. Liquid column chromatography has been used mostly for the cleanup of sample extracts, but modern high-speed methods have recently been applied to the resolution and determination of compounds not volatile or stable enough for gas chromatography. Ancillary methods used in combination with these chromatographic techniques for pesticide identification will also be reviewed. The attempt has been made to cite only the most widely used and reliable analytical methods available as of spring, 1973. Where possible, the methods have been compared and their limitations and some precautions that should be observed in using them are given.

A. Importance of Pesticides and Their Determination

Pesticides may be classified according to either their use (e.g., as insecticides, herbicides, fungicides, rodenticides) or their chemical type (organohalides, organophosphates, carbamates, organosulfurs, inorganics, metalloorganics, anilines, ureas, phenols, amides, triazines, organic acids, quinones, and other miscellaneous chemicals). Compounds of the first three of these chemical types are probably the most important and are the ones that will be stressed in this paper. Chlorinated hydrocarbon compounds are not acutely toxic, but they are persistent in the environment and are stored in fatty tissue. Organophosphorus compounds are acutely toxic but are generally nonpersistent and not stored in fatty tissue. The carbamate compounds are relatively low in acute toxicity and are nonpersistent. The pharmacological action of both the phosphorus and carbamate pesticides is based on their cholinesterase-inhibiting property.

Approximately 90,000 formulations of about 900 chemical pesticide species were being produced in amounts totaling more than 2 billion pounds annually as of early 1972 according to L. A. Richardson of the U.S.E.P.A. Because of the widespread occurrence of pesticides in the environment, the availability of sensitive methods for the analysis of residues of these compounds is extremely important. These analytical methods allow research on the prevalence of pesticides in various environmental media (food, water, soil, air) and in man (tissue, blood, excreta), somatic and genetic effects on humans, disposition of the chemical agents (metabolism, reaction, degradation), and removal of contaminants by chemical, physical, and thermal means. Residue analysis is also vital for surveillance and law enforcement and for investigations of accidental or intentional pesticide contamination.

B. Pesticides in the Environment and in Humans

The advent of gas chromatography has made possible the detection of the persistent pesticides, particularly DDT, at extremely low levels in widespread locations of the environment and in human tissues. It is not generally clear if harmful pharmacological effects result in man or animals from this low level contamination. Human exposure to the nonpersistent phosphorus and carbamate pesticides may be detected by identifying their hydrolysis products (e.g., urinary *p*-nitrophenol for compounds such as parathion and EPN and urinary 1-naphthol for carbaryl). This review will focus on analyses of residues of pesticides and metabolites in human and environmental samples.

II. PESTICIDE CHEMISTRY AND LITERATURE

A review of pesticide chemistry has been written by Benson,[1] and a complete volume of *Residue Reviews* by Mel'nikov was devoted to this topic.[2] A series of monographs edited by Zweig[3] on the chemistry of pesticides began to appear late in 1972. In many cases, equations for pertinent pesticide reactions are given in sections below.

The literature of pesticide chemistry through May, 1970 was reviewed by Benson.[4,5] The literature of pesticide residue analysis is reviewed in April of each odd year in the Applied Reviews issue of *Analytical Chemistry*. A series of books edited by Zweig and published by Academic Press

contains detailed procedures for the analysis of pesticide formulations and residues.

III. GENERAL ASPECTS OF RESIDUE ANALYSIS

A. Sampling

Methods for collecting and preparing a valid analytical sample of the proper size that is random and representative have been reviewed in Chapter 1, Section I of the book by Zweig and Sherma.[6] General guidelines for preparing, compositing, and reporting routine samples of raw and processed agricultural products, meats and poultry, marine products, and milk are described in Sections 140 to 143, Volume I, of the *Pesticide Analytical Manual.*[7]

It is important that the sample delivered to the analytical chemist has not undergone degradation of any pesticide originally present and has not become contaminated with impurities which might interfere with subsequent analysis. Plastic containers may introduce traces of impurities such as polyethylene into samples and should be avoided if analysis will be made by electron-capture GC. Glass bottles, jars, and vials of the appropriate size with caps lined with Teflon® or aluminum foil are generally recommended as sample containers for tissue, blood, water, environmental, and agricultural samples.

Proper storage of samples is also a vital consideration. Agricultural, environmental, and tissue samples which will be extracted within 24 hr are stored in a refrigerator (+2 to +4°C), while those to be analyzed later are deep-frozen (−12 to −18°C). Samples to be analyzed for organophosphate pesticides should be frozen immediately in tightly sealed containers unless analysis is to begin within several hours. Analysis should not be delayed beyond about 12 hr, even when samples are frozen, if at all possible. Blood samples that are to be separated for analysis of serum are centrifuged as soon as drawn; serum is stored in a refrigerator if analysis will be made within 72 hr or in a freezer for longer periods.

B. Cleaning of Glassware

Because the residue analyst is continually involved with the analysis of very small traces (parts per billion) of pesticides using very sensitive but not specific analytical methods, contamination from glassware, reagents, and the sample itself, which could interfere with the analysis or even completely mask the pesticide, must be carefully considered. The preparation of scrupulously clean glassware will be considered here, and reagents and sample cleanup in later sections. New glass received from the manufacturer or glass in use should be cleaned by the following steps:

1. Rinse with acetone.
2. Soak in a hot (50°C or higher) bath of a suitable synthetic detergent (e.g., Alconox®). The detergent should be checked to be sure it will not contribute background contamination by electron-capture GC detection.
3. Rinse with hot tap water.
4. Soak in sulfuric acid-dichromate cleaning solution at 40 to 50°C (observe rigid safety precautions). Commercial substitutes such as Chem-Solv® 2157 (Mallinckrodt®) are safer to use and apparently as effective.
5. Rinse with hot tap water.
6. Rinse with distilled water.
7. Rinse with acetone.
8. Just before use, rinse with the solvent to be used in the analysis.

As soon as possible after a piece of glassware has come in contact with a sample containing pesticide, it should be rinsed with acetone to remove surface residues.

To avoid possible recontamination during drying and storage of cleaned glassware, large glass items (beakers, etc.) are inverted and dried in neoprene-coated metal racks. Small items (glass stoppers, etc.) are wrapped in aluminum foil, dried in an oven, and stored in the foil.

Pipets are washed as above, preferably using a commercial automatic or semiautomatic, self-contained washing unit. Clean pipets are stored in commercial stainless steel storage tubes.

C. Preparation and Storage of Analytical Pesticide Standards

Primary standard pesticides are available from the manufacturer or a government agency such as the Pesticide Repository, Perrine Primate Laboratory, Perrine, Fla. Purities are usually at least 95% but may be lower in some cases. Toxicity levels and relative stabilities are important factors which dictate the methods of handling and storing pesticide standards of various classes.

Concentrated stock (secondary) standards for

chromatographic analyses are prepared at a level of 200 ng/μl by dissolving 20 mg of pesticide in a 100-ml volumetric flask. For IR work, a range of 10 to 25 μg/μl will be required. A micro or semimicro analytical balance and scrupulously clean glassware are employed. Stable, low-toxicity pesticides are weighed into a beaker or cupped aluminum foil. For highly toxic compounds use a closed weighing bottle and rubber surgical gloves, and avoid inhaling any vapors. Alternatively, the primary standard can be weighed directly into a 50-ml volumetric flask, which will fit onto the pan of a Mettler analytical balance. If the purity of the primary standard is less than 99%, apply an appropriate correction factor when calculating the concentration of the stock standard. Hexane, benzene, and isooctane are solvents that will dissolve most common pesticides. Dioxane is useful for triazines and methylene chloride for carbamate insecticides. Concentrated standards of chlorinated compounds are stable for at least six months when stored in a deep freezer; organophosphate standards should be remade at least every four months.

It is usually necessary to prepare standards of intermediate concentrations by dilution of the concentrated standards and then to prepare the working standards by dilution of the intermediate standards. The solvent for these standards is usually isooctane or hexane. For example, to prepare a working standard containing 20 pg/μl, dilute 1 ml of a solution containing 200 ng/μl to 100 ml to prepare a solution containing 2 ng/μl, then dilute 1 ml of this to 100 ml to prepare the required solution. It would be impractical to prepare the final solution from the concentrated standard in one dilution; it would likewise be impractical to prepare an original secondary standard at a concentration low enough to allow only one subsequent dilution.

To minimize effort, working standards are mixtures of as many compounds as possible, the actual combinations being dependent upon the compounds of interest and the ability of the analytical method to resolve them. Compounds are present in varying concentrations to provide an appropriate range for the determination of each constituent of the sample. Intermediate and working standards are stored in a refrigerator.

It should be stressed that the accuracy of all analytical results is dependent on the accuracy with which standard solutions are prepared, and it is impossible to overstate the importance of proper preparation of standards for reliable quantitative results.

D. Solvents and Reagents

Commercial solvents designated "pesticide quality" or "distilled in glass" can usually be used without further treatment. Reagent- or technical-grade solvents require distillation in an all-glass still for most analytical applications. In either case, solvents should be tested for interference in electron-capture GC by concentrating 100 ml of the solvent to about 1 ml and injecting 5 μl into the gas chromatograph. Tests for substances not detected by this procedure but causing pesticide degradation and loss are made by carrying known amounts of standards through the analytical method in the absence of any sample substrate.

Recoveries of some phosphate pesticides from Florisil columns are low if peroxides are present in ethyl ether eluents. Ether is tested for the presence of peroxides by reaction with KI; if present, peroxides are removed by extraction with water, after which the 2% ethanol normally present in ether, which is also removed by the partitioning, is replaced.

Distilled[8] or deionized water is extracted twice with benzene or isooctane before use in analytical procedures. Solid Na_2SO_4, NaCl, and glass wool are Soxhlet extracted (50 cycles) with benzene and oven dried at 130°C before use. Various adsorbents, such as Celite 545 and MgO, require washing prior to use.

E. Cleanup of Samples

Pesticide residue analysis usually consists of three basic steps: extraction of the pesticide from the sample, cleanup or separation of the residues from interfering material co-extracted from the sample, and finally qualitative and quantitative determination of the residues. Burke[9] has reviewed general aspects of residue analysis.

Cleanup and preliminary fractionation of the residue mixture is most often accomplished by chromatographic elution of the extract through a column of an active adsorbent. Florisil (PR grade), a synthetic magnesium silicate which has been very widely used for this purpose, is purchased in an activated state, stored in capped amber bottles, and heated at least 5 hr at 130°C before use. Florisil has proven to be nonuniform[10,11] and to require pretesting of the adsorptive properties of

each batch prior to use. A simple and rapid test method[12] is based on the adsorption of lauric acid from hexane solution to aid in the weight adjustment of the adsorption column. A more reliable method requiring the elution of two standard mixtures containing a total of 19 pesticides and GC analysis of the eluates is described in Section 3D of *Analysis of Pesticide Residues in Human and Environmental Samples.*[13] The proper procedures for sampling and storing Florisil are also given. The latest trends indicate that partially deactivated[14] alumina and silica gel and gel permeation chromatography[15] are becoming increasingly popular for cleanup and separation procedures.

Selective detectors, such as the FPD for phosphorus-containing pesticides (Section VI-B), allow the injection of relatively unclean samples without giving rise to interfering GC peaks. Other problems may arise because of dirty samples, however, such as pesticide decomposition in the inlet system or on the column caused by build-up of extraneous material.

F. Evaporation of Solutions

Solutions of purified extracts are concentrated to a volume of approximately 5 ml using a Kuderna-Danish concentrator fitted with a 3-ball Snyder reflux column and a calibrated collection tube. The tip of the tube is held in a steam bath, and a boiling chip is added. For concentration to small volume (0.4 to 0.1 ml), a 2-ball micro-Snyder or a modified micro-Snyder (for solvents boiling above 65°C) column is used. Impure extracts are evaporated in the same way or by use of a rotary vacuum evaporator with the water bath at room temperature. Extracts contained in a beaker or a centrifuge tube immersed in a water bath at 40°C can be evaporated under a stream of purified dried nitrogen or air adjusted to such pressure to cause a gentle depression on the surface of the solution.

It is important to avoid decomposition of pesticides during evaporation. Burke et al.[16] and Chiba and Morley[17] found that severe losses of pesticides can occur during evaporation, even in the presence of coextractives. They suggested slow evaporation at low temperature and avoiding evaporation to complete dryness. Dieldrin and DDT were lost when an extract was evaporated in the presence of light.[18] Carbamate pesticides are reportedly susceptible to considerable loss when concentrated in a Kuderna-Danish apparatus, and rotary vacuum evaporation at 50 to 55°C with addition of a "keeper" solution was recommended.[19] The author has found that evaporation after addition of 5 drops of keeper solution (1% paraffin oil in hexane) under a nitrogen stream is the best approach, if feasible, for carbamates as well as most other pesticides.

G. Analytical Laboratory and Equipment

A pesticide residue laboratory should be planned so that it has reasonable temperature and humidity controls, an adequate ventilation system, an assembly line layout if large numbers of samples are to be analyzed, and adequate refrigerated storage. There should be separate areas for the preparation and subsequent analysis of samples. The equipment and methods described briefly below are most widely used for residue analysis.

1. Flat-bed Chromatography

In paper chromatography (PC) and thin-layer chromatography (TLC), mixtures of pesticides are separated by differential migration on a paper sheet or adsorbent layer when washed with a developing solvent. The separated spots are detected by applying a reagent which causes the pesticide zones to become colored or fluorescent. The minimum detection level is around 10 ng for TLC and somewhat higher for PC. These methods are good tools for separation, identification, cleanup, and semi-quantitation.

2. Gas Chromatography (GC)

Separations are achieved by selective partitioning of the pesticides between a carrier gas and a column of liquid-coated inert material. GC is an excellent tool for separating residues and determining their constituents. Its specificity and sensitivity depend upon the detection system employed. Burke[9] has reviewed the general requirements that a gas chromatograph should fulfill to be suitable for residue analysis.

Electron capture (EC) detector – Functions by monitoring the decrease of current caused by the presence of compounds that absorb electrons from a calibrated pool produced by the ionization of

nitrogen carrier gas; sensitive* to picogram quantities but quite nonselective; useful for quantitation but not very useful for identification.

Microcoulometric (MC) detector – Functions by pyrolyzing compounds and electrometrically titrating the specific element of interest; selective for halogen, phosphorus, sulfur, and nitrogen-containing pesticides and sensitive to low nanogram quantities; useful for both quantitation and identification.

Electrolytic conductivity detector – Functions by pyrolyzing the compounds and measuring the electrical conductance of an aqueous solution of the water-soluble products; selective for chloride-, sulfur-, and nitrogen-containing pesticides and sensitive to low nanogram quantities; useful for both quantitation and identification.

Flame photometric detector (FPD) – Functions by exciting atoms or molecules (POH, S_2) in a hydrogen flame and detecting the light emitted at specific wavelengths; sensitive to about 1 ng, selective for sulfur- and phosphorus-containing pesticides; useful for both quantitation and identification.

Thermionic detector – Functions by measuring the electrical response caused by thermally excited ions formed by burning compounds in a hydrogen flame incorporating an alkali metal salt; sensitive to low nanogram quantities, selective for phosphorus- and nitrogen-containing pesticides; useful for both quantitation and identification.

Mass spectrometer – Functions by disintegrating compounds, detecting the fragments, and identifying them from their mass-charge ratios; specific for all compounds, sensitive to low ng quantities; useful for identification and quantitation of all compounds.

3. Infrared Spectrophotometer

This functions by exposing compounds to IR energy and detecting the wavelengths absorbed. It is sensitive to low μg to high ng quantities, specific for all compounds, and useful for identification.

4. Liquid Chromatography

This is used for sample cleanup and pesticide analysis (see Section XI).

H. Chromatographic Techniques

The theory of chromatography and the general laboratory techniques to be used for residue analysis can be found in standard reference works on gas chromatography,[6,20,21] liquid column chromatography,[22] paper chromatography,[23] and thin-layer chromatography.[24]

For the liquid phases used in pesticide analysis, a rotary vacuum technique with very slow flask rotation has proved to be the preferred method for preparing GC column packings. An excellent column packing technique has been described by Applied Science Laboratories.[25] The most widely used supports for pesticide analysis are of the diatomaceous type. Column support materials[26-28] and their deactivation[29] have been reviewed and evaluated. Almost any standard syringe technique can be employed for injecting samples for GC or spotting samples for TLC so long as it is consistently used for unknowns and standards. The solvent-flush technique[30] is probably the method to be recommended if maximum reproducibility is desirable. The rate of sample injection must be standardized for EC-GC.[31]

Tentative qualitative identifications of pesticide residues from chromatographic results are made by comparing migration speeds (retention times in column chromatography, R_F values in flat-bed methods) of samples and standards. Such identifications must be confirmed by additional methods (see Section XIV below). Quantitative determinations of residues[32] by GC are usually performed by the external standardization technique:[6] the height or area of the peak for each unknown in the sample is compared with that of the peak of a similar, known amount of each compound injected just before and/or after the unknown. Only one standard concentration is required if the linearity of the detector response has been established. Internal standardization is used primarily for the analysis of pesticide formulations. Data processing by means of a time-shared computer system may be employed for routine GC pesticide analyses.[33] In PC and TLC, quantitative interpretation is based on comparing the spot sizes or intensities obtained with standards and samples. Results of

*Most pesticide scientists define GC sensitivity as the amount of pesticide which will provide a peak whose height corresponds to 10% or 50% of full-scale deflection, and the minimum detectable amount as the amount of pesticide that will give a signal at least 2 times background (noise) from the baseline.

pesticide analyses are ordinarily reported in terms of parts per million (ppm) of pesticide found in the sample, where ppm equals μg/g, ng/mg, or pg/μg:

$$\text{ppm} = \frac{\text{amount } (\mu g) \text{ of pesticide determined by comparison with standards}}{\text{g sample taken} \times \frac{\text{volume sample actually used in analysis}}{\text{final extract volume}}}$$

IV. DETERMINATION OF CHLORINATED HYDROCARBON PESTICIDES AND METABOLITES BY ELECTRON-CAPTURE GAS CHROMATOGRAPHY (EC-GC)

A. Instrumental Considerations

The source of the electron flux for the electron-capture detector is usually either ^{63}Ni or ^{3}H. The nickel detectors can operate at a higher temperature (300 to 400°C) than those with a tritium source (225°C maximum by Federal regulations), and some workers[34] have analyzed biological samples without cleanup using the nickel detector. Even though detector contamination would be decreased because of the higher temperature, contamination of the column would be expected to cause a rapid deterioration in column performance. It is also claimed[35] that the ^{63}Ni detector has a greater linearity in the low-sensitivity range.

With DC (as opposed to pulse voltage) operation, nitrogen is used as the carrier gas. As the radioactivity in the source decreases, so does the sensitivity of the system. Periodic measurement should be made of the background signal profile to gain an indication of the condition of the detector. It is important that the correct polarizing voltage be supplied to the detector to achieve maximum peak response with minimum overshoot in the trailing edge of the peak. The linear range of the detector must be determined, and concentrations of samples and standards chosen to be within this range when quantitative runs are made.

B. Column Technology

An early study[36] of conditions for the efficient analysis of chlorinated pesticides by electron-capture GC recommended: glass column 6 ft long by 4 mm i.d., packed with 10% DC-200 on 80 to 90 mesh Anakrom® ABS, conditioned 1 to 5 days at 250°C, 200°C column and detector temperatures, 120 ml/min flow of nitrogen carrier gas. These chromatographic conditions plus the use of the more polar mixed phase 15% QF-1/10% DC-200 (1:1) with the same parameters are still recommended in the FDA *Pesticide Analytical Manual,* Section 311.[7] However, the use of other liquid phases at lower loadings with slower flow rates now seems to be preferable. Most residue analyses can be adequately performed on 4- to 7-ft columns made of glass or metal; in the latter case, any catalytic activity from the column walls must be obviated.

An excellent combination of working columns recommended by Thompson[37,38] for the analysis of a large number of chlorinated pesticides in miscellaneous sample substrates is as follows:

1. 1.5% OV-17 (phenyl methyl silicone)/1.95% QF-1 (trifluoro propyl methyl silicone), liquid phases premixed* and coated on Chromosorb W, HP, 100 to 120 mesh, 200°C, 60 ml/min carrier flow rate. This is an efficient column material which completely separates all usual pesticides found in tissue samples except *p,p'*-DDE and dieldrin (ca. 75% separated). *p,p'*-DDT is eluted in 20 min or less.

2. 4% SE-30 (methyl silicone)/6% OV-210 (or QF-1), liquid phases premixed and coated on Chromosorb® W, HP, 80 to 100 mesh, 200°C, 70 ml/min flow rate. This packing gives no separation between lindane and β-BHC but good separation between *o,p'*-DDT and *p,p'*-DDD.

3. 5% OV-210 (or QF-1) on Chromosorb W, HP support, 100 to 120 mesh, 180°C, 60 ml/min. Provides full separations of commonly found BHC isomers and fair separation between *p,p'*-DDD, *p,p'*-DDT, and heptachlor epoxide.

4. 3% DEGS (polyester) on Gas Chrom® P support, 80 to 100 mesh, 195°C, 70 ml/min. Gives excellent separations of BHC isomers, complete peak separations between all pesticides usually found in tissues, and an unusual elution sequence (β-BHC after *o,p'*-DDT, *p,p'*-DDT before *p,p'*-DDD; see Figure 1), which makes it useful for confirmation of peak identities.

Relative retention times for more than 50

*FDA mixed-phase columns are prepared by coating the phases separately on the support and mixing the individually coated supports before packing, e.g., 10% DC-200/15% QF-1, mixed 1:1 by weight.

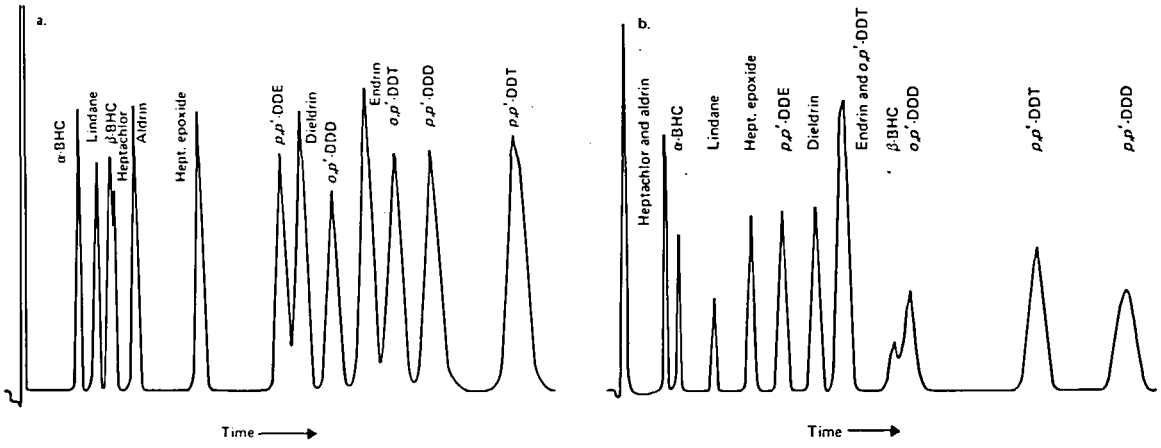


FIGURE 1. Typical chromatograms of a mixture of 13 chlorinated pesticides on (a) 1.5% OV-17/1.95% QF-1 and (b) 3% DEGS columns. Figures reproduced from reference 38 with permission of the authors and the Assoeiation of Official Analytical Chemists.

chlorinated and phosphate compounds on these 4 phases and on 10% DC-200, 3% OV-1 (both methyl silicones), 2% OV-1/3% QF-1, and 5% DC-200/7.5% QF-1 have been published,[38] along with peak-height response values by electron-capture detection relative to aldrin. The extensively used DC-200/QF-1 column was found least satisfactory for residue analysis. Similar retention data, usually relative to aldrin or parathion, for numerous pesticides have been tabulated[6,39] for columns containing DC high-vacuum silicone grease, SE-30/QF-1, GE-SE-52, Apiezon® L grease, GE-XE-60, SE-30, OV-17, and DC-11. Columns containing mixtures of three liquid phases have been designed for special purposes, e.g., 10% DC-200/7.5% QF-1/3% XE-60 (1:1:1, previously coated) and 3% OV-61/7.5% QF-1/3% XE-60 (1:1:0.5) for the separation of hexachlorobenzene, hexachlorocyclohexane isomers, and chlorinated insecticides.[40] Various precoated and pretested packings are available from certain commercial sources.

Columns are conditioned (made ready for use) by heat curing, silylation treatment, and injections of concentrated pesticides (priming). Heat curing is accomplished in the oven of the chromatograph with the column attached to the inlet port but not to the detector, or in a special conditioning oven. The columns numbered 1 to 4 above are conditioned with nitrogen carrier gas flowing through the column at 60 ml/min under the following respective conditions: 245°C, 20 hr; 245°C, 72 hr; 275°C, 20 hr; 237°C, 20 hr. After heat curing, treatment with Silyl 8® can improve efficiency and resolution characteristics of new columns, especially in terms of response of endrin. Four 25-μl injections of Silyl 8 are made at 30-min intervals at the temperature and flow rate to be used for operation. Again, the outlet of the column is not attached to the detector. Allow 1 hr after the final injection. Next make six 10-μl injections, spaced 15 min apart, of a mixture of the pesticides of interest at a level of 200 ng/μl. It has often been noted that column performance is also improved over the course of injecting biological samples into the column.

After conditioning, columns may be evaluated by chromatographing a complex chlorinated pesti-

cide mixture to check for resolution and response, a pure *p,p'*-DDT standard to check for breakdown to *p,p'*-DDD or *p,p'*-DDE, and a pure endrin standard to observe formation of extra peaks (breakdown). An efficient new 6-ft column should have about 3,000 total theoretical plates,[21] computed for the *p,p'*-DDT peak. This compound should elute in 16 to 20 min from all columns.

A properly prepared and maintained column can provide service for many months. If biological samples are routinely injected, off-column injection, with frequent changing of the glass injection insert, will prolong column life.[37]

C. Analysis of Human or Animal Adipose Tissue and Fatty Foods

The recommended procedure for the analysis of tissues, which is described in detail in Section 5,A, (1) of *Analysis of Pesticide Residues in Human and Environmental Samples,*[13] is a modification of the widely used Florisil method first described by Mills.[41-43] The sample is ground with sand and anhydrous Na_2SO_4, then extracted with petroleum ether. The pesticides are partitioned from petroleum ether into acetonitrile, 2% NaCl is added, and the residues are partitioned back into petroleum ether. The petroleum ether is dried by passage through a column of Na_2SO_4 and concentrated, and the residues are cleaned up and fractionated by elution through a 4-in. x 0.25-in. Florisil column with 200 ml of 6%, and then with 200 ml of 15%, diethyl ether in petroleum ether. Fractions are analyzed by GC after appropriate concentration. The recoveries of most chlorinated pesticides range from 85 to 100%. The common chlorinated compounds and PCB's are recovered in the 6% fraction, with the exception of dieldrin and endrin. Some compounds, such as malathion and endosulfan, require elution of the Florisil column with 200 ml of 50% ether-petroleum ether following the 15% mixture for their recovery. Inclusion of a surfactant such as Span 80 in the Mills extraction procedure reduces losses of some pesticides due to binding on the tissues.[44]

Similar procedures used for the recovery of nonionic organochlorine residues in fatty foods are described in Section 211, Vol. I, of the FDA *Pesticide Analytical Manual.*[7] Included in the *Manual* are methods for animal and fish tissues,[45,46] cheese,[47] whole and dried eggs,[48,49] feeds and feeding materials, grains, milk,[50] oils, and oilseeds. For oils that form emulsions during petroleum ether-acetonitrile partitioning, partition chromatography of the petroleum ether extract on Florisil with 10% water-acetonitrile as the eluting solvent is an alternative method.[51] With some samples, additional cleanup, especially of the 15% ether fraction, may be required. This can be accomplished by alkaline hydrolysis with KOH followed by passage through a 10-g MgO-Celite column with elution by 100 ml of petroleum ether (or perhaps either treatment alone), elution through a Celite column treated with H_2SO_4, or passage through a second Florisil column. Other successfully used supplemental cleanup methods for fat samples include channel layer TLC[52,53] and use of an alkaline precolumn[54] in the GC determinative step.

In order to obtain more efficient cleanup by the Florisil column and recovery of a larger number of pesticides, a new elution system consisting of different mixtures of methylene chloride, hexane, and acetonitrile was developed[55] as replacement for the 6, 15, and 50% ethyl ether-petroleum ether eluents (which do not completely elute many phosphate pesticides). Table 1 shows the elution sequence of 50 chemicals known to be completely recovered. The system provided superior removal of fatty substances when cleanup of butter fat, corn oil, and a variety of other samples was tested.

Adsorbents other than Florisil have been used for the initial cleanup of fatty samples. Tolbert[56] employed a magnesia-Celite column eluted with acetonitrile-water (8:2) to purify chlorinated pesticides in oil. Holden and Marsden[57] obtained excellent results with a single-stage cleanup of hexane animal tissue extracts on partially deactivated alumina and silica columns. Columns containing 2 g of alumina, activated at 800° and deactivated with 5% H_2O, were most efficient for cleanup, while silica gel columns (120° activation, 5% H_2O) were more effective for the differential elution of pesticides with hexane. Kadoum[58,59] also used microcolumns of activated silica gel for cleanup of pesticides in animal and various other samples.

Low-temperature precipitation of fats, oils, and waxes in foods and biological sample extracts can be carried out on an improved apparatus,[60,61] and Micro Cel-E, a synthetic calcium silicate solid support with a large surface area, has been used to isolate chlorinated pesticides from large quantities

of fats and oils prior to cleanup by column chromatography.[62]

TABLE 1

Chemicals* Recovered 90% > Through Florisil Column, Using Methylene Chloride, Acetonitrile, Hexane Eluting Mixtures[55]

Eluent A: 200 ml of 20% Methylene Chloride in Hexane

Aldrin	Lindane
α-BHC	Mirex
β-BHC	Octachlor epoxide
Chlorbenside	Perthane
Chlordane (technical)	Polychlorinated biphenyls
o,p'-DDE	Quintozene (PCNB)
p,p'-DDE	Strobane
o,p'-DDT	*o,p'*-TDE
p,p'-DDT	*p,p'*-TDE
Dicofol (divides between A and B)	*p,p'*-TDE olefin
Heptachlor	Tecnazene (TCNB)
Hexachlorobenzene	2,3,7,8-tetrachlorodibenzo-*p*-dioxin
Isobenzan (Telodrin)	Toxaphene

Eluent B: 200 ml of 50% Methylene Chloride-0.35% Acetonitrile-49.65% Hexane

Bulan	Folpet (divides between B and C)
Carbophenothion	Heptachlor epoxide
CDEC	Methoxychlor
Dichlone	Octachlorodibenzo-*p*-dioxin (divides between B and C)
Dichloran	Methyl parathion
Dicofol (divides between A and B)	Ovex
Dieldrin	Parathion
Endosulfan I	Prolan
Endosulfan II	Ronnel
Endosulfan sulfate	Tetradifon
Endrin	
Ethion	

Eluent C: 200 ml of 50% Methylene Chloride-1.5% Acetonitrile-48.5% Hexane

Captafol (difolatan)
Captan
Diazinon
Folpet (divides between B and C)
Malathion
Octachlorodibenzo-*p*-dioxin (divides between B and C)
Sulphenone

*For formulas of pesticides mentioned throughout this review, the reader is referred to *Dictionary of Pesticides*, published yearly by Farm Chemicals, Meister Publ. Co., Willoughby, Ohio.

D. Analysis of Nonfatty Foods

The recommended method is that of Mills, Onley, and Gaither.[47,63] Extract pesticides from the sample with acetonitrile or 35% water-acetonitrile, filter, dilute an aliquot of the acetonitrile phase with water, and extract the pesticides into petroleum ether. Cleanup is on a Florisil column eluted with mixed ether-petroleum ether as above. Fractions are concentrated prior to GC analysis. Section 212 of the FDA *Pesticide Analytical Manual*[7] describes in detail methods for high-moisture products, intermediate-moisture and dry products,[64-66] and fruits and other nonfatty samples of high sugar content.[67] A rapid screening method for 16 organochlorine pesticide residues in wheat has been reported in which extraction is by ball-milling with 3% ethyl ether in hexane and cleanup is on a Florisil column (2% H_2O) eluted with 1% ether in hexane.[68]

Saha[69] determinated chlorinated pesticides in wheat by electron-capture GC as above but with cleanup on a 15-g magnesia-Celite column eluted with 250 ml of benzene-hexane (15:85). Sissons et al.[70] extracted chlorinated residues from a wide range of vegetables with acetone-hexane and cleaned up the pesticides on Nuchar-Attaclay or alumina prior to EG-GC. Interferences from certain vegetables are best removed on a column of $AgNO_3$-treated alumina.[71]

E. Micromethod for Determination of Chlorinated Pesticides in Human Tissues

A method using 1.6 g of Florisil in a size B Chromaflex (Kontes) column was developed by H. F. Enos but never published in the open literature. The method has been widely tested and is described in detail in Section 5A2a,b of *Analysis of Pesticide Residues in Human and Environmental Samples.*[13] Samples (500 mg) of liver, kidney, bone marrow, adrenal, and gonads are extracted three times with 2.5-ml portions of acetonitrile, centrifuging each time and collecting the supernatants in a 25-ml round-bottom test tube. Add 7.5 ml of 2% Na_2SO_4 solution, mix, and extract three times with 2-ml portions of hexane. Combine the extracts in a 10-ml evaporative concentrator, evaporate to 300 μl under a micro-Snyder column,[16] and fractionate the pesticides on the Florisil column by elution with 12 ml of hexane followed by 12 ml of 1% methanol in hexane (fraction 1, contains aldrin, heptachlor, DDE, DDT, heptachlor, and PCB's) and then 12

additional ml of 1% methanol in hexane (fraction 2, contains dieldrin, endrin, ethyl and methyl parathion, ronnel, etc.). Several pesticides, including α-BHC, lindane, diazinon, and DDD, split between fractions. The fractions are concentrated as required and analyzed by EC-GC.

Analysis of brain tissue requires incubation (for 30 min at 60 to 65°C) of the combined concentrated hexane extracts with 0.3 ml of acetic anhydride and 0.3 ml of pyridine, addition of 9 ml 2% Na_2SO_4, and reextraction with 2- to 3-ml portions of hexane prior to concentration and chromatography on Florisil.

The present author has found that addition of 25 ml of 2% Na_2SO_4, instead of 7.5 ml, and extraction with 5 ml of hexane followed by two 2-ml portions of hexane, affords better recoveries of several pesticides, including aldrin and BHC.

F. Analysis of Human Blood or Serum

A rapid survey method for chlorinated insecticides and related materials in blood was published by Dale et al.[72] Modified procedures were published in Volume III, Section HE 213.2 of the FDA *Pesticide Analytical Manual,*[7] and Section 5A3a of *Analysis of Pesticide Residues in Human and Environmental Samples.*[13] Two ml of serum is extracted with 6 ml of hexane in a round-bottom tube on a low-speed rotating mixer. If an emulsion forms, the layers are separated by centrifugation. A 5-ml aliquot of hexane is transferred to a concentrator tube connected to a micro-Snyder column. The extract is concentrated on a steam bath to a volume consistent with the expected pesticide concentration. Minimum detectable limits are 1 to 2 ppb for common chlorinated pesticides.

A more recent method for residues in whole blood makes use of acetone-hexane (1:9) extraction of acidified samples and batchwise cleanup of the concentrated extracts with silica gel prior to GC analysis.[73]

G. Monitoring Human Exposure to PCP and HCP

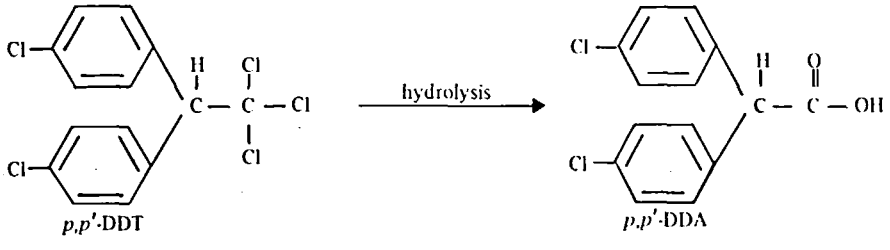
Pentachlorophenol (PCP) and its sodium and copper salts are widely used as wood dressings, herbicides, and antimicrobics. Hexachlorophene (HCP) is a foliage fungicide, plant bactericide, soil fungicide, and acaracide. Human exposure to these compounds has been monitored by GC analysis of blood and urine. Cranmer and Freal,[74] Bevenue et al.,[75] and Rivers[76] determined PCP in human urine and blood after formation of alkyl ethers, and Barthel el al.[77] injected underivatized PCP onto a GC column containing 3% DEGS + 2% H_3PO_4. HCP in urine, blood, tissues, and agricultural samples has been determined by EC-GC after methylation[78,79] or silylation,[80] and without derivatization.[81]

A method for determining PCP and HCP in human adipose tissue of the general population at the 5- and 10-ppb level, respectively, is based on the use of their ethylated derivatives.[82] Tissue (200 mg) is ground with 1 ml of hexane, 0.5 ml of 10% NaOH is added, and the sample is centrifuged. The hexane layer and two more hexane extracts (1.5 ml) are discarded. Concentrated HCl (0.5 ml) is added and three extractions are made with 1.5-ml portions of ether. The combined extracts are ethylated with diazoethane for 20 min, evaporated, and extracted with hexane. The hexane extract, containing ethylated PCP and HCP, is transferred to a silica-gel microcolumn (1 g, 1% H_2O) and eluted with 8 ml of 10% benzene-hexane (elutes PCP) and 8 ml of 60% benzene-hexane (elutes HCP). Fractions are analyzed by EC-GC using a 2-ft x 1/4 in. 2% SE-30 column (HCP) and a 6-ft. x 1/4-in. 4% SE-30/6% QF-1 column (PCP), both at 200°C. Blanks must be run through the entire procedure with each set of samples and standards.

Low levels of PCP (17 to 52 ppb) were found in all general population samples analyzed by this procedure, and HCP (10 to 80 ppb) was found in most samples. The effects of these low levels of exposure are not presently known.

H. Determination of *p,p'*-DDA in Human Urine

A predominant metabolite of *p,p'*-DDT, namely bis(*p*-chlorophenyl)acetic acid (*p,p'*-DDA), is excreted in urine:



p,p'-DDT *p,p'*-DDA

The excretion level of this metabolite has been shown to be a sensitive indicator of exposure to *p,p'*-DDT. The metabolite is determined[83] by extracting urine three times with an equal volume of 2% acetic acid in hexane. The combined extracts are evaporated to near dryness so that no residual water or acetic acid remains. The dry extract is treated with BF_3-methanol reagent to convert free *p,p'*-DDA to its methyl ester. After heating at 50°C for 30 min, the reaction is quenched with water and the reaction mixture is extracted with three 5-ml portions of hexane. The volume of the combined hexane extracts is adjusted and the *p,p'*-DDA methyl ester is determined by EC detection after Florisil cleanup or by microcoulometric detection without cleanup. DDA elutes in the second fraction from a Florisil microcolumn (Section E, above).

An OV-210 column at 175 to 180°C is recommended for GC analysis. Urine from high-exposure donors will contain a small amount of *p,p'*-DDE, which will be completely separated from DDA on this column. Urine from general population donors will yield as little as 8 ppb of DDA.

I. Determination of Chlorophenoxy Herbicides

A method[84] has been developed for the determination of trace quantities of 2,4-D, 2,4,5-T, and their degradation products, 2,4-dichlorophenol and 2,4,5-trichlorophenol, in human and rat urine. The procedure involves acid hydrolysis of the phenolic conjugates, extraction of the free phenols and acids, ethylation with diazoethane, cleanup of the derivatized urine extract on a 1-g column of silica gel (1.5% water) eluted in turn with 20%, 40%, 60%, and 80% benzene-hexane, and EC-GC determination of the fractions using 20% OV-101 or 4% SE-30/6% QF-1 (or OV-210) columns at 175°C. Ethylated 2,4-DCP and 2,4,5-TCP elute in the 20% fraction, and 2,4-D and 2,4,5-T in the 60% plus 80% fraction.

Average recoveries of the herbicides and phenol degradation products from spiked rat urine were 90 to 94%, and limits of detectability 0.01 to 0.10 ppm. Urine samples from occupationally exposed people were analyzed and found to contain 0.2 to 1.0 ppm of 2,4-D and 0.05 to 3.6 ppm of 2,4,5-T.

The optimum conditions for the silylation of acidic herbicides have been studied.[85] The TMS esters are chromatographed on a 3% SE-30 column at 230°C, with detection by electron capture.

Errors introduced by basic reagents used to adjust the pH of the sample mixture during the cleanup and isolation steps of analyses of plants, animal tissue, and water have been studied.[86]

J. Alternative Selective Detectors for Chlorinated Pesticides

Coulson[87] developed an electrolytic conductivity detector capable of detecting organochlorine pesticides without interferences in low ng amounts. Combustion in the presence of a large excess of hydrogen produces HCl, which is determined by measuring the increased electrical conductance of a stream of water originally deionized by passing through an ion-exchange column. The platinum catalyst originally proposed is no longer used in the pyrolysis tube.[88]

Although the sensitivity of the detector is generally 5 to 10 times lower than that of the electron-capture detector, its superior selectivity promises to lead to its wider use for determining and identifying residues of chlorinated pesticides. The detector also responds selectively to nitrogen, sulfur, and phosphorus compounds under certain conditions, and most applications of this detector so far have been for the analysis of nitrogen-containing pesticides. Other selective detectors for chlorinated pesticides are the microcoulometric detector (next section) and copper-sensitized,[89] indium-sensitized,[90] and Na_2SO_4-sensitized[91] flame photometric detectors. A dual-flame FPD detector for simultaneous determination of P-, S-, and Cl-containing compounds has been constructed.[92]

V. DETERMINATION OF CHLORINATED HERBICIDES AND INSECTICIDES BY GC WITH THE MICROCOULOMETRIC DETECTOR

A. Operational Considerations

The microcoulometric (MC) detector is described in detail in Chapter Two of the Treatise by Zweig and Sherma.[6] Chlorine-containing pesticides in the GC column effluent are pyrolyzed under oxidative conditions to Cl^-, which is stoichiometrically and automatically titrated with Ag^+ in a constant-concentration coulometric cell. The

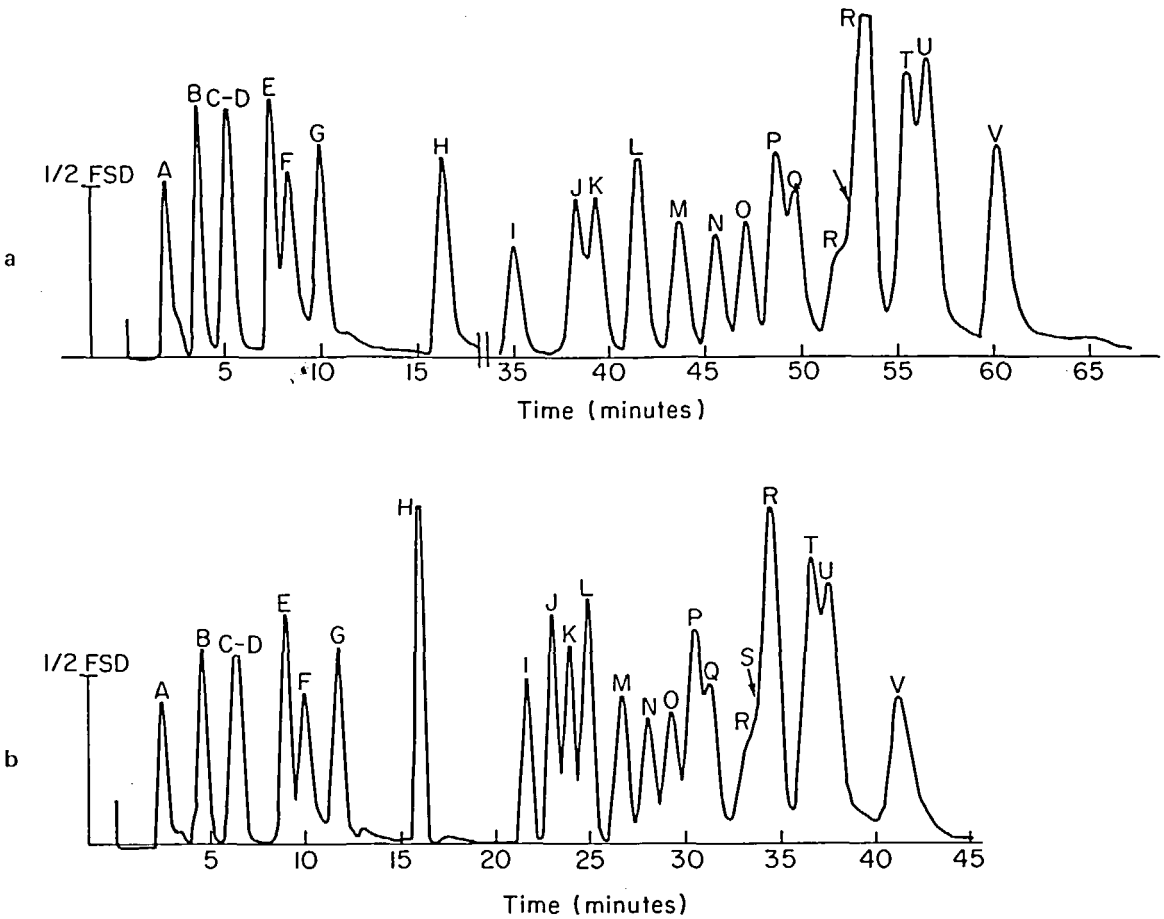


FIGURE 2. (a) Linear programmed-temperature chromatogram of a mixture of 22 pesticides. Column: 20% DC-200 on 30 to 60 mesh acid-washed Chromosorb P, 6 ft x 3/16 in. i.d.; injection port 250°C, column started at 50°C and programmed at 3.7°C/min; nitrogen flow rate 120 ml/min; microcoulometric detection. (b) Nonlinear programmed-temperature chromatogram of the same mixture under the same conditions except that the column started at 50°C and was programmed at 3.7°C/min to 95°C, 30°C/min between 95° and 200°C, and 3.7°C/min to completion. Compounds: (A) methylene chloride (0.75 μg), (B) ethylene dichloride (2.25 μg), (C) 2,3-dichloropropene (1 μg), (D) dichloral urea (1.25 μg), (E) ethide (2 μg), (F) ethylene dibromide (4 μg), (G) monochlorobenzene (2.4 μg), (H) *p*-dichlorobenzene (2.4 μg), (I) TCNB (1 μg), (J) Isopropyl ester 2,4-D (3 μg),(K) lindane (1 μg), (L) isobutyl ester 2,4-D (4 μg), (M) heptachlor (1 μg), (N) aldrin (1 μg), (O) heptachlor epoxide (1 μg), (P) Ovex (5 μg), (Q) DDE (1.5 μg), (R) DDT (4 μg), (S) Trithion (5 μg), (T) methoxychlor (7.5 μg), (U) Tedion (4 μg),(V) Co-Ral (20 μg). Reproduced with permission from Burke, J. A., *J. Assoc. Offic. Agr. Chem.* 46, 198, 1963.

detector can also be made selective to P, S, or N under various conditions with the addition of certain scrubber tubes to eliminate interferences. In general, the MC detector is sensitive to 1 ng of chlorine, which is about 10 times less sensitive than the electron-capture detector.

Because of this lower sensitivity, larger samples, greater concentration of the purified extract (to <1 ml), and injection of larger volumes (25 to 50 μl) are usually required. Cleanup procedures must accommodate the larger samples, but because of increased specificity, cleanup often does not have to be as rigorous as for electron-capture detection. Both the microcoulometric and the electrolytic conductivity detector require more maintenance and operator attention than the electron-capture detector. In one comparative study,[93] the electrolytic conductivity detector was preferred to the microcoulometric detector from the standpoints of simplicity, economy, sensitivity, and peak shape for pesticide residue analysis.

The response of the MC detector is linear, it operates over a wide temperature range, and its sensitivity is not affected by carrier gas flow rate, column bleed, or changes of temperature, The detector is, therefore, satisfactory for use with temperature programming whereas the electron-capture detector is not (see Figure 2). Isothermal

GC is most often used, however, and the FDA[7,94] recommends the following conditions for the MC halogen detector (Dohrmann Instrument Co., Model C-100; halogen cell, Model T-200-S): Aluminum or glass columns 6′ x ¼″, packed with 10% DC-200 or 15% QF-1/10% DC-200 (1:1) on 80-100 mesh Gas-Chrom Q or 90-100 mesh Anakrom ABS, 210°C column, 240° injection, 250° transfer line and combustion inlet, 800° minimum furnace, 120 ml/min nitrogen carrier gas, 75-100 ml/min O_2, 50-100 ml/min nitrogen sweep gas. Retention data for numerous pesticides under these conditions have been published.[95,96] The general level of sensitivity with this technique is about 0.01 ppm based on 10-25 g of original sample.

B. Determination of Chlorophenoxy Acids in Foods

Herbicide residues are extracted with acetonitrile (nonfatty foods) or chloroform (fatty foods and liquids), the acids are converted to their sodium salts, acidified, methylated with diazomethane[97] or dimethyl sulfate,[98] chromatographed on a Florisil column, and determined by isothermal EC- or MC-GC. Severe emulsions form during cleanup of the basic phase by extraction with chloroform. The phases must be separated by centrifugation.[99] This procedure[100] is a modification of PTGC methods reported earlier for fatty foods and nonfatty foods[101,102] which did not include Florisil chromatography. The 10-g Florisil column is eluted with 100 ml of 20% methylene chloride in petroleum ether to recover PCP, if present, and with 2.5% ether in petroleum ether for the methylated acids. Columns containing 10% DC-200 or 10% DC-200/15% QF-1 (1:1) were employed. With electron-capture detection, 1 ng PCP, 2,3,6-TBA, 2,4,5-T, and 2,4,5-TP, and 4 ng 2,4-D and 2,4-DB were detected. MCP was not detected at low levels. Thirty ng of each compound was detected by the microcoulometric detector. Residues were satisfactorily recovered from dairy products, meat, fish, poultry, grains, cereals, fruits, vegetables, sugar, and beverages at 0.01-0.05 ppm levels.

Other polar solvents useful for extraction of free acids and esters from plant material by grinding, maceration, or blending include ethanol, acetone, 2-propanol, and acidic ether. Subsequent cleanup steps generally involve several partition steps with organic solvents for esters and acid-base extractions for free acids, followed by esterification in the latter case. Free acids (and phenols) are normally too polar to be gas chromatographed without esterification, for adsorption on the GC column would lead to tailing and reduced peak heights.

C. Determination of Insecticide Residues in Crops and Foods

Chlorinated insecticide residues in four different crops were determined[103] by MC-GC after extraction and cleanup by a batch (non-column) procedure with acid-washed Norit-A. Average recoveries of 13 compounds in four crops ranged between 66 and 104%.

Organochlorine insecticide residues in raw and canned beef and cheese fat were determined by a method in which the sample was dissolved in petroleum ether and mixed with Celite® 545 to give a free flowing powder after removal of solvent. A column was packed with this Celite-fat sorbent, and elution onto a Florisil column was carried out with dimethylsulfoxide. The Florisil column was then eluted with petroleum ether, and this final eluate concentrated and analyzed by MC-GC.[104]

VI. GAS CHROMATOGRAPHIC DETERMINATION OF ORGANOPHOSPHATE* PESTICIDES

A. Detectors

The electron-capture detector will respond to many phosphate pesticides,[38,105] but generally the sensitivity is 15 to 30 times lower than for an equivalent amount of aldrin. Some chlorinated phosphates, such as ronnel, are detected with comparable sensitivity. Phosphate residues in fatty and nonfatty foods cleaned up by elution through Florisil (Sections IV-C and D, above) can be determined using this detector, for example, malathion in the 50% ether-petroleum ether fraction. However, the poor selectivity of this detector strongly contraindicates its use for this purpose.

*The general term *phosphate* is widely used to denote pesticides of all types containing phosphorus, including phosphites, phosphonates, phosphoramides, pyrophosphates, phosphates, and their sulfur analogs. This nomenclature will be used throughout this review for all phosphorus-containing pesticides.

One reason is that organophosphate residues are relatively polar and require polar solvents for elution from cleanup columns. Interfering materials may also be eluted so that the selective detectors described below are preferred.

A microcoulometric detector selective for phosphorus was described by Burchfield et al.[106] Reductive pyrolysis of the pesticide at 950° produces phosphine, which is coulometrically titrated with silver ions to form silver phosphide. A Pyrex scrubber tube containing alumina is placed before the titration cell to remove hydrogen chloride and hydrogen sulfide, which would otherwise interfere. Very little work has been reported concerning the use of this detector for residue analysis.

The KCl thermionic detector[107-109] (alkali flame detector), a hydrogen flame ionization detector incorporating a KCl-coated wire helix positioned concentrically around the flame, produces an enhanced, selective response for phosphorus. This detector, or one or another modified version of it, has been widely used and is still recommended by FDA[7] as the method of choice for the analysis of phosphate residues. Aue[110] has compared the thermionic detector with the flame ionization and flame photometric detectors for pesticide analysis. The author's experience indicates that the flame photometric detector is about as sensitive as the thermionic detector for most phosphate residues and that it is generally more reproducible when actual samples are analyzed on a routine, daily basis. It is predicted that the flame photometric detector will become the clear choice as the primary phosphorus pesticide detector.

Chromatographic parameters for the thermionic detector are similar to those stated above for electron-capture detection. The detector should provide a sensitivity of ½ full-scale recorder deflection for 2 ng parathion. Retention times and sensitivities of some 70 organophosphorus pesticides on 10% DC-200, 10% DC-200/15% QF-1 (1:1) and 2% DEGS columns using the thermionic detector have been reported.[111]

Some 20 organophosphorus pesticides contain nitrogen and could probably be selectively detected by the nitrogen electrolytic conductivity detector.

B. Flame Photometric Detector (FPD)

1. Characteristics

The FPD detector of Brody and Chaney[112] (commercially available from Tracor, Inc., as the "Melpar FPD") operates by monitoring emission bands, which result from burning the effluent in a hydrogen-rich flame, at 526 nm (phosphorus) and 394 nm (sulfur) by a combination of a narrow band-pass interference filter and a suitable photomultiplier tube. Samples require relatively little cleanup because of the selectivity of the detector (factor of 4 or 5 orders of magnitude) for compounds containing P or S.[113,114] The FPD detector is compatible with temperature programming, although most residue analyses have been done under isothermal conditions.

The flame will blow out after each injection unless the solvent is vented, and a silylated Valco valve, No. CV-8HT, is recommended for this purpose. The minimum detectable quantities of S and P are 200 pg and 40 pg, respectively.[115] In routine operation, 400 pg of ethyl parathion should yield a peak height equal to half of full-scale recorder deflection, although the sensitivity can usually be improved well beyond this in most analyses by careful adjustment of operating parameters.

Sulfur impurities can cause a response in the phosphorus mode of operation while phosphorus compounds cause negligible response in the sulfur mode. Maximum utility of the FPD is afforded by a dual photomultiplier arrangement whereby P and S are simultaneously monitored. This arrangement informs the analyst whether a compound contains only P or S, or both, and the P/S ratio is important information for confirmation of identity. The FPD response is linear over a concentration range of about three to five decades; for example, 0.4 to 400 ng for parathion, 526 nm filter. Three-channel operation of a dual FPD has been proposed[92] whereby S (394 nm) and P (526 nm) compounds would be detected and Cl compounds (360 nm) after reaction with In to form InCl.

2. Operational Considerations

The temperature gradient between the column and detector must be kept as low as possible, but the detector cannot be overheated. The detector is always heated before the column. The detector is never heated above 200°C (high temperature model) or 160°C (low temperature model) or the plastic sleeve of the photomultiplier tube may be damaged. The detector base is heated to about 210°C. The GC inlet port is maintained at about

210°C and the transfer line (Valco valve) is about 240°C.

The voltage of the PM tube and the flow rates of the various gases are selected to give the best overall signal, taking into account detector response (peak height), noise, stability, and ease of ignition of the flame. Attenuator settings are usually selected to yield a noise level which is 1% of full scale. The hydrogen flow should be between 150 to 200 ml/min, oxygen at 50 ml/min for initial flame ignition, and air between 30 to 40 ml/min. The column and purge (make-up) nitrogen flows are equal, about 80 ml/min. After ignition, the oxygen and air flows are optimized by injecting an appropriate amount of an early eluting phosphate pesticide, trying different oxygen-air mixtures. The total flow of the air, oxygen, and carrier gas should not exceed 200 ml/min, and a lower total usually produces the most favorable signal-to-noise ratio. The rate of flow of oxygen is typically about a fourth of that of the nitrogen carrier gas. For example, a carrier flow of 80 ml/min may require an oxygen flow of 20 ml/min; the air flow is governed by the size of the flame desired and the 200 ml/min total flow limit. Suggested flow rates to be tested during set up, with hydrogen at 180 ml/min, nitrogen carrier and purge equalized at 80 ml/min, and PM tube voltage at 750 V are oxygen 40 ml/min, no air; oxygen 40, air 40; oxygen 40, air 60; oxygen 20, air 60 and 80; oxygen 10, air 60 and 80. After the optimum air-oxygen ratio is chosen, the voltage is varied on the PM tube to obtain the best overall signal. Different tubes will require different voltages, a value around 810 V being typical.

3. Chromatographic Conditions

Carbowax® treatment[116] of GC columns has been found to improve the sensitivity of the FPD for phosphate compounds by minimizing adsorption and decomposition caused by active sites on the support. Treatment of columns with Silyl-8 should not be used with the FPD.

The Carbowax treatment is accomplished by removing the silanized glass wool plug and 2 in. of column packing from the inlet leg after heat curing, adding a small plug of glass wool and 2 in. of 10% Carbowax on Gas Chrom Q or Chromosorb® W-HP, 80 to 100 mesh, and topping with another plug of glass wool. The column is conditioned for 16 to 17 hr at 230 to 235°C, the Carbowax packing and glass wool plugs are removed, and the original packing and a fresh glass wool plug are returned to the column. Alternatively, a 3-in. section of 1/4-in. glass tubing containing 2 in. of Carbowax packing and plugs of glass wool at the ends can be used as an extension which is connected, through a drilled-out ¼-in. Swagelok male union, to the inlet side of the column to be treated. The other end of the extension is Swagelok-fitted to the oven connection for conditioning in the chromatograph.

This treatment has been found to improve the responses of OV-210 columns, especially to malathion and methyl parathion. Retention times and relative response data for a series of phosphate pesticides on a Carbowax-treated 4% SE-30/6% QF-1 column at 200°C are shown in Table 2.[117] This column is a primary column for general analytical work with the FPD detector. Beroza and Bowman reported retention times[118] for 20 phosphates on 5% columns of DC-200, QF-1, DC-710, and DEGS, and for over 100 compounds[119] on 5% columns of OV-101, OV-17, OV-210, and OV-255 using the FPD detector. The same authors[120] found the thermally stable liquid phase Dexsil 300, coated (5%) on an HCl-washed Chromosorb W support, to be well suited for multiresidue analysis using the FPD (S and P modes). Interfering substances, accumulated in the column because of injection of large samples with minimum cleanup, could be removed without loss of the phase by purging the packing at 400°C. Retention times of 146 pesticides with temperature programming (150-300°C) were reported.

C. Cleanup Procedures for Phosphate Pesticide Residues in Food

1. Florisil Cleanup

A collaborative study[121] of the determination of seven phosphate residues in fruits and vegetables by the Mills-Onley-Gaither method (Section IV-D) gave average recoveries at the 0.5 ppm level of 88.7% (thermionic detector) and 91.2% (electron capture). However, it is reported[122-124] that only 23 of the 70 phosphate pesticides and metabolites tested through this procedure are recovered, and not all recoveries are complete. The AOAC has validated this method only for carbophenothion, diazinon, ethion, malathion, methyl and ethyl parathion, and ronnel in 18 crops.

Beckman and Garber[125] described a new elution solvent system for cleanup and recovery of

TABLE 2

Relative Retention Times and Responses at 200°C on a 4% SE-30/6% QF-1 Column

(Both the relative retention and the relative response refer to a value of 1.00 for ethyl parathion.)

Compound	Relative retention time	Relative response
Diazinon	0.35	2.45
Diazinon-oxygen analog	0.42	1.0
Ronnel	0.57	0.42
Ronnel-oxygen analog	0.58	0.25
Methyl Parathion	0.75	0.71
Methyl Parathion-oxygen analog	0.83	0.10
Malathion	0.81	0.71
Malathion-oxygen analog	0.85	0.063
Ethyl Parathion	(1.00)	(1.00)
Ethyl Parathion-oxygen analog	1.10	0.5
Ethion	1.83	0.71
Trithion	1.90	0.36
Trithion-oxygen analog	1.78	0.12
TEPP	0.08	5.0
Dichlorvos	0.10	5.0
Demeton-thiono	0.22	2.0
Naled	0.28	0.02
Phorate	0.28	4.0
Sulfotepp	0.28	5.2
Demeton-thiolo	0.37	2.0
Dioxathion	0.38	0.5
Disyston	0.40	3.8
Dimethoate	0.49	0.50
Azodrin	0.54	0.083
Dursban	0.68	1.4
Fenthion	0.72	1.56
Sumithion	0.85	0.80
Phosphamidon	1.02	0.16
Merphos	1.23	0.35
DEF	1.25	0.80
Phenkapton	3.04	0.20
Dasanit	3.16	0.03
Imidan	3.91	0.02
EPN	3.95	0.134
Guthion	6.03	0.044
Coumaphos	11.84	0.20

phosphate pesticides on a Florisil column but did not test sample extracts. The pesticides were eluted in turn with 50 ml of benzene, 50 ml of ethyl ether-benzene (1:2), two 50-ml portions of acetone, and 50 ml of methanol. The elution pattern and recoveries of 65 compounds analyzed by thermionic GC (GC columns: 2% SF-96, 2% SE-30; 2% DC-11; 7.5% QF-1/5% DC-200) have been reported.[125]

A comparision of cleanup columns for phosphate pesticides indicates that "all-Florisil" columns are not the best choice, although the Beckman-Garber method was found to be applicable to methyl and ethyl parathion, malathion, malaoxon, and paraoxon residues in apples and lettuce.[126]

2. Sweep Codistillation

Phosphate residues in fruits and vegetables are extracted from the prepared sample by a single

blending with ethyl acetate; an aliquot of extract is concentrated and cleaned up by injecting it into a heated glass tube 24-cm long (Storherr tube[127]) packed with glass wool, followed by repeated injections of ethyl acetate at 3-min intervals. Nitrogen carrier gas sweeps vaporized volatile components through the packed column to the condensing bath and thence through a short Anakrom scrubber tube to a concentrator collection tube. The collected sample is analyzed by thermionic or FPD GC; the cleanup is probably not suitable for electron-capture detection. Recoveries of phosphate pesticides from milk,[128] edible oils,[129] and soybeans, and cottonseed have also been achieved by this technique. Sweep codistillation is especially advantageous for high fat samples.[122]

3. Comparison of Extraction Procedures

Of nine procedures evaluated[130] for the removal of six phosphorus insecticides and metabolites from field-treated crops, Soxhlet extraction of the finely chopped crop with chloroform-10% methanol for whatever time was necessary proved most reliable and efficient. Analysis of extracts was with the FPD detector without cleanup.

4. Alumina Cleanup

A series of phosphorus insecticide residues was extracted from vegetable samples with acetone-hexane (1:4) and cleaned up by a batch procedure with Nuchar-Attaclay and then on a 30-cm column of Brockmann grade V alumina eluted with 30 ml of hexane and 80 ml of 2% acetone in hexane. Analysis was by thermionic GC using 3% columns of OV-17, OV-210, and OV-225. Recoveries ranged between 70 and 96%.[131] The method was not successful when applied to the more polar organophosphorus insecticides. Using alumina (activity II to III) and petroleum ether and 3% acetone-petroleum ether as elution solvents, Renvall and Akerblom[132] eluted only 13 of the 31 organophosphorus compounds they tested.

5. Charcoal Cleanup

A promising general method for organophosphorus pesticide residues in nonfatty foods, developed by Watts et al.[133] and modified by Storherr et al.,[134] is based on the FDA acetonitrile extraction procedure for chlorinated compounds,[7] followed by dilution with methylene chloride to free extracted water, cleanup on a short charcoal column, and analysis by thermionic or FPD-GC. The chromatographic tube (300 x 22 mm i.d.) was packed dry with 1 g of Celite® 545 and 6 g of adsorbent mixture (acid-treated Norit SG Extra charcoal-Sea Sorb 43-Celite 545, 1:2:4) and topped with glass wool. The eluting solution was acetonitrile-benzene (1:1). Forty-one pesticides, including fourteen alteration products, were satisfactorily (85 to 105%) recovered from kale.

Several other cleanup procedures employing columns containing charcoal;[135] charcoal, Florisil, and Celite;[136] charcoal, Celite, alumina, Attapulgus clay, and Na_2SO_4;[137] charcoal, magnesium silicate, Attaclay, Celite, and Na_2SO_4;[138] charcoal and Solka Floc;[139] and charcoal and silica gel[140] have been described for multiresidues of organophosphate pesticides. Since several of these cleanup procedures are also suitable for chlorinated pesticides, columns containing carbon have great potential for use in development of multiresidue procedures for these two chemical classes, although preparation of mixed columns can lead to problems of reproducibility.

6. Cleanup by Partitioning

Methods[141] for analyzing residues in seven commodity groups (cereals, meats and fish, fruits and preserves, root vegetables, green vegetables, fats, and milk) constituting a total diet included cleanup by solvent partitioning in most cases. Determinations were by CsBr thermionic GC[142] on columns containing either 1.3% Apiezon L and 0.1% Epikote® 1001 or 1.3% butane-1,4-diol succinate and 0.1% Epikote 1001. Thirty-nine pesticides and some metabolites were recovered (70% or better) with a sensitivity of about 0.01 ppm for most compounds.

Kadoum[134] reported the extraction of ethyl and methyl parathion, diazinon, malathion, and Thimet from plant, animal, water, and soil samples by hexane, cleanup by partitioning with aqueous acetonitrile, and analysis by electron capture GC.

D. Elution Pattern of Intact Organophosphates from Silica Gel

The elution pattern of a series of representative phosphate pesticides from a column (Kontes, Size 22) containing 1 g of Woelm silica gel (1.5% water), prewashed with 8 ml of hexane before applying the sample mixture, is as follows:

Eluent	Pesticides eluted
7 ml *n*-hexane	–
8 ml 60% benzene-hexane	Trithion
8 ml benzene	Ethyl parathion
8 ml 8% ethyl acetate-benzene	Malathion, diazinon
8 ml 50% ethyl acetate-benzene	Paraoxon

The presence of compounds in certain fractions plus GC retention times and use of the FPD detector can provide sufficient information for identification of pesticides in various samples.

Kadoum[58,144] reported the use of silica gel for the determination of seven organophosphates in animal, plant, soil, and water extracts, and Bowman et al. have used silica gel extensively (e.g., see Reference 145) to separate parent organophosphate pesticides and metabolites into groups to facilitate identification by GC.

E. Analysis of Alkyl Phosphates

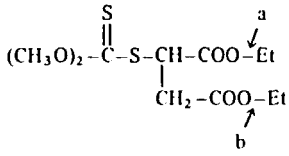
The metabolism and urinary hydrolysis of organophosphate pesticides in mammals result in the excretion of a variety of alkyl phosphates. For example, Azodrin, Gardona, Bidrin, and Phosdrin yield DMP (*O,O*-dimethyl phosphate); Abate, ronnel, and fenthion yield DMTP (*O,O*-dimethyl thiophosphate); Cygon, Guthion and malathion yield DMDTP (*O,O*-dimethyl dithiophosphate); paraoxon and TEPP yield DEP (*O,O*-diethyl-phosphate); coumaphos, Systox, and diazinon yield DETP; and Di-Syston, Thimet, and ethion yield DEDTP. The GC separation and quantitation of these products may be of value in estimating the extent of exposure to the parent pesticide.

The procedure involves acidification of 2 ml of urine with 6 N HCl, extraction of metabolites and hydrolysis products with acetonitrile-ether (1:1), treatment of an aliquot with diazopentane to form volatile trialkyl phosphates, cleanup of the derivatives on a silica-gel column (2 g of gel containing 1.0 to 1.5% H_2O), and GC determination using a dual FPD detector in the P and S modes.

The silica-gel column is eluted with 20 ml of methylene chloride (which elutes amylated DMTP, DETP, DMDTP, and DEDTP), 10 ml of methylene chloride-ether (1:1) which elutes only impurities and should be discarded), and 20 ml of ethyl acetate (which elutes amylated DMP and DEP). A GC column containing 5% OV-210 and operated at 165-175° with a rate of flow of nitrogen carrier gas of 30 to 40 ml/min is suitable for determination of the amyl derivatives. Details are given in Section 6A2a of the *Analysis of Pesticide Residues in Human and Environmental Samples*[13] for this improved version of earlier published procedures.[146-148] The average recoveries of six dialkyl phosphates at the 0.1-ppm level were 98.3% by this method. The excretion of alkyl phosphates in the urine can, therefore, be detected at organophosphate exposure levels much lower than those which result in cholinesterase inhibition. The general class of organophosphate compound involved in the exposure may be deduced by characterizing the metabolite(s) excreted.

F. Determination of Malathion Mono- and Dicarboxylic Acids

The preceding determination of alkyl phosphates is not preferred for monitoring human exposure to the important phosphate insecticide malathion because this pesticide follows another metabolic pathway. Alkyl phosphates are only minor metabolites of malathion,[149] the major metabolites being the mono- and dicarboxylic acids formed after carboxyesterase hydrolysis at *a* and/or *b* to yield either monocarboxylic acid (MCA) or dicarboxylic acid (DCA):



The carboxylic acids of malathion may be determined in urine by the following modification of the alkyl phosphate procedure.

The carboxylic acids are determined[149] by preextracting an aliquot of urine with ethyl ether to recover any intact malathion, followed by acidification and extraction as described above for the alkyl phosphates. The extract is then either methylated or ethylated; the former permits the determination of both carboxylic acids while the latter converts the acids back to malathion. The derivatives can be eluted from silica gel by 10 ml of benzene followed by 10% of ethyl acetate-benzene. The eluents are collected together and analyzed on a 6 ft 4% SE-30/6% QF-1 column at 200°C with a dual-mode FPD. Alternatively, the urine extract is treated with diazopentane and the amyl derivatives determined on a 5% OV-210 column at 200°C with a nitrogen flow rate of 70 ml/min. Retention times for malathion, the amyl

ethyl ester, and the diamyl ester are approximately 4, 8, and 16 min under these conditions. Quantitative recoveries were obtained from rat urine spiked to 0.01-1.0 ppm. Because of the similarities in the methods, both the alkyl phosphates and carboxylic acids of malathion can be determined in one urine sample.

Metabolite	Intact pesticide
p-nitrophenol (PNP)	EPN, Ethyl and Methyl Parathion, TOK, Preforan
p-nitrocresol (NC)	Fenitrothion
2-chloro-4-nitrophenol (C-4-NP)	Dicapthon
2,5-dichloro-4-bromophenol (D-4-BP)	Methyl and Ethyl Bromophos, Phosvel
3,6-dichloro-4-iodophenol (D-4-IP)	Ciba-9491
3,5,6-trichloro-2-pyridinol (T-2-P)	Ethyl and Methyl Dursban
2,4-dichlorophenol (DCP)	VC-13, Zytron, Supona, Genite, TOK, 2,4-DEP
2,4,5-trichlorophenol (TCP)	Ronnel, Lindane, Agritox, Gardona, 2,4,5-T, Silvex, Erbon

A method has been devised for the determination of these phenols together with PCP: DNOC (a dinitrophenol insecticide, fungicide, and herbicide used as the sodium salt); and the phenoxyacid herbicides 2,4-D, 2,4,5-T, and silvex in urine.[150] A 1- to 5-ml sample is treated with one-fifth of its volume of concentrated hydrochloric acid, and the mixture is refluxed at 100°C for 1 hr. The phenols are extracted with ethyl ether, ethylated with diazoethane, and concentrated, and the ethyl ethers are chromatographed on a 2-g silica-gel column (2% H_2O). The column is eluted with 8 ml of 20% benzene-hexane followed in turn by 10 ml each of 40, 60 and 80% benzene-hexane and finally by 10 ml of benzene. The first fraction contains DCP, T-2-P, TCP, D-4-BP, D-4-IP, and pentachlorophenol (PCP). The concentrate of this fraction is analyzed on a 4% SE-30/6% OV-210 column at 175°C, using 60 ml/min of nitrogen and an EC detector; the pesticides are eluted in 10 min in the order listed above. The 40% fraction is discarded and the 60 and 80% fractions are combined, concentrated, and analyzed under the same GC conditions. PNP, *p*-NC and C-4-NP, 2,4-D, silvex, 2,4,5-T, and DNOC are contained in this fraction and separated on the GC column in this order. Some DNOC may also be found in the final benzene fraction. Recovery was found to be 85 to 98% at 0.01 to 0.1 ppm, with a sensitivity of 0.01 to 0.3 ng for the individual phenols.

G. Determination of Halo- and Nitrophenols in Urine

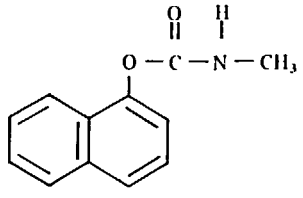
Another approach to the problem of determining exposure to organophosphorus and other pesticides involves determination of halo- and nitrophenol metabolites. A list of pesticides and their metabolites is shown below:

H. Determination of Parent Pesticides and Metabolites as a Single Compound

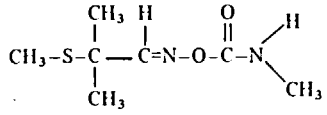
Residues of fenthion, disulfoton, and phorate may each consist of the parent insecticide and five metabolites formed by oxidation of thionophosphate and sulfide groups in each molecule. A fast, simple approach to approximating the total residues of these compounds is offered by oxidizing the insecticide and any metabolites to the oxygen analog sulfone with *m*-chloroperbenzoic acid. After removal of the acid on an alumina column, the sulfone is determined by FPD-GC (526 nm) with a sensitivity of 0.001 ppm.[151]

VII. GAS CHROMATOGRAPHIC DETERMINATION OF CARBAMATE INSECTICIDE RESIDUES

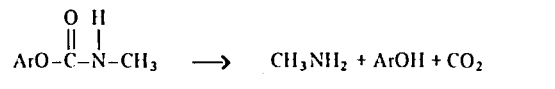
Important carbamate insecticides include the aromatic N-methyl carbamates such as carbaryl



and the carbamoyl oxime Temik



This section will emphasize the determination of the aromatic N-methyl carbamates, which hydrolyze in the following manner:



Ar = aryl

Other important pesticidal carbamates are the N-phenyl carbamate herbicides, which hydrolyze to anilines. The determination of these compounds will be discussed briefly in a later section.

A. Detectors

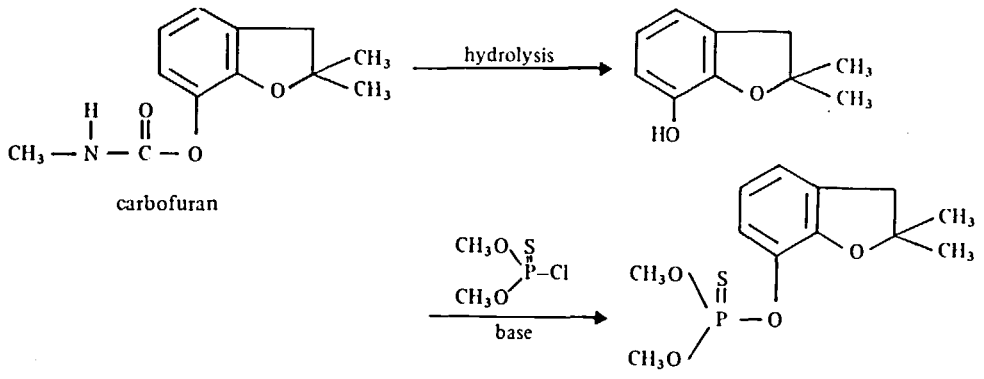
The first use of the microcoulometric detector for the specific determination of nitrogen compounds was reported in 1966 by Martin[152] for the analysis of petroleum mixtures. Nitrogen compounds were quantitatively pyrolyzed to ammonia in a stream of hydrogen over a nickel catalyst, and the ammonia was then titrated automatically with coulometrically generated hydrogen ions. A BaO scrubber tube removes interfering pyrolysis products. The operation of the detector for the determination of carbamate pesticides (detection range 3-200 ng nitrogen) was fully described by Cassil et al.,[153] and lower levels of detection are apparently possible if modifications are carried out.[154]

Coulson[155] used his electrolytic conductivity detector to determine carbaryl by measuring the increase of conductance due to the addition of the ammonia pyrolysis product to deionized water. A scrubber containing strontium hydroxide on quartz wool traps the interfering compounds HCl, H_2S, and PH_3. Patchett[156] has reported a detection limit of 0.1 ng nitrogen for this detector. The conductivity detector is less complex and requires less daily maintenance than the microcoulometric detector, and it is more sensitive for nitrogen. However, it requires somewhat cleaner samples and lacks the stoichiometric capability of the MC detector, which allows direct calculation of the amount of material coulometrically titrated. The electrolytic conductivity detection of *s*-triazine, urea, and carbamate herbicides has been evaluated.[157]

A thermionic detector modified[158,159] by adjusting the position of the collector electrode above the flame and by use of a rubidium salt has a selective response for nitrogen compared to hydrocarbons but little selectivity compared to phosphorus. A detector with an Rb_2SO_4 bead has been used for determinations of *s*-triazine herbicides at 0.2-1.0 ng levels,[158,160] and a detector with a KCl-$RbSO_4$ (1:1) tip was used to determine about 30 ng of carbamate herbicides.[161] A comparative study[162] of the RbCl thermionic detector and the conductivity detector indicated that their responses to nitrogen compounds were of the same order (100 to 200 ng substituted ureas, 7 to 15 ng triazines, 50 to 80 ng carbamates, or 35 to 50 ng thiolcarbamates, for half of full-scale deflection[157]), but the use of the conductivity detector was recommended because of its superior selectivity and ease of operation.

Carbamates containing sulfur can be determined using detectors selective for this element: the microcoulometric sulfur detector[163] and the FPD[164] in the sulfur mode have been used to determine Temik and its metabolites in sugar beets, and residues of Mesurol and Ciba®-10573 in apples were determined by means of a microwave emission detector[165] monitoring the emission due to atomic sulfur.

Many indirect methods have been devised in which intact carbamates or their hydrolysis products are converted to appropriate derivatives for sensitive electron-capture detection, and some of these are discussed in section VII C below. Using a similar approach, Bowman and Beroza[166] effected the alkaline hydrolyses of carbofuran, carbaryl, Baygon, and Banol and coupled the resulting phenols with dimethyl chlorothiophosphate, thus introducing a thiophosphoryl group permitting determinations of quantities below 0.5 ng by FPD-GC in the phosphorus mode (see equation on top of the following page). The hydrolyzed phenol was separated by steam distillation before coupling. The same authors determined Mabam[167] by hydrolysis to the phenol by means of a phosphoric acid plug placed at the head of the GC column. A similar procedure was used for Mesurol and five of its metabolites[168] after hydrolysis with sodium hydroxide. Since each of these carbamates contains sulfur, no further derivatization was required for detection by the FPD detector (S mode).



In summary, the electrolytic conductivity detector at present holds the greatest promise as the primary nitrogen-selective detector for residue analysis. However, analytical procedures for carbamates and metabolites based on derivatization and electron-capture detection will continue to be most popular because of their sensitivity and the specificity built into the procedures by the presence of the derivatization and liquid chromatographic steps. If a sensitive nitrogen-selective FPD detector is developed in the future, the situation might be drastically changed.

B. Direct Methods of Analysis

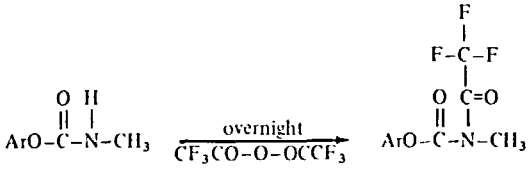
Determinations of intact underivatized carbamate residues by GC are hampered by the fact that many of the compounds are readily decomposed[169,170] on GC columns under usual operating conditions. However, Riva and Carasino,[171] using a KCl thermionic detector, showed that, with proper column conditioning, 300 ng of carbaryl could be chromatographed with less than 10% thermal decomposition. Storherr[19] is employing similar GC methods, combined with the acetonitrile extraction and charcoal cleanup methods described by Storherr et al.[134] for organophosphates, to develop a multiresidue carbamate analytical method. He has found a column containing 5 to 6% DC-200 on 80 to 100 mesh Chromosorb W (HP) at a temperature of 170 to 180°C to be optimum for chromatography. With the thermionic detector, sensitivities ranged from 8 ng of Eptam to 50 ng of carbaryl for 20 to 40% full-scale deflection.

C. Derivatization Methods of Analysis

This section will review methods involving derivatization of the amine moiety (H-N-R or R-N-R), the phenol moiety (ArOH), or the intact aromatic *N*-methyl carbamate insecticides.

Ralls and Cortes[172] brominated carbaryl in a sealed tube with a solution of bromine in carbon tetrachloride. The resulting electron-capturing derivative made it possible to detect carbaryl in green beans at the 1-ppm level. Fishbein and Zielinski[173] converted carbaryl and Zectran (as well as *N*-phenyl carbamate and urea pesticides and α-naphthol) to the trimethylsilyl (TMS) derivatives to retard thermal breakdown and improve peak symmetry:

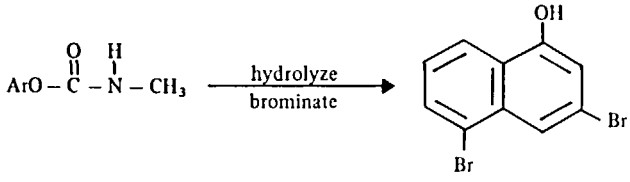
Lau and Marxmiller[174] acetylated two intact Landrin isomers, using trifluoroacetic anhydride, to produce derivatives amenable to electron-capture gas chromatography. Linear response was obtained over the range 0.1 to 0.4 ng. The same method can be used to detect carbofuran and its 3-keto and 3-hydroxy metabolites at the 0.5-ng level:[175]



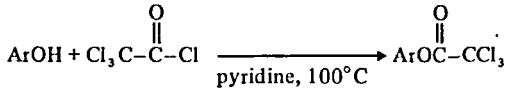
Sieber[176] reported that methyl carbamate insecticides react quantitatively and rapidly with pentafluoropropionic anhydride (PFPA) to give stable N-perfluoroacetylated derivatives that were stable under GC conditions and detectable at subnanogram levels. Shafik et al.[177] modified this technique for the determination of carbamate

insecticides in human fat and blood (see details in Section F). Sullivan et al.[178] achieved greater than 99% conversion of carbaryl to N-acetyl carbaryl by reaction with acetic anhydride in the presence of methanesulfonic acid at 4°C followed by heating to 97°C for 30 min. However, the flame ionization detector employed was not selective or sensitive enough for residue analysis.

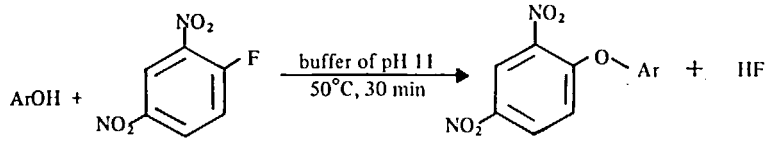
Phenols resulting from alkaline hydrolysis of carbamate insecticides have been analyzed in various ways. Gutenmann and Lisk[179] determined carbaryl by hydrolysis, bromination, and esterification in a single step to produce brominated 1-naphthyl acetate. The detection limit was below 0.5 ng by electron capture and response was linear from 0.8 to 1.6 ng of carbaryl. Van Middelem et al.[180] determined carbaryl, Mesurol, and UC-10854 by hydrolysis and bromination, but not esterification:



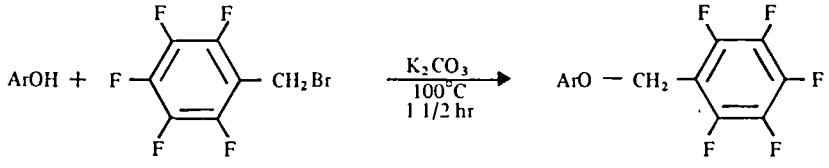
The sensitivity and the range of linear response were similar to those of the methods involving the esterified derivatives. Zectran and Matacil, both of which contain tertiary amino groups, were not successfully determined. Bache et al.[181] studied the bromo- and chloromethyldimethylsilyl derivatives of phenols derived from carbaryl, Mesurol, and Zectran and found them to allow sensitive EC detection, but no residue methods were developed. Butler and McDonough[182,183] followed hydrolysis by treatment of the resulting phenol with trichloroacetyl chloride to form electron-capturing trichloroacetates:



Carbaryl, carbofuran, and MC-A-600 were detected in various crops with a sensitivity of 0.01-0.10 ppm. The sensitivity of detection of the trichloroacetyl derivative of carbaryl was found[184] to be four times that for the brominated derivative.[179] This method can be used to convert metabolites of 3-keto and 3-hydroxy metabolites to electron-capturing derivatives.[175] Argauer[185] monochloroacetylated the phenols liberated by hydrolysis from a series of carbamates and found the minimum detectable quantities to range from about 0.5 to 2.5 ng of carbamate. Shafik et al.[186] used this approach to determine as little as 20 ppb of 1-naphthol in human urine (details are given in Sec. E). The chloroacetylated phenols of Baygon, Landrin, Matacil, Zectran, and carbaryl may be separated on a 2% OV-17/8% OV-210 column at 175°C with a nitrogen flow of 60 ml/min in 16 min in the order stated above.[82] Cohen et al.[187] determined residues of propoxur, butacarb, carbaryl, and Mesurol in vegetables and river waters by forming electron-capturing 2,4-dinitrophenyl ethers after hydrolysis:



Free phenols do not interfere if removed by selective oxidation. Pentafluorobenzyl aryl ethers are formed by reaction of aromatic carbamate phenols with pentafluorobenzyl bromide (PFBB):



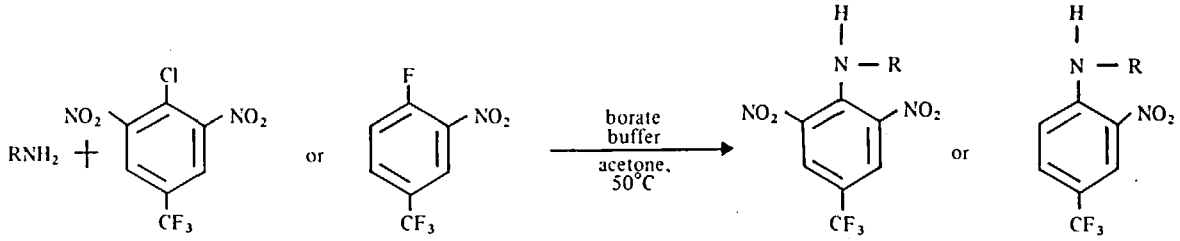
The elution pattern of the pentafluorobenzyl ethers from an l-g silica-gel column containing 1.5% H_2O is given below:

Phenol Derivative	Eluting solvents: 20% B-H	40% B-H	60% B-H	80% B-H	B	20% E-B	1% A-B
Landrin-2,3,5	+						
Landrin-3,4,5	+						
Baygon		+					
Pyrocatechol		+					
Mesurol		+					
Matacil						+	
Zectran						+	
Carbofuran		+					
3-OH-carbofuran							+
3-Keto-carbofuran					+		
1-Naphthol	+						

B = benzene; H = hexane; E = ether; A = acetonitrile

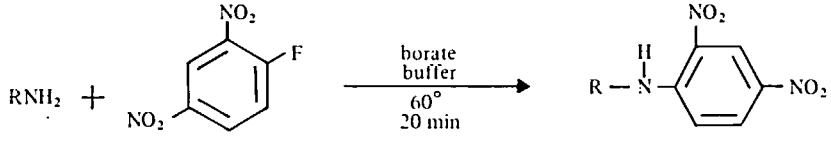
Separation of the pentafluorobenzyl ethers is obtained on a 5% SE-30 column, operated at 175°C with 60 ml/min of nitrogen, in 20 min in the order Baygon,® Landrin® (2, 3, 5), carbofuran, Matacil,® Zectran,® carbaryl, and Mesurol.® In the absence of K_2CO_3, anilines react with PFBB to produce substituted amines.[82]

Crosby and Bowers[188] formed substituted anilines by reacting amine hydrolysis products with two different reagents:



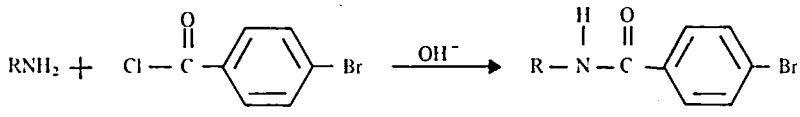
Secondary amines and phenols also react to give corresponding products. The derivatives are detected down to 50 pg by electron-capture GC.

Holden et al.,[189] in a similar approach, used 1-fluoro-2,4-dinitrobenzene to form dinitroaniline derivatives:



[R_2NH moities also react, including some herbicides]

The same reagent (1-fluoro-2,4-dinitrobenzene) was used by Cohen and Wheals[190] to form the 2,4-dinitrophenyl derivatives of aromatic amines resulting from the hydrolysis of urea and carbamate herbicides (see below). Tilden and van Middelem[191] coupled 4-bromobenzoyl chloride with the amines from hydrolyzed carbaryl and Matacil. The method is probably also applicable to N,N-dimethylcarbamates. The derivative was detectable by electron capture at the 0.2-ng level and 0.2 ppm of carbaryl was detected in plant material:



(benzamide)

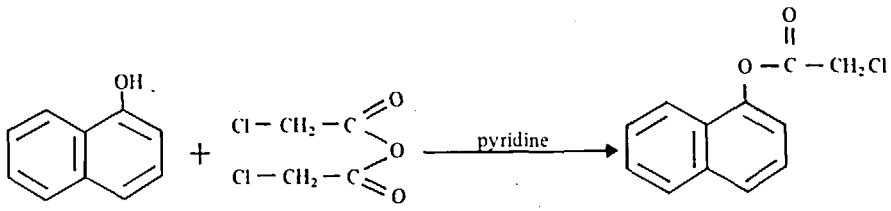
D. Extraction Methods and GC Columns

Methods for extracting carbamate insecticides from plant material and GC columns for the analysis of the insecticides and metabolites have been reviewed.[175] Extraction by blending the sample with methylene chloride, acetone, chloroform, or acetonitrile (or these solvents plus anhydrous Na_2SO_4) is widely used. In some methods for carbofuran,[153,192] Mesurol,[168] Zectran,[193] and possible conjugated hydroxy metabolites, hydrolysis with a strong acid during extraction is included. Liquid phases which have been used for the determination of various carbamates and derivatives include 5% QF-1, 10% DC-200, 0.5 to 20% SE-30, 2 to 5% DC high-vacuum silicone grease, 2% XE-60, 2% Reoplex® 400, 3% Carbowax 20M, 5% DC-11, and 5% Carbowax 20M/10% DC-200 or SE-30 (1:1).

E. Determination of 1-Naphthol in Urine

Humans exposed to the insecticide carbaryl excrete quantities of 1-naphthol, conjugated either as the sulfate or glucuronide.[194] The method[186] described below for the specific and sensitive determination of 1-naphthol in urine serves for measuring human exposure to this important and widely used pesticide.

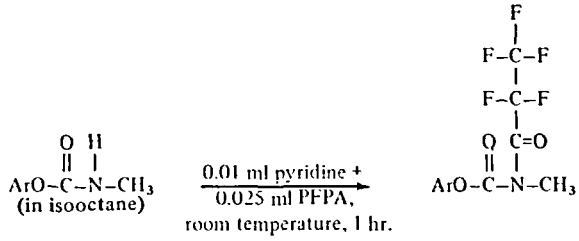
A small sample (about 5 ml) of urine is acidified with hydrochloric acid and refluxed on a steam bath to accomplish hydrolysis. The 1-naphthol present is extracted with benzene and derivatized with chloroacetic anhydride solution:



After cleanup on a 1-g column of silica gel containing 1.5% water by elution with 10 ml of benzene-hexane (2:8) (discard) and 10 ml of benzene-hexane (6:4) (which contains the chloroacetate derivative), the derivative is determined by EC-GC versus a 1-naphthol standard similarly derivatized. Using a 1.5% OV-17 1.95% QF-1 column, the retention time of 1-naphthyl chloroacetate is 0.92 relative to aldrin. As little as 50 pg of the derivative should be detected.

F. Determination of N-Methylcarbamate Insecticides in Blood Serum and Human Fat

A procedure has been developed[177] for the simultaneous determination of 0.04 to 1 ppm of Baygon, Landrin, carbofuran, Matacil, Zectran, carbaryl, and Mesurol in blood serum and human fat. The N-methylcarbamates are extracted with acetonitrile and partitioned into methylene chloride. The extract is then cleaned up by liquid-column chromatography. Gel permeation chromatography[195] on Bio Beads SX-2 with elution by methylene chloride, followed by chromatography through a charcoal adsorbent mixture[134] column is used for fat samples, while silica gel containing 20% water or charcoal is used for blood samples, depending on the level of pesticide present. The purified intact carbamates are derivatized with pentafluoropropionic anhydride (PFPA) in the presence of catalytic amounts of pyridine:



The derivatives are separated on a 6-ft column containing 5% SE-30 on Chromosorb W (HP) operated at 170°C with a rate of flow of nitrogen carrier gas of 30 ml/min. The derivatives are eluted between 4 and 16 min in the order listed above. Amounts of carbamates between 20 and 80 pg can be detected by electron capture under these conditions.

VIII. GAS CHROMATOGRAPHIC DETERMINATIONS OF MISCELLANEOUS CLASSES OF PESTICIDES

A. Sulfur-Containing Compounds

Many pesticides containing sulfur also contain phosphorus and/or chlorine and can therefore be

detected by the FPD in the phosphorus mode or by electron capture as described above. If it is desired to base detection on the sulfur atoms, four possibilities exist. Flame photometric detection at 394 nm is selective for sulfur-containing compounds. Response in nonlinear and less sensitive than when the P mode of the detector is employed. A helium-plasma microwave emission spectrometric detector monitoring the 5453.8-Å sulfur line has been designed[196] but is seldom used for residue analysis. The electrolytic conductivity detector operates by pyrolyzing the effluent compounds under oxidative conditions to form SO_2, which dissolves in the deionized water to form conducting strong acids. Venting removes most of the interfering CO_2, while any that does dissolve is only slightly ionized. Halides as HX are removed by a silver-wire scrubber. The microcoulometric detector operates by titration of the SO_2 produced by pyrolysis, with coulometrically generated iodine; sodium azide is added to the cell to remove halogens and strong oxidants. Microcoulometry is sensitive to about 1 ng of sulfur.

Procedures for the extraction and Florisil cleanup of thiophosphate pesticide residues in fruits and vegetables for sulfur microcoulometric detection have been described by Nelson.[197] Relative retention data for sulfur-containing pesticides detected by sulfur microcoulometry have been reported for 15% QF-1/10% DC-200 (1:1),[198] 10% DC-200,[96] 15% DC-200,[199,200] and 10% QF-1[199] liquid phases.

B. Triazine Herbicides

Liquid phases used for the successful separation of mixtures of s-triazine herbicides include 0.5% Apeizon L[201] at 200°C and ethylene glycol adipate[202] at 140°C, both on 120 to 150 mesh glass beads, Reoplex 400[203] on 80 to 90 mesh kieselguhr, 2.5% Versamid 900[204] on 60 to 80 mesh Diatoport® S, 5 to 10% Carbowax 20M and 5% GE-XE-60.[205] Ebing[206] has studied the factors that influence the reproducibility of retention data for triazines obtained by temperature-programmed GC with packed columns, and Henkel and Ebing[204] reported the separation of eleven triazines by temperature programming on a Versamid® column.

Triazine residues in crop materials have been determined[205] by extraction with chloroform, transfer to benzene, cleanup on a 12-g column of alumina (Activity V) eluted with 75 ml of hexane (discard) and then with 150 ml of benzene-hexane (1:1) to recover the triazines, evaporation, and SO_2 and Cl microcoulometric GC on a 5% Carbowax 20M column to determine 0.25 μg of triazine in the equivalent of 5 g of crop extract (0.05 ppm). A similar method for prometryne, atrazine, and simazine residues in milk involves extraction by a modified Mills procedure and removal of butterfat by acetonitrile partitioning prior to alumina cleanup and GC. Details are given by the Geigy Chemical Corporation in Section 120.222, Vol. II, of the FDA *Pesticide Analytical Manual.*[7]

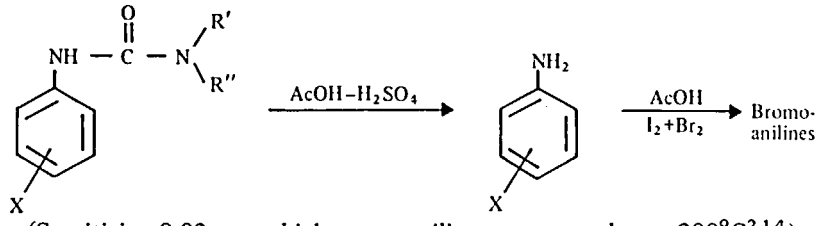
Triazines can be analyzed using the electron-capture detector or detectors selective for S, N, or Cl atoms, depending upon the particular compounds of interest. The Rb_2SO_4 alkali-flame detector has been used to determine residues of atrazine and simazine in corn (50 ppb), soil (20 ppb), and water (1 ppb) without cleanup. Less than 0.5 ng of triazine was detectable.[207] Prometone, prometryne, and propazine (0.1 to 0.2 ppm) were determined[204] in soils using the flame ionization detector, although electron-affinity detection would have been equally suitable. Successful silylation of hydroxy simazine, hydroxy propazine and hydroxy atrazine was achieved with bis(trimethylsilyl) trifluoroacetamide. Analysis of GC on a column of 10% OV-17 or 0.5% neopentyl glycol sebacate showed a sensitivity of 1 ppm on corn and soil.[208] Due to the very polar nature of hydroxy triazines, cleanup may be performed on a Dowex 50 ion-exchange resin (H^+ form) eluted with 4 M NH_4OH in 50% ethanol and then with 1 M HCl,[209] or on columns of acidic alumina or silica gel.[208] A sulfur-selective FPD detector was used for the determination of the sulfur-containing triazine Bay 94337 to eliminate the need for the stringent, time-consuming cleanup required for EC detection.[210]

A mechanized all-glass GC sample-introduction system for triazine herbicides has been designed,[211] and the automation of extraction, cleanup (by solvent partitioning), GC determination, and calculation of results has also been reported.[212]

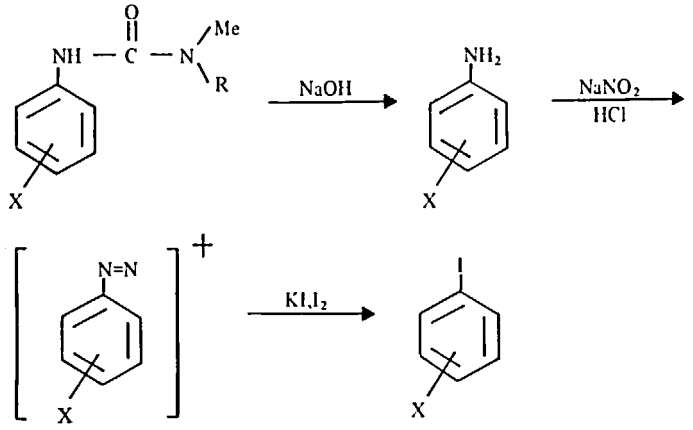
C. Urea and Carbamate Herbicides

GC methods for phenyl-substituted urea and carbamate herbicides are often based on hydrolysis followed by determination of the corresponding anilines. Kirkland[213] determined 0.1 to 1.5 ppm

levels of monuron and diuron in soils, fruits, and vegetables by steam distilling and extracting the anilines and separating them by programmed temperature GC on a 20% Apiezon L grease column. The relatively insensitive flame ionization detector was used in this early work. Halogenated aniline derivatives have been formed to improve senitivity by allowing use of the electron-capture detector in determinations of herbicides such as CIPC, monuron, diuron, and neburon:

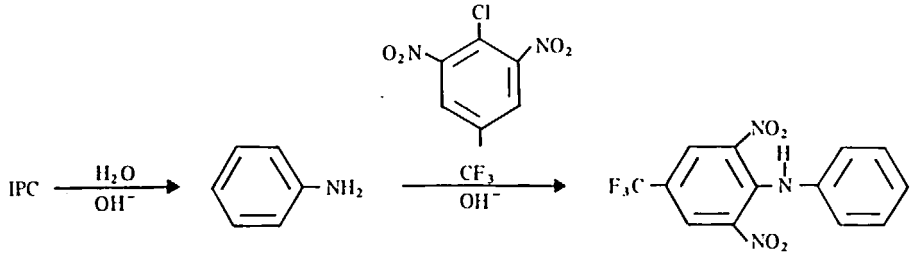


(Sensitivity 0.02 ppm; high-vacuum silicone grease column, 200°C[214])



(Sensitivity 0.01-0.05 ppm; QF-1 column, 150°C[215])

A method for the determination of N-Phenylurea herbicides in soil extracts is based on thermal decomposition of the compounds catalyzed by tetraethylammonium hydroxide followed by programmed temperature gas chromatography of the anilines (180 to 250°C, 6°/min) on a 1.5% Versamid 900 column.[216] IPC, molinate, EPTC, pyrazon, and CDEC were determined after hydrolysis by reaction of aniline with 4-chloro-α,α,α-trifluoro-3,5-dinitrotoluene



followed by EC-GC using a 3% SE-30 column at 150° to 205°C.[188] The 2, 4-dinitrophenyl derivatives of hydrolysis products such as aniline, 3- and 4-chloroaniline, 4-bromoaniline, and 3,4-dichloroaniline have been formed by reaction with 1-fluoro-2,4-dinitrobenzene on a silica-gel thin layer; the derivatives are eluted with acetone and separated on a 1.0% XE60/0.1% Epikote 1001 column at 215°C. Ten herbicides were determined in waters, soil, and plant materials at the 0.001 to 0.05 ppm level by this procedure.[190] A recent study[217] of derivatives for the EC-GC determina-

TABLE 3

Parent aniline	Pesticides yielding this degradation product	PFPA Derivative: Response (nm/mg)	PFPA Derivative: Relative retention
Aniline	IPC, Carbetamide, Fenuron, Siduron	3.4	0.40
m-Chloroaniline	CIPC, Barban	3.5	0.98
p-Chloroaniline	Monuron, Monolinuron, Urox	3.3	1.00
p-Bromoaniline	Metabromuron	2.3	1.48
3-Chloro-4-methylaniline	Chlortoluron	2.5	1.50
3,4-Dichloroaniline	Propanil, Diuron, Neburon, Linuron	1.7	2.45

tion of anilines resulting from the metabolism of various anilide, carbamate, and urea pesticides concludes that reaction with pentafluoropropionic anhydride is the best choice. Recoveries between 85 and 90% were made from urine spiked with mixtures of anilines at levels of 1.0 ppm and 0.1 ppm. Extraction was with benzene, cleanup after derivatization of the extract was by elution through a 1-g silica-gel column (3% H_2O) with 20% benzene-hexane, and electron-capture gas chromatography was on a 5% SE-30 column at 150°C with a nitrogen carrier flow rate of 60 ml/min. Response and relative retention data are shown in Table 3; it is clear that conditions must be found that give better separation than those chosen in this preliminary study.

The direct chromatography of twelve intact substituted urea herbicides was studied by McKone and Hance[218] who found a 5% E-301 methyl silicone column at 150°C gave best results. Using these conditions, McKone[219] determined linuron, benzomarc, chlorbromuron, diuron, fluometuron, metobromuron, methoxymarc, and neburon in soils at the 0.05- to 1-ppm level by electron-capture detection. A multiresidue method for the GC determination of carbamate herbicides in foods was developed by Onley and Yip.[161] Samples were extracted with ethanol (fruits and vegetables) or ethanol-water (6:4, for low-moisture samples such as soybeans and alfalfa), and the carbamates were partitioned into petroleum ether, concentrated, and cleaned up on a 4:1 MgO-cellulose column eluted with 2% methylene chloride in petroleum ether and 1% ethanol in petroleum ether. The fractions were concentrated and chromatographed on a 15% QF-1/10% DC-200 (1:1) column at 160°C with a KCl-Rb_2SO_4 (1:1) thermionic detector and electron-capture detector (for Sirmate). An FPD detector (sulfur mode) and 5% OV-101 column (155°C) was used to confirm S-containing compounds. Avadex®, CIPC, Eptam, Ordram®, Ro-Neet, Sirmate, Sutan®, Tillam®, and Vernan® were detected with a sensitivity of about 0.02 ppm.

Both all-glass and stainless steel GC systems may be successfully used for the determination of intact urea herbicides. N-aryl carbamate herbicides are, in general, more thermally stable than N-methyl carbamate insecticides, and are therefore more amenable to direct determination by GC. However, decomposition of compounds of either class can easily occur under certain conditions.

Thier[220] has developed a determination of carbamate, urea, and amide herbicide residues in plant material. The pesticides are extracted with acetonitrile and partitioned into petroleum ether and dichloromethane (8:2), and the organic phase is cleaned up on a Florisil column. The purified extract is first examined for chlorinated and phosphate insecticides by EC-GC, and then the herbicides are brominated and determined under the same conditions.

D. Phenolic Pesticides (see Section VI-G)

Dinitrophenols, like chlorophenoxy acids, are usually determined after conversion to volatile methyl ether derivatives by reaction with diazomethane. The optimum methylation procedure for acids and dinitrophenols involves addition of isooctane to the reaction vessel and a temperature of 70°C.[97] DNP herbicides are extracted from crops with chloroform and subjected to a two-

column cleanup step on acid-Celite and Florisil.[221]

Mixtures of methylated dinitrophenols have been separated on columns containing 10% DC-200 at 150°C[221,222] and 5% DC-high-vacuum silicone grease at 200°C;[223] and a mixture of 2,3,6-trichlorobenzoate, four chlorophenoxy methyl esters, and three dinitrophenol methyl esters on a 10% DC-200/15% QF-1 (1:1) column at 185°C.[97] Free phenolic pesticides have been chromatographed at 200°C with reduced tailing on 3% columns containing neopentylglycol succinate, butanediol succinate, or cyclohexanedimethanol succinate, each mixed with 1% orthophosphoric acid.[224] Phosphoric acid, which acts as a "chaser" for the polar pesticides, is often added to GC columns used for phenols.[225]

Chlorophenol pesticides have been chromatographed as N-(trichloroacetyl)carbamate, *m*-fluorosulfonylbenzoate ester, or chlorophenylfluorosulfonylbenzenesulfonate derivatives on columns containing 5% OV-17 (225°C) and 3% SE-30 (200°C) columns.[226]

Thier[227] has proposed a multiresidue analytical method for determining all acidic herbicides (carboxylic or phenolic) using reagents and procedures similar to those in the familiar insecticide multiresidue procedures. Nineteen compounds including phenoxyalkanoic acids, benzoic acid derivatives, phenols, and other acids were studied, and the procedure included extraction with acetonitrile, separation from neutral compounds by partitioning at controlled pH, methylation, and gas chromatography. Confirmation was obtained by TLC after column chromatography on Florisil and by derivatization followed by GC. Recoveries from various foods at 0.1-1.0 ppm levels ranged from 70 to 110%.

E. Uracil Herbicides

Bromacil has been determined using a 20% SE-30/0.2% Epon-1001 mixed-phase column and microcoulometric detector with temperature programming (100-300°C).[228] For simultaneous determination of terbacil and bromacil, a more-polar 5% XE-60/0.2% Epon® 1001 column (100-225°C) was employed.[229] More recently, electron-capture detection has been successfully employed for the determination of uracils.[230-232]

F. Fumigant Mixtures

Multiple residues of organic fumigants (methyl bromide, carbon disulfide, chloroform, ethylene dichloride, carbon tetrachloride, and ethylene dibromide) in grains are determined[233] by refluxing the whole or ground grain in acid medium and the volatile fumigants are dried (Chromosorb W drying tube), collected in cold isooctane, and determined by EC-GC on a 30% DC-200 column at 70°C. This procedure was used to analyze a series of commercially fumigated grain samples.[234]

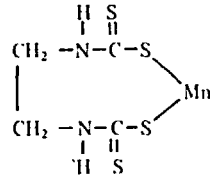
Other methods have been published for determining fumigant multiresidues in wheat and flour with a flame ionization detector;[235] trichlorochloroethylene, CS_2 and CCl_4 in cereal grain by EC-GC;[236] ethylene oxide, ethylene glycol, diethylene glycol, and ethylene chlorohydrin residues on date fruit with an FID;[237] and phosphine uptake by foodstuffs; insects and solvents using MC (5-ng detection limit), thermionic (20 pg), and FPD (2 pg) detectors.[238] A review of advances in fumigant residue analysis has been published.[239]

G. Organomercurial Fungicides

Alkyl-, alkoxyalkyl-, and arylmercury pesticides are determined as dithizonates on 1.2 to 1.5-meter columns containing 2% polyethylene glycol succinate at 170 to 180°C[240] using electron-capture detection. Organomercurial residues are extracted from fruits and vegetables using a slightly alkaline solution of cysteine hydrochloride in 2-propanol, the extract is washed with diethyl ether or toluene, and the residues are extracted with a diethyl ether solution of dithizone. The extract is dried by passage through a Na_2SO_4 column and concentrated prior to GC or TLC.

H. Ethylene Thiourea (ETU)

Ethylene bisdithiocarbamate fungicides, such as maneb



yield ETU as a degradation product, residues of which can be determined by GC. Determination in apples in the 0.01 to 1.0 ppm range has been carried out by extraction from samples with methanol followed by treatment with benzyl

chloride in refluxing 50% aq. methanol to form 2-benzylmercapto-2-imidazolinium chloride, extraction of interfering substances, extraction of the derivative as the free base, and trifluoroacetylation to yield a compound determined by EC-GC.[241] Residues in fruits, vegetables, and milk (0.02-10 ppm) have been determined by extraction with ethanol-$CHCl_3$ (2:1), cleanup on a cellulose column, derivatization with 1-bromobutane, and GC using a KCl-Rb_2SO_4 thermionic detector.[242] The flame photometric detector was employed for determinations of ETU in vegetable and fruit crops.[243]

IX. PAPER CHROMATOGRAPHY (PC)

The first multiresidue analytical method available to pesticide chemists was based on PC.[41,42,244] Since the advent of GC, PC has been used mainly for confirmation of residues and occasionally for semiquantitative determinations. PC has now been largely displaced for these purposes by TLC, which will give generally faster and more efficient separations with better spot definition and greater sensitivity. Despite these advantages, which are often only minor considerations, PC is still used for residue analysis by many workers, especially in countries outside of the United States, and new methods, mostly for the determination of individual residues in specific samples, continue to be published (see, e.g., refs. 245, 246). The major advantages of PC relative to TLC are its low cost, reproducibility, and convenience. The general methodology for the PC of pesticides has been described in several treatises.[23,247-250]

A. Chlorinated Insecticides

Chlorinated insecticides in fatty samples are extracted with petroleum ether, partitioned into acetonitrile, and cleaned up on Florisil and MgO-Celite columns as for gas chromatography.[42,251,252] Nonfatty food samples are prepared by the Mills-Onley-Gaither procedure.[63]

Separations are achieved by ascending development on 8 x 8-in. Whatman No. 1 paper, prewashed with water[253] or aqueous $AgNO_3$ followed by water[244] and air-dried, which is impregnated with refined soybean oil or mineral oil (5% in diethyl ether). Mobile solvents are methanol, methylcellosolve, or acetone mixed with 25% water. Over 114 pesticides have been examined in these aqueous solvent systems.[254,255] Non-aqueous chromatographic systems composed of paper impregnated with 10% 2-phenoxyethanol in diethyl ether or 35% N,N'-dimethylformamide in ether with isooctane as the solvent have also been used.[255] Dieldrin and endrin are not separated in these systems but are resolved on paper impregnated with 5% Union Carbide® Lubricant 50 HB 5100 and developed with isooctane.[256]

Detection of spots is usually made by spraying with silver nitrate-2-phenoxyethanol reagent followed by irradiation for 4 to 5 min under UV light. Sensitivity ranges from 0.01 to 2 μg of chlorinated pesticide per spot.[255,256] Other detection tests used to a limited extent involve spraying with KOH and $Fe_2(SO_4)_3$,[23] treatment with methyl yellow followed by exposure to UV radiation,[257] and treatment with indophenol blue and a weak aliphatic organic acid.[258]

Quantitative estimations are carried out by visual comparison of separated sample spots (size and/or intensity) with a series of standard concentrations run on the same paper, elution of spots (located by spraying guide strips) with acetone and analysis by the Schechter-Haller colorimetric method,[259] weighing sprayed spots,[260] and photoelectric densitometry (maximum spot density method).[261]

A novel method of development, based on the acetonitrile cleanup of crude insect tissue directly on the paper described by Menn et al.,[262] has been reported by Major and Barry[263] for determination of chlorinated insecticides and malathion on fruits and vegetables at the 5 ppm level. Samples are stripped by the Mills procedure,[41] and aliquots of strip solution are spotted on paper and developed twice with acetonitrile to within 1 in. of the top of the paper. The pesticides are thereby carried away from fats and waxes and concentrated on the top line. The paper is dried, turned through 180°, standards are spotted on the new origin line, now one inch from the bottom of the paper, and development is carried out according to Mills.[41]

B. Organophosphate Insecticides

Multiresidues of organophosphate insecticides and metabolites in fruits and leafy vegetables are extracted with acetonitrile, transferred into ethyl acetate, and cleaned up on a charcoal-Celite

column eluted with 25% chloroform in ethyl acetate and then chloroform or benzene.[264] Another procedure uses cleanup of the acetonitrile extract on polyethylene-coated alumina and magnesol columns.[265] Guthion has been chromatographed after acetone extraction and chloroform partitioning without chromatographic cleanup.[266]

For one-dimensional chromatography of organophosphates, the paper (usually Whatman® No. 1, 20 or acetylated paper[267]) has been impregnated with 10 or 30% formamide in acetone and developed with isooctane,[268] with 2 or 8% mineral oil and developed with methanol-H_2O-0.5% NH_3 or 70% aqueous acetone,[269] with 5% silicone 550 and developed with acetone-ethanol-water (1:1:2) or 10% aqueous acetone,[265] and with 5% vaseline and developed with acetone-ethanol-H_2O (1:1:2).[270] Two-dimensional separations have been carried out on paper impregnated with 20% formamide in acetone and developed with benzene-chloroform (6:4 followed by 9:1);[271] paper impregnated with 20% DMF and developed with isooctane, then reimpregnated with 10% mineral oil and developed with 50% aqueous DMF[268] or 40% aqueous acetonitrile;[264] and paper impregnated with 30% DMF in acetone and developed with hexane, then reimpregnated with 5% liquid paraffin and developed with 50% DMF.[266] Dursban® and its metabolites have been separated on non-impregnated paper with eight different solvents.[272] Separations have also been carried out on Whatman 3MM paper, of which the lower third was impregnated with 10% light mineral oil and the upper two thirds with 10% Epon resin 828. The developing solvent was 35% aqueous acetonitrile.[273]

Many commonly used chromogenic reagents for organophosphate insecticides have been reviewed.[274] The most useful reagents are N-bromosuccinimide-fluorescein,[275] bromine-fluorescein-UV light,[268] UV light alone,[268] ammoniacal silver nitrate,[268] $AgNO_3$-bromophenol blue-citric acid,[264] metanil yellow after bromination,[276] DCQ reagent,[277] ferric chloride-salicylsulfonic acid,[269] bromine-Congo Red,[278] bromine-KOH,[265] Dragendorff reagent,[270] and enzymatic detection following bromination.[267,279,280] The last of these is the most sensitive, detecting less than 1 μg of most organophosphates and sometimes as little as 5 ng.[250]

Organophosphate residues can be determined by digestion of the paper chromatographic spot by oxygen-flask combustion followed by chemical determination of orthophosphate.[281,282] PC has a distinct advantage over TLC when such digestion procedures are to be used since the paper can be readily digested with the sample, whereas elution of separated solutes from thin layers would often be required.

A comprehensive general scheme for determining organophosphorus residues in foodstuffs has been devised by Bates.[283] The pesticides and metabolites were extracted with acetone, water was added, and the residues were partitioned into chloroform. Solvent was removed, the extract was taken up in acetone, and fats and waxes were frozen out at -80°C. Further cleanup was achieved by column chromatography. Polar compounds were developed on formamide-impregnated paper twice in the same direction with hexane, then at right angles with benzene-chloroform (9:1). Non-polar pesticides were separated on DMF paper developed with hexane and then with 50% aqueous DMF at right angles. Spots were detected with bromine-4-methylumbelliferone or $AgNO_3$-bromophenol blue, excised, and determined by a digestion-molybdenum blue total phosphorus method.

C. Carbamate Insecticides

Carbaryl residues in wine have been determined, after extraction and cleanup by solvent partitioning, by ascending development on Whatman No. 20 paper with 20% aqueous methanol. Carbaryl has an R_F value of 0.65 and 1-naphthol, the metabolic breakdown product, has one of 0.37 (Figure 3). Detection is accomplished by spraying with a mixture of equal volumes of 1.5 N-methanolic NaOH and 1-butanol (1:1) to hydrolyze carbaryl to 1-naphthol followed by air drying and dipping into 0.01% *p*-nitrobenzene-diazonium fluoborate in methanol-ether (1:1) to yield blue spots in which the dye can be determined by the maximum-spot-density method. The detection limit was 0.5 μg carbaryl.[284]

Dimetan,® Pyrolan,® dimetilan, and isolan have been chromatographed on Whatman No. 4 paper coated with 10% glutaronitrile in acetone, with diisopropyl ether saturated with glutaronitrile as the solvent. Orange-red spots were formed by spraying with 15% aqueous KOH followed by steaming, spraying with 1 N acetic acid in

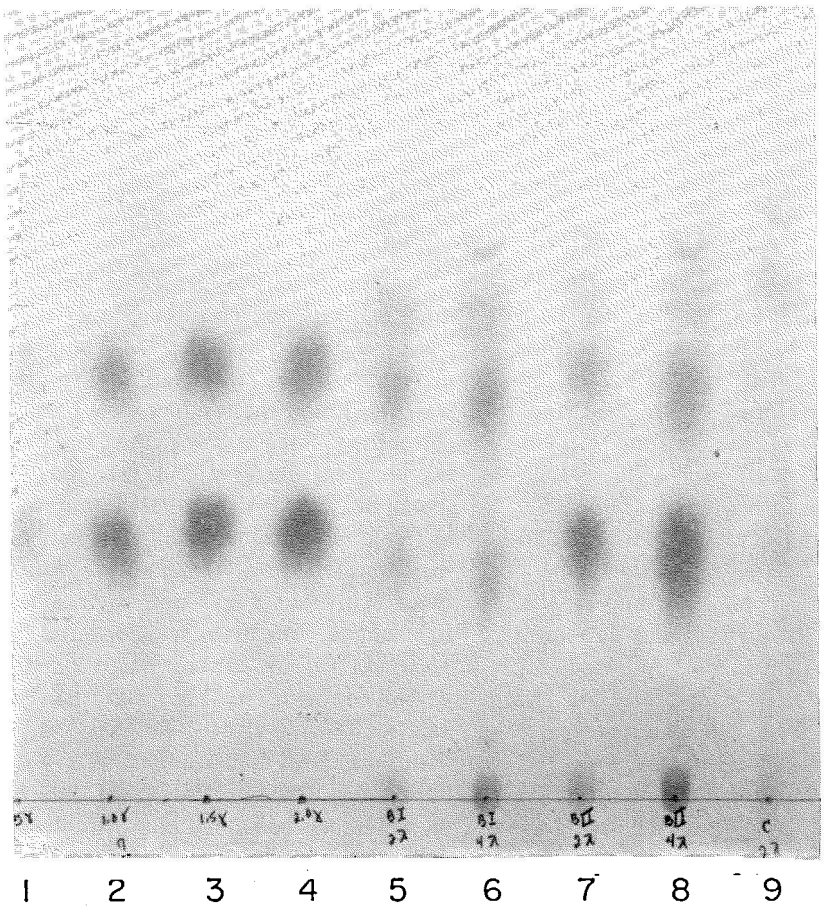


FIGURE 3. Separation of carbaryl and 1-naphthol by one-dimensional paper chromatography. Reproduced from reference 284 with permission of the authors and the American Chemical Society.

methanol, drying, and spraying with 0.1% *p*-nitrobenzenediazonium fluoborate in methanol.[285]

D. Herbicides

1. Chlorophenoxy Acid Herbicides

Residues of 2,4-D, MCPA, 2,4,5-T, 2,4,5-TD, and various esters of 2,4-D have been determined in wheat samples. After extraction and cleanup on a Florisil column and esterification of acids to the corresponding methyl esters with diazomethane, the following solvent systems were used:

	Immobile		Mobile
Methylated acids	10% Mineral oil in ether	or	50% Acetonitrile in H_2O
	35% DMF in ether		Isooctane
2,4-D Esters	10% Mineral oil in ether		65% Acetonitrile in H_2O

Spots were visualized with $AgNO_3$ and UV light.[101] Alternatively, bromcresol purple (0.04%) in formaldehyde-ethanol (1:5) followed by exposure to ammonia vapors produces yellow spots on a purple background.[286]

2. Triazines

Atrazine and simazine residues in corn may be determined with a sensitivity of 0.1 ppm by extraction with $CHCl_3$-NH_3 (20:1), transfer to petroleum ether, cleanup on a Celite 545-10% aqueous $HClO_4$ (1:1 w/v) column and then a

Florisil column, and chromatography on paper impregnated with 15% methanol in petroleum ether and developed at once with trichloroethane-methanol-isooctane (20:5:75). Spots (R_F 0.7 and 0.5, respectively) are detected with $AgNO_3$-2-phenoxyethanol reagent and UV light, and semi-quantitatively estimated by visual comparison with standards.[287]

3. 3-Aminotriazole (ATA)

ATA and metabolites are extracted from plant material and cleaned up,[288,289] chromatographed[288-291] on Whatman No. 1 paper with 80% phenol (R_F ATA, 0.78), 80% pyridine (0.72), methanol-formic acid-H_2O (80:15:5) (0.60), or *n*-butanol-acetic acid-H_2O (27:7:17) (0.55), and detected with azo dye plus phenol (yellow spot)[289] or azo dye plus H-acid (red).[288]

4. Urea Herbicides

A method for the determination of monuron in plant tissue is based on hydrolysis, steam distillation, and extraction with controlled pH to recover *p*-chloroaniline, chromatography on Whatman No. 1 paper with methanol-isoamyl alcohol-benzene-2 N HCl (35:17.5:35: 12.5), and detection of the amine with Ehrlich reagent or by diazotization and coupling.[292,293] Quantitative analysis is made by spectrophotometry at 560 nm.[294] The methods should be amenable to other herbicides with aniline moieties.

Nine substituted urea herbicides and/or their TCA salts and TCA (trichloroacetic acid) can be separated by use of paper impregnated with mineral oil and developed with 25% THF in water or untreated paper developed with acetic acid-isooctane (1:3). Detection is by screening under UV light and with $AgNO_3$-2-phenoxyethanol chromogenic reagent plus UV light.[295]

E. Fungicides

Dithiocarbamate fungicides are chromatographed on glass fiber paper impregnated with 0.5 to 20% formamide in acetone with chloroform, petroleum ether, or hexane-$CHCl_3$ (3:1) as the mobile phase.[296] Unmodified paper with butanol-acetic acid-H_2O (4:1:1)[297] or butanol-ethanol-1.5 N $(NH_4)_2CO_3$ buffer[298] has also been used to separate a variety of fungicides. Detection of fungicides is made by use of Grote Reagent,[298] sodium azide-iodine reagent,[296,298] or bioautographic detection (sensitivity 0.1 μg for thiram, ziram, and ferbam.).[297]

X. THIN-LAYER CHROMATOGRAPHY (TLC)

Since methods for the analysis of pesticide residues by thin-layer chromatography have only very recently been reviewed in great detail by this author[299] through 1971, this section will be limited to a description of the most important and recent multiresidue methods for several widely-used classes of pesticides. The techniques for TLC are very similar to those for ascending PC, and these have been described for pesticide analysis by Kovacs[300,301] and Getz.[302]

TLC is mainly used for the confirmation of residues tentatively identified by GC. In addition, TLC may be used for screening residues, semi-quantitative analysis when a fast estimation of the residue level is desirable, when a gas chromatograph is not available, or if the pesticide of interest in nonvolatile or unstable during GC. TLC is also occasionally used for cleanup purposes prior to determination by gas[303,304] or thin-layer[305] chromatography.

The reader is referred to a paper by Walker and Beroza[306] for data on many types of insecticides in a large number of the TLC solvents.

A. Chlorinated Insecticides

Extracts of fatty and nonfatty foods, cleaned up on a Florisil column as described above in Sections IV-C and D, are spotted on prewashed alumina-coated plates and developed with heptane (for the 6% ether-petroleum ether eluate) and 2% acetone in heptane (for the 15% eluate). Detection is provided by spraying with $AgNO_3$-2-phenoxyethanol reagent and exposing to high-intensity UV light to produce brown to purplish-black spots. Conventional 8x8-in. glass plates, commercial precoated TLC sheets, or 3-1/4 x 4-in. microslides[307] may be employed. Complete details of these methods and R_F values[308] for numerous compounds in these two solvents, as well as an alternate system consisting of immobile DMF on alumina and isooctane solvent, are contained in Sections 410 and 411 of Volume I of the FDA Pesticide Analytical Manual.[7] Silver nitrate can be incorporated into acid-washed Al_2O_3 before coating the plates so that only exposure to

UV light is required for the spots to be visualized. This procedure is detailed in Section 412 of the Manual.[7] Silica-gel layers developed with isooctane-methylene chloride (9:1) are also excellent for separation of organochlorine residues.[302]

A diphenylamine-$ZnCl_2$ detection reagent proposed by Faucheux[309] has the advantage of producing spots of various colors with different chlorinated insecticides, thereby aiding identification. However, the sensitivity of this reagent is lower than with $AgNO_3$, for which maximum limits of 10 to 50 ng are usually achieved.

Abbot et al.[310] have described a semiquantitative screening method for organochlorine residues in fats and vegetables. The sample is cleaned up by a dimethyl sulfoxide partitioning process, by chromatography on a small column of alumina, or by a combination of these. TLC is performed on alumina layers incorporating $AgNO_3$, residues are identified by their R_F-values and estimated (down to 0.01 ppm) by comparing the sizes of the spots with those obtained from standards.

A TLC method for confirmation of chlorinated residues in serum and adipose tissue is described in Section 12B of *Analysis of Pesticide Residues in Human and Environmental Samples.*[13] An extract from 50 g of serum, cleaned up on Florisil and concentrated to 100 μl before spotting for TLC, will produce a visible spot at 2 ppb (assuming that 10 ng of chlorinated pesticides can be detected). An adipose tissue extract from 5 g, concentrated to 500 μl, will give a readable spot at 10 ppb. The method involves TLC of the 6% and 15% Florisil column eluates on alumina layers, with additional prior cleanup of the 15% fraction on an alumina microplate developed with acetonitrile.

The TLC of DDT and 14 related compounds (including DDE and DDD isomers, DDE, Kelthane,® etc.) was studied[311] on precoated alumina and silica plates using 33 solvent systems and 16 variations of the $AgNO_3$-2-phenoxyethanol-UV detection method. Eleven compounds were separated on a layer of aluminum oxide developed three times with *n*-hexane in one direction and twice with *n*-hexane-ethyl ether-acetic acid (90:10:1) at right angles.

B. Chlorophenoxy Acid Herbicides

Food extracts containing methylated chlorophenoxy acids[100] are cleaned up on Florisil and chromatographed on alumina layers using hexane saturated with acetonitrile as the solvent. Cleaned-up extracts containing free chlorophenoxy acids are developed for a distance of 3.5 cm on a precoated silica gel sheet with cyclohexane-acetic acid (10:1), then the sheet is dried and developed for 15 cm in the same direction with benzene-petroleum ether (3:1). Spraying with $AgNO_3$ chromogenic spray produces black spots in both cases. Details of both methods and R_F-values are given in Sections 421 and 422 of the FDA Manual.[7] Maximum sensitivity is about 50 ng for the methylated acids and 0.1-0.5 μg for the free acids. Chromotropic acid plus $AgNO_3$ is an alternate detection reagent.[312]

C. Organophosphate Insecticides

Storherr's[134] charcoal adsorbent mixture and the column and batchwise charcoal procedures,[264,313] with supplemental partition cleanup for oily samples[314] and alumina and partition cleanup for non-fatty foods, as described by Getz and McCully,[302] are recommended for cleanup of phosphate residues prior to TLC. Florisil columns eluted with 6, 15, and 50% ether-petroleum ether may be used for those pesticides recoverable from this adsorbent (see Table 201-A, FDA *Pesticide Analytical Manual*[7]). Cleanup by partitioning[315] and sweep codistillation[316] proved adequate when detection was made by the enzyme inhibition technique. Microcolumns of alumina, Florisil and carbon eluted with either 20% methylene chloride or 20% ethyl acetate in isooctane have provided adequate cleanup of crop extracts for TLC.[122]

Successful one-dimensional chromatographic systems include silica gel layers developed with isooctane-acetone-chloroform (70:25:5) in tanks without liners,[302] alumina layers supporting dimethylformamide and developed with methylcyclohexane,[7] and silica gel developed with 20% acetone in hexane.[317] A two-dimensional procedure developed by Gardner[318] has the significant advantage of specificity, obtained by oxidation of the pesticides with bromine vapor before development in the second direction. Silica-gel layers with toluene, 25% heptane in ethyl acetate, or ethyl acetate as solvents were used along with the Storherr charcoal cleanup procedure[134] and enzymatic detection to identify 18 pesticides in crops at the 0.01:ppm level. Polyamide[319,320] and Florisil[321] layers have also been used for one- and two-dimensional TLC.

Several chromogenic reagents are used to detect organophosphorus insecticides. Spraying with a solution of $AgNO_3$ and bromcresol green or bromphenol blue in acetone followed by spraying with 0.01% citric acid solution or a citrate buffer of pH 4.0 detects thiophosphoryl compounds as blue or magenta spots.[264,313] Treatment with p-nitrobenzylpyridine and tetraethylenepentamine permits the visualization of all organophosphorus insecticides as blue or magenta spots.[322] Enzyme inhibition techniques are becoming increasingly important for the selective and sensitive (e.g., 1 pg of paraoxon[323]) detection of enzyme inhibitors such as organophosphate and carbamate insecticides. An example of the principles involved in these techniques is as follows:

$$\underset{\text{Indoxyl acetate substrate}}{C_6H_4NHC{:}COOCH_3} + H_2O \xrightarrow[\text{e.g., plasma, serum}]{\text{Enzyme}} \underset{\text{Indoxol}}{C_6H_4NHC{:}COH} + \underset{\text{Acetic acid}}{CH_3COOH}$$

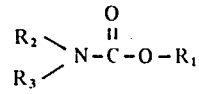
$$2(\underset{\text{Indoxol}}{C_6H_4NHC{:}COH}) + O_2 \longrightarrow \underset{\text{Indigo blue}}{C_6H_4NHCOC{:}COCHNH_4C_6} + 2H_2O$$

These two reactions will occur on the thin layer to produce a blue background color. At the locations of the inhibiting pesticides. the first reaction will not occur so that colorless spots will be evident. Instead of using a chromogenic substrate, the acetic acid produced in the first reaction can be detected by spraying with a pH indicator such as bromothymol blue. When this is done, the spots containing inhibiting pesticides appear blue and the background yellow (acid color of the indicator). A complete review of the analysis of pesticides by the TLC-enzyme inhibition technique has been written by Mendoza,[324] and more general reviews of enzyme inhibition methods, including surveys of application to pesticide residue analysis, by Gardner[325] and Villeneuve.[326] Flavones applied after bromination serve as fluorogenic spray reagents for organophosphorus pesticides on silica-gel layers.[327]

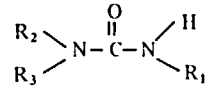
A reflectance scanner designed by Beroza et al.[328] used in conjunction with an automatic sample spotter[329] is recommended[302] for the determination of phosphate residues separated by TLC or PC. When developed further, this approach would seem to have great potential for the accurate and precise analysis of residues of all pesticide classes. The *in situ* fluorimetric analysis of organothiophosphorous pesticides after spraying with 1,2-dichloro-4,5-dicyanobenzoquinone[330] or quenched palladium(II)-calcein indicator solution[331] has also been successfully achieved.

D. Carbamate Pesticides and Related Compounds

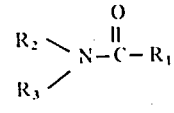
Carbamates are represented by the general formula



In phenylcarbamate herbicides, R_1 is an alkyl group, R_2 is a phenyl or substituted phenyl group, and R_3 is hydrogen. In methylcarbamate insecticides, R_1 is an aryl group or heterocyclic ring, R_2 is a methyl group, and R_3 is either hydrogen (N-methyl carbamates) or a second methyl group (N,N-dimethyl). Phenylurea herbicides are represented by

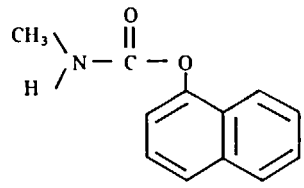


where R_1 is a phenyl or substituted phenyl group and R_2 and R_3 are alkyl or alkoxy groups. Anilide herbicides have the structure

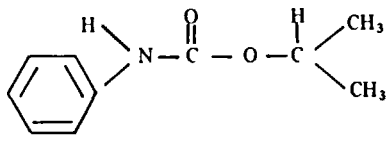


where R_1 is an alkyl group and R_2 and R_3 are hydrogen and substituted aryl groups. Specific examples are given on top of the following page.

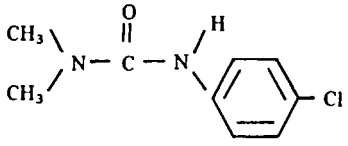
The analysis of crop extracts for substituted urea herbicides and metabolites has been carried out by TLC.[332] Samples were extracted with acetonitrile, chlorinated insecticides and the metabolite 3,4-dichloronitrobenzene were removed by extraction with petroleum ether for analysis by GC, the substituted ureas and other



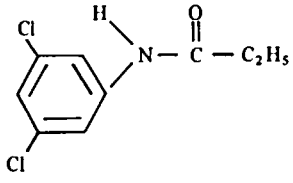
Sevin: N-methyl carbamate insecticide



IPC: N-phenyl carbamate herbicide



Monuron: Urea herbicide



Propanil: Anilide herbicide

metabolites were extracted with methylene chloride followed by CCl_4 and the extracts were combined and concentrated and cleaned up on a MgO-cellulose-Florisil column. Two eluates were obtained from the column: one contained the substituted urea herbicides, aniline metabolites, and 3-(3,4-dichlorophenyl)-1-methoxyurea, and the other contained four other metabolites with the urea moiety. The second fraction was further cleaned up on a Florisil column and both fractions were finally analyzed by TLC on alumina layers developed with dioxane-butyl ether-benzene (1:20:40), methanol-$CHCl_3$-benzene (1:9:25), or dioxane-methylene chloride-butyl ether-benzene (1:10:10:26). Spots were detected under UV light or with dimethylaminobenzaldehyde chromogenic spray for additional confirmation.

Another excellent approach for the analysis of urea herbicides in plant and soil samples involves separation of the corresponding coupled anilines on cellulose plates. The samples are hydrolyzed, steam distilled and extracted according to standard procedures,[333] the compounds are diazotized and coupled with N-ethyl-1-naphthylamine, and the resulting azo dyes are chromatographed with dimethylformamide-0.5 N HCl-ethanol (6:2:2). Aniline and its 4-chloro-, 4-bromo-, 3,4-dichloro-, 3-chloro-4 bromo-, and 3-trifluoromethyl-derivatives were characterized by this method.[334]

Other adsorbents that have proven suitable are polyamide for carbamates,[335,336] and silica gel for carbamates, phenylureas,[336-338] and anilides[339] Detection methods for carbamates and related compounds include UV absorption,[339,340] enzyme inhibition methods,[324] *p*-nitrobenzenediazonium fluoborate-KOH,[335] bromine-fluorescein,[335] Rhodamine B-UV,[335] $AgNO_3$-UV,[335] pinacryptol yellow,[335] $NaNO_3$-1-naphthol,[337] Bratton-Marshall Reagent,[341] $KMnO_4$-UV,[342] diphenylpicrylhydrazyl,[343] vanillin,[343] and flavone reagents.[344] Quantitative analysis is achieved by UV spectrophotometry after elution[337] or by *in situ* fluorimetry. For the latter technique, nonfluorescent pesticides are caused to undergo a reaction which degrades the compound to a fluorescent product (e. g., carbaryl conversion to 1-naphthol by reaction with NaOH[340]) or produces fluorescent derivatives (e.g., dansyl[345-348] or 4-chloro-7-nitrobenzo-2,1,3-oxadiazole[349] derivatives).

Solvent systems and techniques have been reported that yield reliable and reproducible R_F-values for the identification of pesticide residues. These methods have been applied to identification of 16 chlorinated[350] and 61 herbicidal carbamate, urea, triazine, and uracil type pesticides.[351]

The official AOAC and FDA method for the determination of the important carbamate insecticide carbaryl in fruits and vegetables (0.05-2 ppm) is based on TLC.[352] Residues are extracted by blending with methylene chloride, cleaned up rapidly by partitioning, and the extract is developed on an alumina layer with acetone-benzene (1:4). Carbaryl is detected as a blue spot (R_F 0.52-0.60) by spraying with *p*-nitrobenzenediazonium fluoborate chromogenic reagent and deter-

mined by differentiating color intensities between sample and standard spots.

The chromatography of carbamates has been reviewed by Fishbein and Zielinski.[353]

E. Triazine Herbicides

Triazine herbicide residues extracted from vegetables and soil samples with ethyl ether are cleaned up by elution through a basic Al_2O_3 column and a $NaHSO_4$ column.[354] Separations of triazines on silica-gel layers are obtained with toluene-acetone (85:15),[354] CCl_4-absolute diethylamine (90:15),[354] benzene-acetic acid-H_2O (50:50:3) (for hydroxy triazine derivatives[355]), chloroform-isopropyl ether (3:2),[356] CCl_4-nitromethane (1:1),[357] ethyl acetate-petroleum ether (3:7),[358] benzene-chloroform-ethyl acetate (5:4:1),[358] or chloroform-methanol (98:2)[359] as the chromatographic solvent. The last reference lists R_F-values for eight triazines in 91 different solvent systems. Triazine spots are detected by tolidine-KI reagent after chlorination,[355] observation under UV light,[354] $AgNO_3$-UV,[354,356] and Brilliant Green-bromine.[357] Quantitative analysis has been carried out by visual comparison of spot colors[357] and quenching,[358,360] UV reflectance spectroscopy after spot removal,[361] *in situ* scanning of fluorescence quenching,[361] and liquid scintillation counting for labeled triazines.[359]

The chromatography of triazines has been reviewed by Fishbein.[363]

F. Dinitrophenols

The dinitrophenol pesticides DNOC, DNBP (dinoseb), DNAP, and DNOCHP (DN-111) have been separated on cellulose layers impregnated with mineral oil-acetic acid-ethyl ether (5:2:93) by development with methanol-acetic acid-water (73:2:25). Methyl ethers formed by reaction with diazomethane were separated when the immobile phase was 10% mineral oil in ether and the mobile phase was methanol-acetic acid-H_2O (30:25:45). The spots appear orange under UV light after the layers are sprayed in turn with acidic $SnCl_2$ and p-dimethylaminobenzaldehyde reagents.[364]

Several substituted dinitrophenol pesticides were separated on silica-gel layers developed with petroleum ether-ethyl ether-formic acid (90:10:2) and on cellulose layers supporting ethyl oleate with 40% aqueous ethanol as solvent. Spots were detected under UV light or by spraying with 2% ethanolic KOH.[365]

A TLC procedure for identification and quantitation of 2,4-D, dicamba, MCPP, 2,4-DB, DNOC, and DNBP in various crops at a sensitivity of $\leq$ 0.05 ppm has been described.[366] After extraction and cleanup by alkaline hydrolysis and partition, the herbicide residues were converted to the nitro derivatives by reaction with 2% $NaNO_2$ in concentrated H_3PO_4. The derivatives were chromatographed on silica-gel layers with benzene-acetic acid (85:15) and visualized as pink, purple, and blue spots by reducing nitro groups to amines followed by diazotization and coupling with the Bratton-Marshall reagent.

G. Dithiocarbamate Fungicides

For the TLC determination of ethylenethiourea (a degradation product of ethylenebisdithiocarbamate fungicides) in tomato foliage, soil, and water, samples were extracted with dioxane and two chromatographic solvents, namely chloroform-*n*-butanol-methanol-H_2O (100:5:1:0.5) and dioxane-formalin-acetic acid-H_2O (3:1:1.5:1), were used for silica-gel TLC.[367]

Residues are extracted from leaves by shaking with chloroform and concentrates are developed on silica gel with benzene for dimethyldithiocarbamates and benzene-methanol-acetic acid (48:8:4) for ethylenebisdithiocarbamates. Spraying with copper (I) chloride-hydroxylamine reagent permits the detection of 2.5 μg of pesticide.[368]

Residues of dialkyldithiocarbamates and metabolites in animal material and plants are extracted with chloroform, chromatographed on alumina layers with heptane-benzene-acetone (10:1:22.5) and detected by spraying with sodium azide reagent. Spots can be eluted in 0.2 N NaOH and analyzed by UV spectrophotometry at 250 to 280 nm.[369]

H. Organomercurial Fungicides

Dithizonate derivatives prepared after extraction of residues from fruits and vegetables may be separated on silica gel or alumina by development with hexane-acetone (19:1). The sensitivity of visual detection of the naturally red or yellow derivatives is about 2 μg.[240]

I. Miscellaneous Classes

The TLC of pyrethrins has been described by Stahl and Pfeifle[370] and that of methylenedioxy-

phenyl synergists has been described by Fishbein et al.[371]

J. Multiclass TLC Studies

This section will review studies in which TLC was applied to the analysis of pesticides of diverse chemical classes.

A screening method for chlorinated, phosphate, and carbamate pesticides in vegetables at the 500-ppb to 0.05-ppm level employed extraction, chromatography on $AgNO_3$-impregnated alumina layers in a special chamber at controlled humidity, and detection by exposure to UV light for chlorinated pesticides and cholinesterase inhibition for phosphates and carbamates.[372] Screening methods for carbamate and phosphate insecticides in foods[373] and chlorinated and phosphate insecticides in tobacco[374] have been reported. Spray reagents described for the general detection of pesticides include 0.05% fisetin in isopropanol followed by inspection under UV light,[375] hydriodic acid,[376] and diphenylamine-HCl.[377] Numerous pesticides have been chromatographed and detected by electron-donor-acceptor complexing reagents.[380] Five herbicides representing four different classes were determined by the ring-oven technique after separation by TLC.[381]

XI. HIGH-SPEED LIQUID CHROMATOGRAPHY

High-speed liquid chromatography (LC) is a new and exciting approach to the separation and determination of poorly volatile and heat-labile pesticides. With analyses performed by LC, there is usually no need to form derivatives or to extensively clean up sample extracts. The most widely used LC detectors, the ultraviolet photometer and the refractometer, are nondestructive, thus facilitating collection of separated compounds for further confirmation. The contribution of the mobile liquid phase to the selectivity of the chromatographic system makes it possible to perform many complex separations. A disadvantage of LC at the present time is that detector sensitivity is not adequate for applications that require determinations of picogram quantities of pesticides. Complete, automated LC instruments with high-pressure (5000-psi) pumping systems are available from various commercial sources.

The theory, principles, and practice of LC have been described in two recent books.[382,383] The analysis of pesticide residues by LC has been thoroughly reviewed by Horgan.[384] Two excellent papers on LC detectors have been published by Conlon[385] and Veening.[386] Articles on column technology[387] and high-performance column-packing materials[388] are also available.

Lindane residues in isooctane extracts of spinach and fish have been determined without cleanup by injection into a liquid chromatograph containing a 3 ft x 2.3 mm i.d. stainless steel column packed with 10% (w/w) β,β-oxydipropionitrile on 37 to 50-μ Porasil C as support with isooctane carrier liquid and a differential refractometer detector. Fractions were collected and lindane was confirmed by TLC. The sensitivity of this system was such that about 200 μg of injected lindane standard gave about 1/3 scale deflection. The amounts of samples injected were 150 μl of spinach extract (representing 15 g of spinach) and 20 μl of fish extract (representing 1.8 g of fish).[389]

Synthetic herbicide mixtures have been separated by reversed-phase LC. IPC, CIPC, and two thiocarbamates were separated in 4 min on a 2 ft x 2.3 mm-i.d. Corasil/C_{18} column at 79°C with 50% aqueous methanol as solvent flowing at 1.5 ml/min (1,750 psig pressure) and a UV (254 nm) detector. 1-Naphthaleneacetamide and the methyl ester of 1-naphthaleneacetic acid were separated under the same conditions in 2 min, as was a mixture of 7 ng of fenuron, 12 ng of monuron, and 5 ng of diuron.[384] Corasil/C_{18} is a packing material (Waters, Inc.) consisting of a Corasil base with a C_{18} silane chemically bonded to the surface. Corasil consists of a solid glass core with a thin porous silica surface. The same column packing separates PCB isomers when eluted with acetonitrile-water.[390]

LC has been used to detect and isolate impurities in the pesticide Abate and to determine Abate in water after a simple extraction with chloroform. Separations were carried out on a Zipax (Dupont®) support coated with 1.0% β,β-oxydipropionitrile with heptane as the carrier liquid. Abate was eluted in about 3 min and was detected with a sensitivity of about 1 ng with a UV detector.[391]

Parathion and methyl parathion residues were determined in crop extracts at the 0.01-ppm level by LC using a polarographic detector.[392] The column consisted of silanized diatomaceous earth

coated with isooctane, and the carrier liquid was water-ethanol-acetic acid-NaOH-KCl (60:1:38.8:0.80:0.21:0.09 w/w) saturated with isooctane.

Nanogram quantities of the fungicide hexachlorophene have been detected by ultraviolet LC using a stainless steel, column 2 ft long and 2.3 mm i.d. packed with Sil-X silica, 36-40 μ (Nester Faust) with hexane-*n*-butyl chloride (55:45) solvent flowing at 0.7 ml/min (200 psi). The hexachlorophene was derivatized to its di-*p*-methoxy benzoate or dianisate ester after extraction and cleanup. Preliminary studies indicated a sensitivity of 0.03 ppm (40 ng detected) for hexachlorophene in blood serum or other body fluids.[393]

LC obviously has also a great potential for pesticide formulation analysis, e.g., the assay of dust formulations of methomyl insecticide.[394]

XII. AIR ANALYSIS

Pesticides are introduced into the atmosphere by volatilization from sprayed crops and soils, by direct spraying of crops at ground level or from aircraft, and by emission from pesticide manufacturing or formulation plants. In order to gather information on the extent of human exposure to airborne pesticides, the Division of Pesticide Community Studies of the U. S. Environmental Protection Agency, in cooperation with the EPA Perrine Primate Laboratory, Perrine, Fla., has set up a National Air Monitoring Program with some 40 air-sampling sites throughout the United States. The findings in the first two years of this monitoring program, which have been reviewed,[395] indicate that the predominant airborne pesticides are malathion, DDT and its metabolites, BHC, dieldrin, ethyl and methyl parathion, and diazinon.

The National Air Monitoring Program currently uses a collecting system composed of ethylene glycol in a Greenburg-Smith type impinger, developed by Midwest Research Institute,[396] interfaced with the FDA Florisil multiresidue analytical procedure.[397] Descriptions of the air-sampling unit and procedures for collecting samples, reporting results, evaluating ethylene glycol, and performing the analyses are contained in *Analysis of Pesticide Residues in Human and Environmental Samples*, Section 8.[13]

Although some 24 chlorinated and four phosphate pesticides are being routinely monitored with success by the existing analytical method, it has the limitation that only those pesticides extracted from ethylene glycol by hexane and subsequently eluted from the Florisil column by the mixed ether eluents may be identified and determined. Carbamates and numerous phosphate pesticides[398] would not be determined by this system because of these limitations.

A multiresidue, multiclass analytical method for chlorinated, phosphate, and N-methyl carbamate insecticides in the ethylene glycol trapping solvent has been developed to overcome the difficulties with the FDA multiresidue procedure in the air-monitoring scheme.[399] Although this method has to date only been tested with a limited number of compounds, it is apparently suitable for the recovery, identification, and determination of many more pesticides than the present method. The procedure involves partitioning and extracting the pesticides from 100 ml of ethylene glycol, after adding 600 ml of 2% Na_2SO_4 solution, with 40 ml of methylene chloride, fractionation and cleanup by elution through an l-g silica-gel (20% H_2O) column with 10 ml of hexane, 15 ml of 60% benzene-hexane, and 15 ml of 5% acetonitrile-benzene, and determination of chlorinated pesticides by EG-GC, phosphates by FPD-GC, and carbamates by EG-GC after derivatization with pentafluoropropionic anhydride (see Section VII, F above). Excellent recoveries (90 to 110%) of most of the 27 compounds tested were obtained at the lowest levels studied, 1 to 4 ng/m^3 of air. Problems were encountered with several compounds, especially Baygon, Matacil, and Zectran, at these low levels because of background interference from the ethylene glycol. It is expected that this new multiresidue approach involving extraction with methylene chloride and cleanup with silica gel will be adaptable for the determination of pesticide residues in samples other than air, although additional cleanup steps (e.g., gel-permeation chromatography) may be necessary for especially difficult samples such as human fat. To this end, it was found that all of the pesticides studied were quantitatively extracted by methylene chloride from acetonitrile (a common solvent for extraction of pesticides from food, fat, blood, and other environmental samples) when the ratio 4 ml acetonitrile :25 ml 2% Na_2SO_4 is used and extraction is carried out with 5 ml of methylene chloride followed by two more 2-ml portions.

Alternative, perhaps superior, approaches have

been proposed for air sampling of pesticides, and still others are now being actively studied. The more convenient sampler devised by the Syracuse University Research Corporation[401] utilizes much higher sampling rates than the MRI collector and glass beads coated with cottonseed oil in place of ethylene glycol. Another approach is exposure of a nylon chiffon cloth screen, saturated with 10% ethylene glycol in acetone, to the atmosphere for five days, followed by Soxhlet extraction of the pesticides from the cloth with hexane-acetone (1:1), cleanup with alumina and Florisil column chromatography, and GC determination.[402] Results with the screen are only qualitative as the amount of air passing through is variable and not measured. Support-bonded chromatographic phases[403] appear to be efficient collecting media which should provide little background interference, and their uses for pesticide analysis should be studied.

XIII. ANALYSIS OF WATER AND SOIL SAMPLES

"Grab" (instantaneous) samples of water may be collected in wide-mouth glass (never plastic) bottles if the sample size dose not exceed 2 liters. Samples of 1 gal or more may be collected in the bottles in which commercial pesticide-grade acetone is supplied. Bottle caps should be Teflon-lined. The size of the sample required is determined by the sensitivity of the method employed for each analysis. In addition, enough sample should be collected to permit running duplicate, spiked, and background analyses. Due to the instability of many pesticides in water, samples should be extracted and analyzed at once. If samples must be stored, they should be placed in a cool, dark place, preferably a refrigerator, and holding time and storage conditions should be reported with the results. Continuous sampling may also be carried out, e.g., by the use of carbon adsorption cartridges. Methods for collecting open-ocean marine-organism samples without contamination have been elaborated.[404]

Sample-collection glassware should be scrupulously clean, as described above in Section III,B. Precautions for standards, reagents, and solvents should also be followed as described above. There have been two studies[405,406] of the contaminants that are contributed to samples by organic solvents, glassware, plastic ware, cellulose extraction thimbles, filter papers, silica gel, and other chemicals and materials, and their authors suggest methods for overcoming these problems for sensitive analyses by EC-GC.

Hexane or hexane-ether are usually suitable solvents for the extraction of chlorinated pesticides from water. Phosphates may be extracted with hexane or petroleum ether,[407] or with chloroform for more polar compounds, and carbamates with methylene chloride. Chlorinated and phosphate pesticides have been extracted together with benzene. A systematic approach based on *p*-values for choosing the pH, solvent, and number of extractions for quantitative liquid-to-liquid extraction of pesticides from water has been applied to organophosphates.[408]

Extracts of soil, water, and sediment often contain large amounts of elemental sulfur, which interferes in the GC analysis of early-eluted compounds by EC or FPD detection. A chemical desulfurization method in which extracts are refluxed with a copper-aluminum alloy (Raney® copper powder) is effective in removing such interference.[409] Addition of metallic mercury to precipitate sulfur has also been recommended.[410]

A. Organochlorine and Phosphate Pesticides

An excellent review of methods for the extraction, cleanup, and determination of chlorinated insecticides in waters and soils has appeared.[411] Pesticides are usually extracted from mud and soil samples in a Soxhlet extractor. Water samples are extracted in a separatory funnel, small bench-type continuous extractor, large continuous extractor,[412] or by reversed-phase liquid-liquid extraction using an impregnated filter.[413] Cleanup is seldom required prior to GC if the pesticide concentration exceeds 50 to 100 ppb. When it is required, cleanup may be effected by column chromatography, TLC, liquid-liquid partition or a chemical technique.

For EC-GC, a one-liter water sample is drained into a 2-l separatory funnel (Teflon stopcock) and extracted twice with 60 ml of 15% ethyl ether in hexane. Thirty-five ml of saturated Na_2SO_4 is added to the sample, and extraction is carried out with 60 ml hexane. Each extract is passed through an Na_2SO_4 column and the combined extracts concentrated to 5 to 6 ml in a Kuderna-Danish evaporator and then to 1 ml in a warm water bath with a stream of air or nitrogen. If the sample contains thermally unstable phosphates, a stream

of air is used exclusively.[414] An exploratory injection of 5 to 10 μl is made, and then dilution, further concentration, and/or cleanup is carried out as indicated by the sizes of pesticide peaks, the presence or absence of interfering peaks, and the background level. Cleanup can be accomplished by acetonitrile partitioning (often useful for high-organic waste-water samples) or Florisil chromatography, using 6% and 15% ether in petroleum ether as eluents.

The sample extract or eluates from the Florisil cleanup may be subjected to TLC in order to confirm residues detected by GC or to clean up residues especially high in organic content prior to GC. For these purposes, silica-gel layers developed with CCl_4 are used, and pesticides are visualized as dark spots after spraying with Rhodamine B (0.1 mg/ml in ethanol) and viewing under UV radiation of short wavelength. When GC is to follow TLC, adsorbent areas known to contain the pesticides are scraped loose of the plate and sucked with mild vacuum into a medicine dropper plugged at the tip with glass wool, and the pesticides are eluted with ether-petroleum ether (1:1).

For MC-GC or electrolytic conductivity GC, 2 to 3-liter samples of water are usually taken because of the lower sensitivities of these detectors. Cleanup will not usually be required with these selective detectors.

Details of all the above procedures will be found in the EPA water analysis manual.[415]

Similar methods are used for the analysis of chlorinated and organophosphorus insecticides in soils and house dust (vacuum cleaner dust bags). Samples (1 to 2 g) of soil or dust are extracted in a Soxhlet apparatus with 10% or 50% acetone-hexane and cleaned up, if necessary, by elution through an alumina column with hexane and then through a Florisil column with ether-petroleum ether. Details of this procedure are found in Section 11 of *Analysis of Pesticide Residues in Human and Environmental Samples.*[13] Soxhlet extraction or extended shaking with hexane-acetone (1:1) was found to give the highest recoveries of 11 chlorinated pesticides from 3 (wet) soil types in a comparative collaborative study.[416]

Cleanup and separation of pesticides extracted from water have been successfully carried out on silica-gel microcolumns. Silica gel grade 950 deactivated with 1% water and eluted with 10% and 60% benzene in hexane was used prior to GC analysis of eight chlorinated compounds extracted by petroleum ether from Iowa river water;[417] silica-gel columns eluted with hexane, 60% benzene in hexane, benzene, and 50% ethyl acetate in benzene were used for separating 50 chlorinated and phosphate pesticides and PCBs extracted from Italian surface waters by continuous extraction[412] with petroleum ether (2 stages) and benzene (3rd stage) into four groups;[418] and cólumns of silica gel and alumina deactivated with 5% water and eluted with various solvents were used for the cleanup and recovery of 18 chlorinated and phosphate pesticides extracted from water.[419]

Chlorophenoxy herbicides and their esters are determined in water by adjusting the sample to pH 2, extracting with benzene or diethyl ether, methylating the acids with diazomethane or BF_3-methanol, followed by EC-GC or MC-GC.[420] 2,4,5-T has been determined by electron-capture detection as the *n*-butyl ester after extraction from soil by shaking with ether-chloroform-glacial acetic acid (25:25:1).[421] The retention time of this ester does not coincide with that of any coextractive. These herbicides have also been determined in water and soil by paper chromatography.[422]

The suitability of TLC for determining residues in different soils without cleanup was found to vary with the chromatographic solvent and soil type. The lower limits of detectability for DDT, DDE, aldrin, and dieldrin were 0.1, 0.2, 0.5, and 1 μg, respectively, in sandy or clay soil.[423]

B. Anilide Herbicides

Propanil, dicryl, Karsil, 3,3′,4,4′-tetrachloroazobenzene, azobenzene, Ramrod, and 2,4-dichloroaniline in soil were extracted by blending with acetone and determined in the filtrate using a flame ionization detector and a 5% UC-W98 column at 150-250°C.[339] The concentrated extract was also analyzed by TLC on silica-gel sheets containing fluorescent indicator and developed with benzene. Detection was obtained under UV light.

C. Carbamate and Urea Herbicides

These pesticides are extracted from acidified water using chloroform or methylene chloride[337] and from soils[424] with acetone and separated and detected as described in Section X,D. Eight urea herbicides were recovered at 76 to 104% levels after shaking for one hour with methanol.[219] Ten

phenylurea herbicides in soil samples were subjected to alkaline hydrolysis and steam distillation-extraction followed by conversion to iodinated derivatives which were detected by GC with a minimum detectability of 0.01 to 0.05 ppm.[215] Also, ten ureas and carbamates have been determined in soils by EC-GC after conversion to 2,4-dinitrophenyl derivatives on a TLC plate.[190]

D. Triazine Herbicides

Triazines are extracted with methylene chloride from water adjusted to pH 9 with ammonia and with diethyl ether from soil treated with ammonia. In the latter case, cleanup is achieved by solvent partitioning prior to TLC as described in Section X,E.[357] GC determination of triazines at the 0.001-ppm level in water was carried out by extraction with methylene chloride, concentration of the extract, and chromatography on a 2% NPGS column at 195°C using a RbBr thermionic detector. This method was superior to ultraviolet spectrophotometry and cathode-ray polarography at this level.[425] When column cleanup of triazines is required, a deactivated (ca. 15% water) alumina (slightly basic or neutral) column eluted with approximately 10% ethyl ether in carbon tetrachloride is recommended.

E. Uracil Herbicides

Bromacil, terbacil, and 3-butyl-6-methyl uracil are extracted from soil with 1.5% NaOH as described for GC[231] and chromatographed on silica gel with benzene-hexane-acetone (5:2:1) followed by chloroform-acetone (9:1) (two-dimensional TLC). Spraying with 0.5% Brilliant Green in acetone followed by bromination permits the detection of the pesticides with a sensitivity of 0.1 ppm based on a 100g sample.[426]

Bromacil and isocil, along with various carbamates and ureas, were extracted from neutral water samples with methylene chloride, developed on silica gel with hexane-acetone (7:3) or chloroform-nitromethane (1:1), and detected at the 0.1-ppm level by spraying with Brilliant Green solution followed by bromination, treatment with 2 N H_2SO_4 and dichlorofluorescein, and exposure to UV light.[427]

F. Miscellaneous Herbicides

Nineteen acidic, neutral, and basic herbicides were determined[428] in soils at the 1-ppm level and in water at the 0.1-ppm level by two-dimensional silica-gel TLC. Acidic herbicides were developed with chloroform-acetic acid (19:1) and benzene-hexane-acetic acid (5:10:2), and neutral and basic herbicides with hexane-acetone (150:45) and chloroform-nitromethane (1:1). Acidic herbicides are separated from neutral and basic compounds by extraction of samples adjusted to appropriate pH values (basic conditions for extraction of neutral and basic compounds with organic solvents, acidic conditions for extraction of acidic herbicides). Basic and neutral herbicides were detected as purple spots under UV light on phosphor-containing layers, and acidic compounds were detected as yellow spots by spraying with bromocresol green adjusted to its basic color.

XIV. METHODS FOR CONFIRMING THE IDENTITIES OF PESTICIDE RESIDUES

One of the major tasks facing the pesticide analyst is to obtain convincing identification of a trace residue. If enough residue is present, and it can be isolated in a reasonably pure state, infrared spectroscopy and mass spectroscopy can provide unequivocal identification. More often, very low concentrations of residue are found and other, less certain, methods must be employed.

Considerations of set theory[429] indicate that three independent "equivocal" results are required in order to be confident of the recognition[430] or identity of a pesticide residue. These might be elution in a certain fraction from a liquid chromatography cleanup column, GC retention time,[431] and positive response of a selective GC detector (e.g., microcoulometric detection for Cl or S). Another possible combination that would be a basis for confidence is the GC retention times from a polar column and a nonpolar one plus an R_F-value from PC or TLC or an extraction p-value. Still another would be a GC retention time, a TLC or PC R_F-value, and the GC retention time of a derivative formed by a chemical or photochemical reaction. One further way to improve specificity is by more rigorous cleanup methods.

The dependence or independence of measured values was analyzed by Elgar,[432] who reported that many widely used confirmatory methods may not give independent evidence of identity since they are measuring the same physical property, and that great care must be taken when deciding which to use in combination so that a great deal of

work is not done without gaining additional useful information. Examples of highly correlated values include GC retention times on many stationary phases (highly polar DEGS and a nonpolar phase are strongly complementary); PC or TLC R_F-values using different adsorbents and/or different solvents; *p*-values in different solvent pairs; and TLC, PC, and *p*-values. These combinations may not provide the necessary independent information.

An excellent treatise on pesticide identification is available from the American Chemical Society as No. 104 of its *Advances in Chemistry Series.* Chapters are included on chemical derivatization techniques for confirmation of organochlorine residues by Cochrane and Chau, identification with microcoulometric and electrolytic conductivity detectors by Westlake, identification with flame detectors by Aue, infrared microtechniques by Blinn, and UV spectrophotometry by Aly, Faust, and Suffet. An extensive review of mass spectrometry (MS) and combined GC-MS has been written by Biros.[433]

The mass spectrometer is the most sensitive spectroscopic tool available to the chemist for identification, providing useful data on 100 ng or less of material. For most compounds, MS will provide not only the molecular weight of the sample being ionized but also information about the number and kinds of functional groups, the presence of hetero atoms, and the arrangement of these atoms in the molecule from characteristic fragmentation patterns. Combined GC-MS has been applied to the identification and determination of pesticides in human adipose and liver tissue. The pesticides were extracted, cleaned up by liquid-liquid partitioning, and separated by a silic-gel chromatographic step into two fractions with sufficient resolution on a 3% OV-210 column to permit the identification of seven pesticide residues. Sensitivities were generally 0.3 ppm, or as low as 0.05 to 0.1 ppm when computer-enhancement techniques were applied.[434] A GC-MS system for the identification of multiple chlorinated residues in foods has also been reported.[435]

IR spectroscopy[436] is generally sensitive at the 1-μg level but has been used down to the 0.1-μg level in some applications. It is thus considerably less sensitive than GC or TLC and cannot be used unless enough sample is available to provide a concentration of the compounds being determined that is large enough for IR observation. The potassium bromide micro-pellet technique applied to trapped GC fractions or excised TLC spots is the most sensitive and dependable, but considerable experience is necessary to prepare pellets with low contamination. Details of this technique are available in Section 12E of *Analysis of Pesticide Residues in Human and Environmental Samples.*[13] Stringent sample cleanup prior to GC or TLC is mandatory if IR spectroscopy is to be successfully applied. The IR and UV spectra of 76 reference pesticides have been published recently.[437] IR identification has been made of chlorinated pesticide residues in sludge, soils, fish, and industrial effluents.[438]

Extraction *p*-values[439-442] are a powerful tool for identifying pesticides at the nanogram level. These values, defined as the fraction of total solute partitioning into the upper phase of a two-solvent system, are determined by means of the single or multiple distribution of a solute between equal volumes of two immiscible solvents. Data for 88 pesticides in six different binary solvent systems have been compiled.[440] *p*-Values for most pesticides are appreciably different from those of normal contaminants. The determination of these values is simplified since only relative, rather than absolute, values are required, and the sensitivity is at the level of electron-capture gas chromatography.

Chemical derivatization methods for confirming pesticide residues have been studied in greatest detail by Cochrane and Chau in Canada.[443] As an example, aldrin may be epoxidized to dieldrin (GC retention time 1.9 relative to aldrin on an SE-30 column) by heating with peroxyacetic acid.[444] Other useful derivatives[432] of aldrin are obtained by reaction with HI and $HgBr_2$ for one hr at 100°C (relative retention of product, 4.3); silver acetate, I_2, and acetic acid (7.5); or N-bromosuccinimide and acetic acid, for 1 hr at 100°C (5.4). A good derivative should have a high electron-capture response, and a retention time that is different from (and preferably longer than) that of the parent pesticide. Dieldrin can be confirmed by reaction with HBr and acetic anhydride for 30 min at 100°C to give a bromoacetate derivative with a retention time of 6.0 relative to aldrin.[432] Other confirmation reactions useful at low levels (0.01 to 0.1 ppm) include the dehydrochlorination[445] of DDT, DDD, and methoxychlor to the respective olefins with ethanolic KOH; the formation of HCB penta-

chlorophenyl propyl ether;[446] the oxidation of DDE with CrO_3; the hydrolysis of endrin, dieldrin, heptachlor epoxide, or oxychlordane with concentrated H_2SO_4; the dechlorination of heptachlor and *p,p'*-DDT (to *p,p'*-DDD) by $CrCl_2$; and the UV irradiation of aldrin, heptachlor, chlordane, and other pesticides.[447,448] PCBs and organochlorine pesticides can be differentiated by observing the reactivity of the residues to HNO_3-H_2SO_4 (organochlorine compounds are destroyed, whereas PCBs and toxaphene are unaffected) and by carbon skeleton chromatography.[449]

Residues of organophosphate insecticides may be confirmed by alkaline hydrolysis to their corresponding dialkylphosphates or thiophosphates followed by esterification and determination by FPD-GC,[450] oxidation to corresponding sulfones with $KMnO_4$[451] or *m*-chloroperoxybenzoic acid,[151] or reduction of hydrophilic metabolites with $TiCl_3$.[452]

Other methods which have been used less frequently for qualitative analysis are polarography,[453] carbon skeleton chromatography,[449,454] and GC fragmentation procedures.[455] Nuclear magnetic resonance spectroscopy is useful for elucidating the structures pesticides and their degradation and metabolic products rather than for residue analysis.[456]

XV. DETERMINATION OF POLYCHLORINATED BIPHENYLS (PCBs)

PCBs are nonpesticide environmental contaminants which interfere with most common chlorinated pesticides in GC residue analysis. This is especially true of *p,p'*-DDT, *o,p'*-DDT, *p,p'*-DDE, and *p,p'*-DDD, since prominent PCB peaks have similar retention times to these compounds on many commonly used GC liquid phases. Separation of PCBs from DDT and certain other chlorinated pesticides prior to GC is achieved by column chromatography on silica gel-Celite (4:1). PCBs (plus chlorinated naphthalenes,[457,458] if present) are eluted from the column with petroleum ether prior to elution of pesticides with acetonitrile-hexane-methylene chloride (1:19:80). The method is applicable to samples extracted and cleaned up on Florisil (6% ether-petroleum ether fraction) in the usual way (see Section IV, C, D).[459] Recovery of PCBs is known to decrease with decreasing chlorine content,[460] and contamination of the pesticide fraction with PCBs has been detected by a GC-MS procedure.[435] Other workers have also used silica-gel-column chromatography for the difficult separation of PCBs from *p,p'*-DDE.[461,462] The purity of the nonpolar solvents used in these separations is critical for consistent elution patterns.[463] Column chromatography on alumina has been used to separate PCBs from dibenzodioxins.[464]

Cleanup methods need not be as rigorous for GC-MS techniques as for conventional GC.[465] Less than 10 ng *p,p'*-DDE has been detected and determined in the presence of Aroclor 1254 by monitoring the m/e = 246 peak of the GC effluent.[466]

A human monitoring program for PCB levels in human adipose tissue samples has been carried out since April, 1971, using a semiquantitative TLC procedure. PCBs were found in over 50% of the more than 2,000 samples analyzed, ranging in level from trace amounts to 16 ppm.[467] In this method,[468] adipose tissue is subjected to extraction by petroleum ether, partitioning with acetonitrile, and cleanup on Florisil. A portion of the 6% ether-petroleum ether fraction concentrate is treated with KOH to dehydrochlorinate DDT and DDD to their olefins so that separation from PCBs is not a problem. Oxidative treatment with CrO_3 is then applied to convert any interfering DDE to *p,p'*-dichlorobenzophenone, which has an R_F-value different from that of any of the PCBs on an $AgNO_3$-impregnated alumina layer developed with 5% benzene in hexane. Exposure to UV light permits detection of PCBs, and quantitative estimation is by visual comparison with Aroclor 1240 standards ranging from 25 to 400 ng/μl in hexane. PCB levels (0.4 to 3.5 ppm) found in 140 general population samples in this monitoring program have been confirmed and identified by combined GC-MS.[469] Computer-assisted GC-MS has also been used to identify PCBs in fish extracts after silica gel column chromatography,[470] and DDT and its metabolites have been converted to dichlorobenzophenones prior to GC determination in the presence of PCBs.[471]

TLC on alumina with *n*-heptane as solvent separates PCBs and pesticides.[472] PCB separations have also been made by two-dimensional[473] and reversed-phase TLC.[474] Chlorodibenzo-*p*-dioxins

were separated from pesticides and PCBs by TLC.[475]

The quantitation of PCBs by GC has posed a serious problem since many Aroclor preparations are in existence with different chlorine percentages, the electron capture response of the individual chlorobiphenyls is variable, and chromatograms of environmental samples may be very complex. Quantitation based on commercial PCB preparations have been made using total peak areas,[459] areas of one or more selected peaks,[476] peak heights,[477] and average electron-capture detector responses.[478] GC curves, relative retention times, and response data from 10% DC-200 and 15% QF-1/10% DC-200 (1:1) columns have been compiled for six of these Aroclors used as analytical references.[479] Quantitative standards of Aroclors 1221 through 1260, in which the weight of PCB in each peak is known, have now become available, along with a systematic method for their use in the electron-capture determinations of the constituents of environmental samples.[480] Another recommended approach to the reliable quantitation of PCB mixtures is the use of programmed-temperature GC on SCOT (support-coated open tubular) columns with electrolytic conductivity or microcoulometric detectors, in conjunction with mass spectrometry to confirm the identification of a given chlorine peak and to determine the number of chlorine atoms per molecule (necessary for quantitation by microcoulometry).[481]

PCBs have been recovered from water in the parts per trillion range by liquid-liquid partition between water and Chromosorb W coated with *n*-undecane and Carbowax 4000.[413] Porous polyurethane foam also adsorbs PCBs from water.[482]

A very promising method[483] for PCB determinations involves perchlorination with antimony pentachloride followed by GC measurement of the amount of the resulting decachlorobiphenyl. PCBs are first separated from pesticides by charcoal column chromatography: pesticides are eluted by 25% acetone in ethyl ether, PCBs by benzene. Chlorinated pesticides may be selectively detected in the presence of PCBs by use of a modified Coulson conductivity detector at 600°C with a hydrogen flow of 1 to 2 ml/min.[484]

Other aspects of the chromatographic determination of PCBs are described in a review by Fishbein,[485] and the current status of PCB interference and analysis in terms of the AOAC-FDA multiresidue method for chlorinated pesticides has been detailed by Burke.[486]

XVI. ANALYSIS OF INDIVIDUAL PESTICIDE RESIDUES

In most instances, the procedures discussed above are multiresidue analytical methods which are designed to provide determinations of a number of pesticides of a single chemical class or, less often, of several classes. Procedures developed for the determination of individual residues are usually very similar in nature, and space limitations do not allow coverage of these methods for the hundreds of pesticides in use today. Readers interested in methods for these specific chemicals in specific samples are directed to compilations available in Volume II of the FDA Pesticide Analytical Manual,[7] Volumes 6 and 7 of the series on Pesticide Analysis edited by Zweig[6] for Academic Press, and a review on the analysis of herbicide residues by GC.[487]

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