Journal of Chromatography, 113 (1975) 97–137
Chromatographic Reviews

CHREV. 82

CHROMATOGRAPHIC ANALYSIS OF FUNGICIDES

JOSEPH SHERMA
Department of Chemistry, Lafayette College, Easton, Pa. 18042 (U.S.A.)
(Received July 19th, 1974)

CONTENTS

1. INTRODUCTION

The term "pesticide" (economic poison) is generally taken to include those compounds functioning as insecticides, nematocides, miticides (acaracides), growth regulators, fungicides, and rodenticides. Fungicides are compounds used on farm crops as protective agents against the attack of a fungus. Types of fungicides in use today include soil fungicides, seed protectants, foliage and fruit protectants, and eradicants.

This review will cover chromatographic methods for the separation and analysis of fungicides and metabolites as standards and as residues in various sample matrices. Only organic fungicides will be included, and these will be classified as organomercury compounds, carbon disulfide–amine reaction products (dithiocarbamates), halogen-containing compounds, cationic compounds, and miscellaneous compounds. The difficulty with neat classification of fungicides is indicated by the fact that within some of these classes are compounds of different chemical types, *e.g.*, Dyrene and dichlone both contain halogen but the former is a triazine and the latter a quinone. Some of the newer organic fungicides are "systemic" in nature, that is they are translocated to other parts of the plant than those to which they are applied. However, studies on the mechanism of fungicide action will not be covered in this review, nor will properties, uses, ecological, or toxicological aspects. The analysis of fungicide formulations will be only occasionally mentioned. Formulae and alternate names for all fungicides mentioned will be found in the appendix at the end of this paper.

The literature of fungicide chromatography is covered through May, 1974. The attempt was made to be selective in the choice of cited references and to present only those methods which appear most likely to give satisfactory results in practical analytical situations.

Analytical methods for pesticides usually consist of the following steps: sampling and sample preparation, extraction, liquid–liquid partition cleanup, liquid–solid cleanup, and determinative procedures. The exact nature of the procedures required depends upon the residue of interest and the sample type. Tentative qualitative identification and quantitation of residues is normally carried out using either gas–liquid chromatography (GLC), thin-layer chromatography (TLC) or high-speed liquid chromatography (HSLC), while confirmation of identity is obtained by combination of two or more chromatographic methods, chemical derivatization, or spectrometric analysis. General aspects of chromatographic pesticide residue analysis have been covered in two recent books[1,2] and a review article[3], while other review articles have dealt with the chromatography of herbicides[4], carbamate insecticides and herbicides[5,6], triazine herbicides[7], fumigants[8], and organomercurials[9].

The primary chromatographic method presently employed in pesticide residue analysis is gas–liquid partition chromatography. This is because of the availability of a series of sensitive and selective detectors[10,11] for pesticide compounds containing halogen, P, S, and N atoms. These detectors, which are applicable in fungicide analyses, include the relatively non-specific electron capture detector, and the specific microcoulometric, flame photometric, alkali flame ionization, and electrolytic conductivity detectors. The latter greatly facilitate analysis due to the minimal amount of cleanup of extracts that is required. Cochrane and co-workers have systematically

evaluated and compared these detectors for pesticide analysis in a valuable series of papers[12–16]. Thermal conductivity and flame ionization detectors are normally not useful for residue analysis but may be employed to advantage for pesticide formulation analysis.

2. BEHAVIOR OF FUNGICIDES IN MULTIRESIDUE PESTICIDE ANALYTICAL PROCEDURES

Various systematic schemes have been developed for analyzing residues of a large number of pesticides. Although chlorinated and organophosphorus insecticides and chlorophenoxy acid herbicides have mostly been studied, some fungicides and metabolites have been tested through various portions of these multiresidue procedures. The fungicides PCNB and TCNB were quantitatively extracted (80–100%) from fruits, vegetables, and cereal grains by blending with acetonitrile[17,18]. Fungicides recovered from evaporated acetonitrile extracts by hexane partition included captan, chlorothalonil, Dyrene, folpet, and PCNB[17,18]. Chlorothalonil, captan, HCB, and PCNB were recovered when an acetonitrile extract was diluted with water, sodium sulfate solution was added, and some dichloromethane was added to the hexane before partitioning. A mildly acid buffer was required to prevent hydrolytic decomposition of captan[18]. Dyrene and PCNB were recovered from hexane extracts of butterfat by acetonitrile partition[18].

Pesticides extracted from samples by acetonitrile and then partitioned into hexane may be cleaned up prior to GLC or TLC by chromatography on a 3.0-g Darco G60 carbon-Solka Floc BW40 cellulose column contained in a 100-ml buret. Captan and PCNB are eluted in fraction 1 with 180 ml 1.5% acetonitrile in hexane, folpet with 200 ml chloroform and chlorothalonil and Dyrene with 200 ml benzene[17,18]. Fraction 1 will also contain most chlorinated insecticides (DDT) and polychlorinated biphenyls (PCBs), while fraction 2 will contain organophosphorus insecticides (parathion).

A differential elution scheme was worked out for separating and confirming some 55 pesticides by use of a 15 × 2.5 cm column of Florisil partially deactivated with 2% water and eluted in turn with ten 300-ml portions of hexane containing 0–30% dichloromethane followed by 5–30% ethyl acetate. Six fungicides were included in the study[18] and were eluted as follows: HCB in the hexane fraction, PCNB and TCNB in the two fractions of hexane containing 5 and 10% dichloromethane, Dyrene in the 5% ethyl acetate fraction, chlorothalonil in the 5 and 10% ethyl acetate fractions, and captan in the 20% ethyl acetate fraction. All recoveries were greater than 95%, except captan, which was 80%. The United States FDA multiresidue Florisil cleanup procedure[19] employs activated Florisil columns (4 in. × 22 mm), eluted with light petroleum (b.p. 30–60°C) containing 6–65% diethyl ether. Table 1 shows results for the fungicides tested through this procedure. With an improved elution system, PCNB and TCNB were eluted ≥ 90% from the FDA Florisil column with 200 ml 20% dichloromethane in hexane, DCNA and dichlone with 200 ml 50% dichloromethane–0.35% acetonitrile–49.65% hexane, and captafol and captan with 200 ml 50% dichloromethane–1.5% acetonitrile–48.5% hexane. Folpet divided between the second and third fractions[20].

TABLE 1

ELUTION PATTERN OF FUNGICIDES FROM AN ACTIVATED (1200°F) FLORISIL COLUMN[18,19]

Fungicide	*Diethyl ether (%) in light petroleum (b.p. 30–60 °C) used for elution (200-ml fractions)*	*Recovery (%)*
DCNA (Botran)	6, 15*; 6, 15, 20	<80; >80
Captafol	6, 15	0
Captan	6, 15	0
Captan epoxide	6, 15	0
Chloroneb	6	>80
Chlorothalonil	6, 15	0
Dichlone	6, 15	0
Dinocap	6, 15	>80
Dyrene	6, 15	>80
Folpet	6, 15, 20	>80
Hexachlorobenzene	6	60**; >80***
Hexachlorophene	6, 15, 50	0
Chloronitropropane (Korax)	6, 15	0
PCNB	6	>80
TCNB	6	>80
Tetraiodoethylene	6	>80

* 6, 15 means 6% followed by 15%.
** Fatty foods.
*** Non-fatty foods.

Captan and HCB were recovered ≥ 80% at levels greater than 0.05 ppm from spiked plant and animal tissues and mixtures by a low-temperature (−78°C) precipitation cleanup method used to separate fats, oils, and water from acetone–benzene–1 *N* H_2SO_4 (19:1:1) extracts[18,21].

Another important multiresidue scheme in use today employs deactivated (5% water) silica columns for separation and cleanup of residues prior to GC[22–24]. Elution is two-stage, the first eluent being hexane and the second diethyl ether–hexane (1:9). This system has been used mainly for organochlorine insecticides, PCBs, and a few organophosphate insecticides. Although no fungicide recoveries have been reported, it is likely that many would be quantitatively eluted, especially if a third, more polar solvent were used.

Only a few types of GLC liquid phases have been extensively used for separating pesticide mixtures, and the relative retention times of some fungicides on these phases are shown in Table 2. Temperature and carrier gas flow-rates are chosen for routine analyses with these columns so that the reference compound elutes in approximately the time given. Column temperatures of 180–225°C and flow-rates of 60–120 ml/min are typical for 6-ft. × ¼-in. columns in which the liquid phases are coated on the support (*e.g.*, Chromosorb W AW) at levels of 3–15%.

TLC studies of a series of organochlorine pesticides on 250-μm layers of MN silica gel G-HR included the fungicides chlorothalonil and captan[18]. The pesticides were detected by spraying with the usual $AgNO_3$–2-phenoxyethanol reagent solution followed by exposure to ultraviolet (UV) light. Chlorothalonil had an R_F value of 0.3 when developed with 1% acetone in hexane, while captan remained at the origin

TABLE 2

RETENTION TIMES OF FUNGICIDES RELATIVE TO PARATHION ON SEVERAL GLC LIQUID PHASES[18,19,25]

Compound	RRT_p			
	*NP**	*IP***	*P****	*M*§*
TCNB	28	—	—	20
HCB	43	10	—	22
PCNB	50	—	—	32
Chlorothalonil	52	60	77	64
Tetraiodoethylene	54	—	—	27
Dichlone	54	—	—	46
Parathion	100 (5 min)	100 (20 min)	100 (4 min)	100 (14 min)
Dyrene	120	50	182	79, 96§§, 133§§
Folpet	120	101	—	107
Captan	122	40	—	112
Hexachlorophene	—	130	—	—

* Non-polar phases, *e.g.* DC-11, DC-200, SE-30, OV-1.
** Intermediate polarity phases, *e.g.* OV-210, QF-1.
*** Polar phases, *e.g.* DEGS.
§ Mixed phases, *e.g.* SE-30/QF-1.
§§ Minor peak.

with this solvent and with hexane, benzene–hexane (1:1), and 1% methanol in hexane.

The above indicates the results obtained for a few fungicides which have been tested through parts of several widely used multiresidue pesticide screening procedures. Other fungicides, especially those which are apolar, might be successfully analyzed by these procedures, but in most cases their behavior has not been studied nor has their recovery been validated. The following sections of this review list more specific procedures related to the analysis of certain fungicide residues.

3. ORGANOMERCURY COMPOUNDS

The identification and determination of organomercurial fungicide residues by TLC and GLC of the dithizonates was reported by Tatton and Wagstaffe[26]. R_F values and retention times are shown in Table 3. The method for determination of residues of these fungicides at 0.01- to 5-ppm levels in various foodstuffs involved extraction of the sample with a slightly alkaline solution of cysteine hydrochloride in 2-propanol, washing of the extract with diethyl ether, and extraction of the organomercurials from the aqueous solution using 0.005% dithizone in diethyl ether. The extract was dried by passage through a Na_2SO_4 column, concentrated, and examined by TLC and/or GLC as described in Table 3. Gherardi *et al.*[27] also used dithizonates for the separation of methylmercury and phenylmercury by development on 0.3-mm alumina thin layers (activated at 150 °C for 30 min) with diethyl ether–light petroleum ether (3:7) as solvent. Dithizonates were identified by R_F values and spot colors. Mercury compounds were extracted by the Westöö[28] method from canned tuna fish samples prior to derivatization. Underivatized organic mercury halides were detected

TABLE 3

R_F VALUES × 100 OBTAINED BY TLC AND GLC RETENTION TIMES (min) OF ORGANOMERCURY FUNGICIDE DITHIZONATES[26]

Systems: (1) Silica gel/hexane–acetone (9:1). (2) silica gel/hexane–acetone (19:1). (3) silica gel/hexane–acetone (93:7). (4) alumina/hexane–acetone (19:1). Layer thickness, 250 μm; detection by natural yellow or red colors. (5) 2% PEGS on Chromosorb G AW DMCS, 60–80 mesh; glass column 1.5 m × 3 mm; nitrogen carrier gas; ECD. (6) Same as (5) except 1% PEGS and 1.2-m column length.

Dithizonate	*TLC systems*				*GLC systems*	
	1	*2*	*3*	*4*	*5*	*6*
Methylmercury	64	48	57	89	1.2	—
Ethylmercury	64	51	62	91	2.0	—
Methoxyethylmercury	32	16	25	58	4.9	—
Ethoxymethylmercury	44	23	34	71	4.9	—
Phenylmercury	48	34	46	72	27.0	5.0
Tolylmercury	52	40	53	79	19.5	3.2
Mercury di-dithizonate	19	9	17	19	—	—

by TLC on silica gel under similar conditions with 4,4′-bis(dimethylamino)thiobenzophenone as spray reagent.

Geike and Schuphan[29] studied the detection of organomercury fungicides after TLC by enzymatic and chemical techniques. Urease proved to be most sensitive for detection, with limits of 50–1000 ng for the fungicides and 1–60 μg for impurities found in the fungicides tested. Bovine liver esterase and α- and β-amylase were also inhibited by the fungicides, but detection was generally less sensitive. Chemical detection with sodium sulfide and dithizone was sensitive to 0.5–20 μg of the fungicides, and impurities did not interfere. Diisopropyl ether and chloroform–ethyl acetate (10:4) were suitable solvents for the separation of the nine fungicides studied, namely methylmercury chloride, methylmercury sulfate, methoxyethylmercury chloride, phenylmercury acetate, Merthiolate, Germisan, Quinex, Panogen, and Memmi.

Bache and coworkers[30,31] developed GLC methods for determining organomercuric fungicides at levels of 1 ppb* in crops grown in treated soils. A microwave powered plasma emission detector and columns and conditions as shown in Table 4 were employed.

Ealy *et al.*[32] determined methyl-, ethyl-, and methoxyethylmercury halides in environmental samples by leaching with molar sodium iodide for 24 h, extracting the alkylmercury iodides into benzene, and determination by GLC on a column of 5% cyclohexylenedimethanol succinate on Anakrom ABS at 200 °C with nitrogen carrier gas and an electron capture detector. Good separation of peaks was obtained for the mercury compounds as either chlorides, bromides, or iodides.

The GLC determination of inorganic mercury alone or in the presence of organomercurials (methyl-, ethyl-, and phenylmercury(II) chlorides) in water and a wide range of biological media was studied by Zarnegar and Mushak[33]. The method for inorganic mercury was based on the ability of various organometallics [*e.g.*, pentacyanomethylcobaltate(III)] to react electrophilically with the mercury to yield

* Throughout this article, the American billion (10^9) is meant.

TABLE 4

GAS CHROMATOGRAPHIC RETENTION TIMES AND SENSITIVITIES OF ORGANOMERCURY FUNGICIDES[31]

Compound	*Retention time (min)**	*Sensitivity (ng)***
Dimethylmercury	6.8	0.6
Methylmercury chloride	2.8	0.6
Methylmercury dicyanodiamide	2.8	0.8
Benzylmercury acetate	45.0	8.8
Methylmercury dithizonate	3.0	0.7

* Column for dimethylmercury: 2 ft., Chromosorb 101, 60–80 mesh, 100°C, 80 ml/min carrier gas flow-rate; for other compounds: 6 ft., 20% OV-17/QF-1 (1:1) on Gas-Chrom Q, 80–100 mesh, Carbowax treated, 152°C, 80 ml/min.

** For 50 % full scale deflection.

alkyl and aryl mercurials which were determined by GLC. Co-determination of inorganic mercury and the organomercurials originally present in the samples was carried out by sequential (difference) or simultaneous procedures. An electron capture chromatograph with 18-in. coiled-glass columns containing Durapak Carbowax 400 (low capacity factor, K') on Porasil F (140 or 170°C) or 10% DEGS on Anakrom SD (190°C) and argon–methane carrier gas proved optimal. Low nanogram levels of mercury were detected with this procedure.

4. DITHIOCARBAMATES

The compounds covered in this section include dimethyldithiocarbamates (ferbam, thiram, ziram), ethylenebisdithiocarbamates (nabam, maneb, zineb), the breakdown products ethylenethiourea and carbon disulfide, and dazomet.

The dithiocarbamates constitute the most important organic fungicides used to control plant fungus diseases. Residues of these compounds have traditionally been determined by a CS_2 evolution method with colorimetric read out[34–37]. Alternatively, ferbam, maneb, nabam, thiram, zineb, and ziram residues may be analyzed indirectly by GLC determination of the CS_2 generated when a fruit or vegetable sample and the residue are reacted with 1.5% $SnCl_2$ in 4 N HCl at 60°C in a closed system. An aliquot of head-space gas in the reaction flask is then injected into a chromatograph equipped with a flame photometric detector in the sulfur mode (394 nm) or an electron capture detector. Nanogram quantities of CS_2 may be detected, and fungicides at 3.5 and 7.0 ppm levels were recovered in the range 82–112% from a variety of spiked crops[18,38]. The column used for GLC of CS_2 was 6 ft. × ¼ in., packed with 10% SE-30 or 6% QF-1/4% SE-30 on Chromosorb W HMDS at 50°C. With a carrier flow-rate of 40 ml/min, CS_2 eluted in about 1 min. The electron capture detector (ECD) gave a linear plot for peak height *vs.* nanograms CS_2 injected between 0.14 and 0.69 ng while the flame photometric detector (FPD) gave a linear logarithmic plot between 0.069 and 0.41 ng[18,38].

Zielinski and Fishbein[39] reported that zineb, maneb, and nabam released ethylenethiourea when fungicide samples were injected into a gas chromatograph.

A 6-ft. column of 4% QF-1 at 180°C and a nitrogen carrier gas flow-rate of 86 ml/min gave a retention time of 3.75 min for the thermal decomposition product.

The dithiocarbamates zineb, ziram, and ferbam have been "stripped" or washed from surfaces of some vegetable and fruit samples with chloroform prior to TLC analysis[18,40]. This procedure gave higher recoveries for some compounds compared with extraction by blending. Benzene (A) was the solvent for dimethyldithiocarbamates and acetic acid–methanol–benzene (1:2:12) (B) for ethylenebisdithiocarbamates on silica gel thin layers. Spots were detected with a 2.5-μg lower limit by spraying with cupric chloride–hydroxylamine hydrochloride chromogenic reagent to produce yellow, green, or brown colors. R_F values were as follows[18,41]:

Compound	*Solvent A*	*Solvent B*
Thiram	0.17	0.86
Tetramethylthiuram	0.30	—
Ziram	0.68	0.94
Maneb	0.98	0.98
Zineb	0.95	0.88

The paper chromatography (PC) of ferbam, maneb, nabam, thiram, zineb, and ziram was studied using formamide-impregnated paper developed with chloroform, petroleum ether, or chloroform–hexane (1:4). Reagents for detection included sodium azide–iodine, zincon, and 4-chlororesorcinol + ammonia. These systems allowed separation and identification of individual compounds[42]. Weltzien[43] employed ascending PC with *n*-butanol–acetic acid–water (4:1:1) solvent and bioautographic detection for separation and detection of thiram (0.81; 0.1), Polyram (0.83; 2.0), ziram (0.80; 0.1), ferbam (0.81; 0.1), zineb (streak; 5.0); Urbazit (0.83; 0.1), Brestan (0.91; 2.5), captan (0.89; 2.0), copper oxychloride (0.22; 5.0), Ceresan (0.80; 25), and Cerenox (0.78; 10). R_F values and sensitivities in μg are given in the parentheses.

Vekshtein and Klisenko[44] separated dialkyldithiocarbamates and metabolites on alumina layers with heptane–benzene–acetone (10:1:22.5) solvent, spraying with iodide–azide reagent for detection. Spots were eluted from the layer with 0.2 *N* NaOH and analyzed by UV spectrophotometry at 250–280 nm. Determinations in plant and animal material were made at the 0.02 to 0.1-ppm level by extraction of samples with chloroform (or chloroform + 0.5% NaOH for acid samples) and two-dimensional TLC with carbon tetrachloride–*n*-butanol (100:0.75) as solvent for cleanup. Kosmatyi *et al.*[45] determined zineb in tobacco plants (10 μg/100 g) by a TLC method based on acid decomposition of the fungicide, absorption of the CS_2 produced in methanolic KOH, and chromatography of the resulting methylxanthate on silica gel KSK–alumina (1:1) with acetone–methanol (20:1). The chromogenic reagent was 2% $(NH_4)_2MoO_4$, acidified with HCl, and the size of the spot was measured by densitometry.

Porter[46] used TLC to detect the presence of thiram in wheat seeds. Chloroform seed washings and standards were spotted on silica gel layers and developed with chloroform–carbon tetrachloride (3:1) in an S-chamber. Visualization of thiram was obtained by spraying the layer with starch until it was opalescent and then with sodium azide–starch solution to produce white spots on a blue-black background.

Up to 30 min was required for full color development. The R_F value of thiram was *ca.* 0.19–0.20 and the detection limit was 0.01 μg. Diameters of developed spots were used to estimate sample concentrations relative to standards, all initial zones being limited to 1 mm diameter in size. The separation of thiram, ziram, and zineb was carried out[47] on silica gel layers developed with acetone, respective R_F values being 0.53, 0.44, and 0.38. Reaction with iodine vapors was used for detection at unspecified levels.

Ethylenethiourea (ETU; 2-imidazolidinethione) is a potentially hazardous[48] degradation product of ethylenebisdithiocarbamate fungicides (nabam, maneb, zineb, Dithanes, etc.)[49], which has received wide attention over the past several years. ETU is also a contaminant in formulated EBDC fungicide products. Onley and Yip[50] and Yip *et al.*[51] determined ETU in fruits, vegetables, and milk at 0.02- to 10-ppm levels by extraction with an ethanol–chloroform mixture, cleanup on a cellulose column, and TLC after further cleanup on an aluminum oxide column eluted with methanol–acetonitrile–benzene (3:15:82) or GLC after derivatization with 1-bromobutane. TLC was carried out on alumina layers developed in saturated tanks with methanol–chloroform–benzene (1:5:10). ETU was detected with a sensitivity of 0.5 μg as a blue spot with $R_F \approx 0.23$ by spraying with Grote's reagent. GLC of the bromobutane derivative was carried out on a 6-ft. 30% DC-200/5% SE-30 column to the end of which was connected a 1-ft. anhydrous K_2CO_3 column, both at 200 °C. A KCl–$RbSO_4$ (1:1) thermionic detector[52] gave 50% full scale deflection for about 70 ng ETU derivative. The ETU derivative under the TLC conditions above gave a reddish-purple spot with $R_F \approx 0.43$.

ETU in commercial ethylenebisdithiocarbamate formulations was determined on a 3-ft. column of 2% Carbowax at 220°C with a thermal conductivity detector[53]. Newsome[54] determined ETU residues in apples (0.01–1 ppm) after conversion to the S-benzyl derivative followed by extraction, trifluoroacetylation, GLC-ECD, and confirmation by mass spectrometry. Blazquez[55] determined ETU residues in tomato foliage with a sensitivity of 1 ppm by a silica gel TLC method after extraction with dioxane. Two chromatographic solvents were used: chloroform–*n*-butanol–methanol–water (100:5:1:0.5) and dioxane–formaldehyde–acetic acid–water (3:1:1.5:1). Detection reagents used were iodine–starch and potassium ferricyanide–ferric chloride (1:1). Onley *et al.*[56] employed GLC-FPD (S mode) and sweep co-distillation cleanup for analysis of ETU in food crops. Cook and Leppert[57] determined ETU on potatoes (0.05 ppm for 60-g samples) using HSLC with UV detection after Florisil column and dichloromethane–water partition cleanup of methanol extracts. Haines and Adler[58] used methanol extraction, alumina column cleanup, derivatization with 1-bromobutane and GLC-FPD with a 6-ft. 20% SE-30 column at 200°C for determination of ETU at 0.01-ppm levels in food crops. The volatile derivative formed with ETU and bromobutane was characterized as 2-*n*-butylmercapto-2-imidazoline. Cruickshank and Jarrow[59] studied ETU degradation after UV irradiation on a silica gel thin layer. ETU and its photolysis products were separated with diethyl ether–methanol (9:1) (R_F ETU = 0.50), *n*-butanol–acetic acid–water (4:1:1) (0.71), 2-butanone–pyridine–water–acetic acid (70:15:15:2) (0.85), and benzene–ethyl acetate–diethylamine–methanol (50:40:10:8) (0.57). Detection was made by a combination of fluorescence quench, ninhydrin, and Ehrlich's reagent.

The fungicides dazomet and Vapam are similar to those already mentioned in

this section since they are manufactured from carbon disulfide and amines. Although no specific reports on their chromatographic analysis have been found, it is possible that they can be determined by a CS_2 evolution–GLC method similar to that described above.

5. CATIONIC COMPOUNDS

Two long-chain, nitrogen-containing cationic compounds are important agricultural fungicides, namely dodine and glyodin. No gas chromatographic procedures have been developed for these compounds, and residues are normally analyzed by colorimetric methods. The colorimetric method for dodine[60] involves formation of a complex with bromcresol purple, extraction of the complex into chloroform, and hydrolysis to form the colored product. Glyodin interferes with this method, so a qualitative test for distinguishing between the two fungicides on paper chromatograms was developed[61]. An aqueous solution containing 10% sodium hydroxide, 10% sodium nitroprusside, and 10% potassium ferricyanide was prepared, diluted with three volumes of water and allowed to stand until the color had changed to pale yellow. This reagent when sprayed on chromatograms gave a blue color with glyodin and a red color with dodine.

6. FUNGICIDES CONTAINING HALOGEN ATOMS

Kilgore and White[62] carried out separations of mixed chlorinated fungicides, isomers, and derivatives with both an all-glass isothermal system utilizing an ECD and an all-metal temperature-programmed system utilizing a flame ionization detector (FID). For isothermal separation, a 5% QF-1 column at 180°C was found superior to a 5% DC-11 column, but later peaks were diffuse and tailed. When the 5% QF-1 column was temperature programmed between 100 and 200°C (FID), a complete separation of chloronitropropane, PCP, HCB, TCNB, chloranil, PCNB, DCNA, dichlone, and Dyrene was achieved in about 25 min. Only the rear of the PCP peak exhibited any tailing. Fig. 1 shows the separation of a more complex mixture of seventeen fungicides, including isomers and derivatives, with this system.

Hutzinger *et al.*[63] recorded the 70-eV mass spectra of fifteen chlorinated aromatic fungicides. The characteristic patterns are useful for confirmation of residues of these fungicides tentatively identified by chromatography.

A. Captan, folpet, and captafol (see also Sections 6L and 6P)

These three phthalimide compounds are protective, non-systemic fungicides with closely related structures. Formulation analysis of captan and folpet was described by Crossley[64] using a GLC column of 3% XE-60 supported on Chromosorb G at 200°C with a thermal conductivity detector (TCD). Formulation analysis of captafol has been carried out on a column of 5% QF-1 on Chromosorb G at 260°C, also with a TC detector[65].

Pomerantz *et al.*[66] detected captan, folpet, and captafol residues in a variety of raw agricultural commodities at 0.1- to 2-ppm levels. The residues were extracted

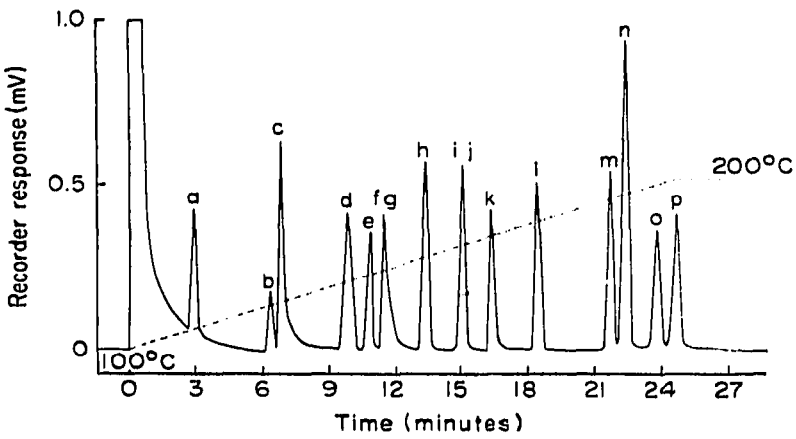

Fig. 1. Programmed-temperature separation of seventeen chlorinated fungicides, isomers, and derivatives on a stainless-steel column, 6 ft. × 1/8 in., containing 5% QF-1 on Chromosorb G (AW-DMCS), temperature programmed between 100 and 200°C at 4°C/min; helium carrier gas; FID; 1 μg of each compound. a = Chloronitropropane; b = 2,4,6-trichlorophenol, methyl ether; c = 2,4,5-trichlorophenol, 2,4,6-trichlorophenol; d = 2,4,5-trichlorophenol, methyl ether; e = 2,4,5,6-tetrachlorophenol, methyl ether; f = 2,4,5,6-tetrachlorophenol; g = PCP; h = HCB; i = PCP, methyl ether; j = TCNB; k = chloranil; l = PCNB; m = 1,2,4-trichloro-3,5-dinitrobenzene; n = dichlone; o = 1,2,3-trichloro-4,6-dinitrobenzene; p = Dyrene.

with acetonitrile, partitioned into dichloromethane–light petroleum (20:80) and purified by chromatography on a Florisil column eluted with dichloromethane–light petroleum (20:80) followed by dichloromethane–acetonitrile–light petroleum (50:1.5:48.5). The fractions were concentrated and analyzed by GLC on a stationary phase of 5% QF-1 on Chromosorb W HP at 155°C with 120 ml/min nitrogen flow-rate and an ECD. Captan and folpet were well separated, with the former eluting in about 12 min. Four nanograms of captan caused one-half full scale detector deflection. Kilgore *et al.*[67] determined captan residues (0.01 ppm) on fruits, vegetables, and cottonseed by GLC-ECD with a 10% DC-200 column at 185°C. Pomerantz and Ross[68] reported retention data for captan, folpet, captafol, and metabolites on 10% DC-200 and 15% QF-1/10% DC-200 (1:1) columns at 210° and 200°C, respectively. Captan residues present in cherries and raw agricultural products were recovered >80% at 1.6-ppm levels by extraction, charcoal cleanup[69], and GLC on a 10% DC-200 column with a Coulson conductivity detector.

Crossley[65] described the determination of captafol residues in crops on a 5% QF-1 GLC column at 190 °C with electron capture detection. When residues were above 5 ppm no cleanup was required, but below 5 ppm silica gel TLC was recommended for cleanup. Some oily crops required additional cleanup by solvent partitioning or Florisil column chromatography (benzene eluent) prior to TLC. The solvent for TLC was 2.5% diethyl ether in benzene, with captafol showing an R_F value of 0.4 after two ascending developments. Detection was with $KMnO_4$ reagent (see Table 5). Twenty-seven pesticides were tested, including captan and folpet, and did not interfere in the GLC step of the procedure. Presumably all three fungicides could be determined by this same overall method.

Baker and Flaherty[70] determined the three compounds simultaneously in

TABLE 5

R_F VALUES AND DETECTION TESTS FOR CAPTAN-TYPE COMPOUNDS[68]

Compound	R_F *relative to captan*	*Color response***			
		*1****	*2*§*	*3*§§*	*4*§§§*
Captan	1.00*	Y/W (≤1)	Y/P (0.2)	—	
Folpet	1.14	Y/W (≤1)	Y/P (1)	—	
Captafol	1.02	—	Y/P (0.2)	OB/W (0.2)	
Captan epoxide	0.70	Y/W (≤1)	Y/P (1)	—	
Captafol epoxide	0.68	—	Y/P (1)	OB/W (0.2)	
Tetrahydrophthalimide	0.19	—	Y/P (0.5)	—	BG/Y (1)
Phthalimide	0.35	—	—	—	BG/Y (1–2)
Tetrahydrophthalimide epoxide	0.08	—	—	—	BG/Y (1–2)

* R_F of captan was 0.61, 154-mm solvent front distance.

** Y/W = yellow spot on white background; Y/P = yellow spot on pink background; OB/W = orange-brown spot on white background; BG/Y = blue-green spot on yellow background; — = no color development. Lower limit of detection in μg given in parentheses.

*** 15 g resorcinol dissolved in glacial acetic acid and diluted to 100 ml; spray and heat for 10–15 min at 110 °C.

§ 1 ml aqueous $KMnO_4$ (1.5 g/25 ml) diluted with acetone to 100 ml.

§§ 0.25 g DMPD dihydrochloride in methanolic NaOH (0.7 g NaOH in 50 ml absolute methanol).

§§§ 10 g $K_2Cr_2O_7$ in 80 ml conc. H_2SO_4 diluted to 200 ml with water; spray and heat for 20–30 min at 130 °C.

fruits at 0.1- to 1-ppm levels by a GLC-ECD method employing a 3% XE-60 phase on Chromosorb W at 190°C. This same column was earlier used by Bevenue and Ogata[71] to separate captan and folpet residues extracted from fresh papayas. Relative retention times on this column as reported by Baker and Flaherty[70] were captan 1.00, folpet 0.82, and captafol 3.34. Several chlorinated and organophosphorous insecticides had similar retention times to captan and folpet on this column, so a 1% SE-52 liquid phase on Chromosorb W at 180 °C was used to separate captan and folpet from these insecticides and to confirm the presence of the fungicides. Residues were initially extracted into acetonitrile, partitioned into hexane–diethyl ether (1:1), and cleaned up on an activated silica gel column eluted with hexane–diethyl ether (1:1). To remove any interfering insecticides prior to GLC, the silica gel column was first washed with hexane–diethyl ether (9:1) to elute the insecticides and then pure diethyl ether to elute the fungicides.

Archer and Corbin[72] detected captan residues at 1-μg levels in prune fruits and blossoms in the presence of captafol by TLC. The residue, after benzene extraction and Florisil cleanup, was spotted on a silica gel H plate and developed with isopropanol–benzene (4:96). Spraying with either resorcinol–glacial acetic acid or pyridine tetraethylammonium hydroxide reagents produced a yellow color with captan. The former spray did not produce a color with captafol and neither spray detected DCNA.

Pomerantz and Ross[68] described a TLC method which differentiates among captan, folpet, captafol and related compounds based on silica gel chromatography with 1% methanol in chloroform as solvent, followed by sequential color develop-

ment of spots with N,N-dimethyl-*p*-phenylenediamine (DMPD), $KMnO_4$, and chromic acid sprays. Table 5 shows these results.

Fishbein *et al.*[73] quantitatively determined captan and captax on silica gel channel chromatoplates by both densitometry at 420 nm and measurement of spot area (width of channel × height of zone). Resorcinol reagent detected captan at R_F 0.35 as a yellow spot with yellow fluorescence under 366 nm UV light (1-μg and 0.5-μg detection limits) after chromatographic development with chloroform. Plots of optical density *vs.* weight and $\sqrt{}$area *vs.* weight were linear between 1–10 μg and 1–20 μg, respectively. Captax was detected by cupric chloride reagent as a yellow spot with R_F 0.25 (2-μg limit) after development with isopropanol–ammonia–chloroform (50:10:40). Densitometry and spot area plots were both linear from 2–16 μg. The feasibility of these methods was demonstrated by the quantitative recovery of 200- and 800-μg amounts of captan from extracts of spiked mouse tissue.

B. Cela W 524

The transport of this systemic fungicide in barley plants was studied by Bruchhausen and Drandarevski[74] using bioautographic TLC against *Cladosporium cucumerinum*. Methanol extracts of plants were developed on silica gel F_{254} layers with water-saturated benzene–ethyl acetate (1:1) followed by diethyl ether in the same direction, thereby separating the fungicide from plant pigment co-extractives. R_F was approximately 0.3 after these two developments, and 0.2 μg of fungicide was detectable.

C. Chloranil

Burke and Holswade[75] reported three peaks for chloranil with retention times relative to aldrin (3.5 min) of 0.37 (major peak), 1.57, and 1.73 on a 6-ft. glass column of 15% QF-1/10% DC-200 (1:1, w/w) on Gas-Chrom Q at 200°C. Five nanograms produced one-half full scale deflection (major peak) with a tritium electron capture detector. The same packing in a 6-ft. aluminum column at 210°C produced one peak with a retention time of 0.44 relative to aldrin (5.5 min) and 8 μg was necessary to produce one-half full scale deflection with a chloride microcoulometric detector. Barrette and Payfer[76] reported chloranil retention times on a 6-ft. 20% DC silicone grease column of 5.2 min at 190°C with 30 p.s.i. (at inlet) helium gas flow and 0.70 min at 230°C with 50 p.s.i. helium.

D. Chloroneb

Formulations of chloroneb were assayed by GLC on a 6-ft. × ¼-in. column packed with 20% SE-30, 200°C, with thermal conductivity detection and biphenyl as internal standard. At a flow-rate of 50 ml/min, chloroneb had a retention time of *ca.* 11 min[77]. Residues of chloroneb and its metabolite 2,5-dichloro-4-methoxyphenol were determined utilizing programmed-temperature chloride microcoulometric GLC after separation from the sample by steam distillation. A 4-ft. × ¼-in. 10% DC-560 silicone oil plus 0.2% Epon Resin 1001 column programmed from 100° to 180°C and a carrier flow-rate of 50 ml/min separated chloroneb and the metabolite with

respective retention times of 10.7 and 9.3 min. Minimum detectability was 0.01–0.05 ppm for both compounds in a variety of crop, soil, food, and biological samples.

TLC of radio-labeled compounds has been used in various studies of the metabolism of chloroneb. Hock and Sisler[78] used silica gel developed with ethyl acetate–methanol (10:1), while Rhodes and co-workers[79,80] employed silica gel developed in chloroform (R_F chloroneb, 0.6–0.8) for the separation and detection of various metabolites.

E. Chloronitropropane

Yaffe *et al.*[81] determined technical and formulated chloropropane by GLC using 20% DC-550 or DC-710 columns at 100 °C and thermal conductivity detection. Cullen and Stanovick[82] determined chloronitropropane on food crops between 10 and 100 ppb by GLC-ECD with a 4% XE-60 liquid phase at 80°C. The residue was extracted with benzene–methanol (2:1) and the filtered and water-washed extract directly injected into the GLC column. Low chromatograph temperatures allowed interfering plant extractives to be trapped in the injection port (held at 120°C), but a Florisil batch cleanup procedure was used when necessary (*e.g.*, for green vegetables). The injection port required daily cleaning immediately after analysis of all crop extracts. The absolute sensitivity of the system was 25 pg of the fungicide, and the retention time was 3 min.

2-Nitropropane, a metabolite of chloronitropropane, was extracted from cottonseed with ethyl acetate and analyzed by GLC-ECD without cleanup at 0.05- to 0.2-ppm levels[83]. With a 6-ft. 5% Carbowax 20M column at 150°C and a carrier gas flow-rate of 60 ml/min, the metabolite eluted in *ca.* 2.5 min while the parent eluted with the solvent. Calibration curves were linear from 0.02 to 0.6 ng and recoveries were 85 ± 11%.

F. Chlorothalonil

The method proposed by the Diamond Shamrock Corp.[61] for analysis of this fungicide and its metabolite 4-hydroxy-2,5,6-trichloro-isophthalonitrile in potatoes at 0.01 ppm (ECD) or 0.02 ppm (microcoulometric detector) includes simultaneous extraction with acidified acetone, cleanup and separation of the residues on a partially deactivated Florisil column eluted with 5% and then 50% acetone in dichloromethane, conversion of the hydroxy metabolite to the methyl ether with diazomethane, and GLC analysis of the parent and the derivative. A 6-ft. column packed with 20% DC-200, a temperature of 275°C, and a carrier (helium) flow-rate of 140 ml/min was recommended for microcoulometric detection and a 5-ft. column of 14% Hi-Vac Silicone at 235 °C and 40 ml/min flow-rate of helium for electron capture detection. The retention time was *ca.* 2.5 min for both the parent (5% eluate) and metabolite (50% eluate) in both GLC systems. The Food and Drug Administration[61] tested the procedure with a 10% DC-200 column (200°C) and an ECD and found a retention time of 0.6 for chlorothalonil relative to aldrin, a sensitivity of about 500 pg for one-half full scale deflection, and 93–119% recoveries at 0.1- and 0.2-ppm levels.

G. DCNA

Beckman and Bevenue[84] determined DCNA residues in fruit with a sensitivity of 0.01 ppm using Florisil cleanup and microcoulometric GLC with a 6-ft. × 5-mm column of 20% DC-11 on Chromosorb P at 210°C. With a carrier flow-rate of 100 ml/min, DCNA eluted in *ca.* 4 min. Cheng and Kilgore[85] used a 5% DC-11 column at 185°C and electron capture detection to determine DCNA in benzene extracts of unprocessed stone fruits without cleanup. Brewerton *et al.*[86] determined DCNA down to 0.1 ppm on fruits and vegetables by GLC-ECD with a 5% QF-1 column at 182°C.

The Upjohn Co.[87] recommends a microcoulometric GLC method for determination of DCNA residues (0.1–10 ppm) in fruits, vegetables, soils, and garlic. The residue is extracted with benzene, transferred into acetonitrile, cleaned up by partition with hexane and additionally Florisil (deactivated with 4–10% water) column chromatography (benzene eluent) for products containing chlorophyll, and determined on a 4-ft. column of 5% DC-200 on Anakrom ABS at 150–160°C. The retention of DCNA was 0.47 relative to aldrin and sensitivity was 2 μg at 64 ohms.

Keswani and Weber[88] studied the TLC of DCNA along with seventeen other substituted nitroanilines and related compounds. Silica gel G layers developed with hexane–acetone (3:1) produced an R_F value of 0.42 for DCNA, which was naturally yellow on the plate and remained yellow after diazotization, α-naphthol, or ferric chloride–potassium ferricyanide detection reagents were applied. Von Stryk[89] separated DCNA and its metabolites *p*-nitroaniline, 2-chloro-4-nitroaniline, and 2,6-dichloro-1,4-diaminobenzene by two-dimensional development on silica gel layers with hexane–acetone (3:0.5) followed by benzene–chloroform (8:2). The R_F values of DCNA in these solvents were 0.32 and 0.28, respectively. Detection was made at the 0.5-μg level by exposing the dried plate to 350-nm UV light. The method was tested on spiked plant substrates which were extracted with benzene and cleaned up on a Florisil column.

The GLC-ECD determination of DCNA and its major metabolite 2,6-dichloro-4-hydroxyaniline in tissue and excreta at 0.1 ppm was described by Moseman[90]. After extraction with hexane or acetonitrile and cleanup, if necessary, by partitioning and on Florisil or silica gel columns, DCNA was chromatographed on 3% OV-1 (180–195°C), 5% OV-210 (155°C), or 4% SE-30/6% OV-210 (185°C) columns, depending upon the sample. DCNA and the phenol metabolite were simultaneously determined in urine after acid hydrolysis, neutralization with base, extraction with benzene, and preparation of the chloroacetate derivative of the metabolite, by GLC on an OV-210 column at 160 °C. Aldrin eluted in *ca.* 4 min, DCNA in 5 min, and the dichloroaminophenol in 6 min with a nitrogen flow-rate of 70 ml/min.

Van Alfen and Kosuge[91] employed preparative silica gel TLC to isolate DCNA metabolites from culture fluids in their study of the microbial metabolism of this fungicide. Metabolites were separated and detected by analytical thin-layer radiochromatography with benzene–diethyl ether (1:1) and chloroform–acetone (7:3) mobile phases.

H. Dichlofluanid

Strawberries treated with this fungicide during growth were analyzed for the

parent and breakdown product dimethylaminosulfanilide by GLC-ECD[92]. Two different 5-ft. × 1/8-in. columns containing 5% DC-11 and 5% QF-1 at 180°C gave retention times of 4.5 and 3.0 min, respectively, with a nitrogen flow-rate of 50 ml/min. The sulfanilide had a retention of 1.5 min on the latter column and was not detected on the former. Residues were extracted by shaking the frozen or canned sample with benzene, the aqueous phase was discarded, and the benzene layer dried over sodium sulfate, filtered, concentrated if necessary, and chromatographed without cleanup. Dichlofluanid was detected at 0.01- to 1.6-ppm levels, while no residues of the sulfanilide were found in any sample tested.

J. Dichlone

Benzene was used to extract dichlone from fruit samples, the extracts were dried over sodium sulfate, and cleanup carried out on a Florisil column, if required[93]. GLC-ECD on a 5-ft. column of 5% QF-1 provided linear calibration curves over a range equivalent to 0.3–5.0 ng dichlone, which eluted in just under 6 min. Samples should be analyzed quickly after extraction to preclude degradation and conversion of the fungicide. Recoveries were poor at lower fortification levels (50% at 0.01 ppm, 90% at 1.0 ppm) and when the length of time between extraction and analysis increased[94].

The fungicides dichlone, Bulbosan, fuberidazole, and chlorothalonil were detected following cellulose and silica gel TLC by spraying the plate with a reagent which forms a π-complex with the pesticides. Various reagents (TNF, CNTNF, TCNE, etc.) were tested with pesticides at 5 μg/spot levels and characteristic colors resulting were tabulated. Neither detection limits nor practical analytical applications were included in this report[95]. A TLC method for 0.01–0.05 ppm dichlone in grape leaves and berries or apples involved extraction, purification by microsublimation, and development on silica gel G using cyclohexane–chloroform (7:3)[96].

J. Dichlozoline

Pack *et al.*[97] analyzed grapes and grape products by extraction with hexane, cleanup of low levels (< 0.5 ppm) of residues in certain samples on a silica gel column eluted with hexane–benzene (1:1), and GLC-ECD on a 5-ft., 2% DEGS (185°C) or 2-ft., 5% QF-1 (150°C) column. With a carrier flow-rate of 30 ml/min, retention times were *ca.* 4 min and 7 min, respectively, and 1 ng gave about one-half full scale recorder deflection. Detection limits were 0.01 ppm and recoveries at 0.01–1 ppm were excellent. A similar GLC-ECD method for analysis of the same products was reported by Mestres *et al.*[98]. The sample was extracted with light petroleum in the presence of sodium sulfate and Celite to reduce emulsions, and the extract was then analyzed by GLC at 0.01- to 0.5-ppm levels. At levels below 0.25 ppm, Florisil column cleanup was required.

K. Drazoxolone

Yuen[99] recommended colorimetry at 400 nm for the analysis of both formulations and residues of drazoxolone. Extracts of certain plant species such as grass

contain pigments which caused high background absorption readings. Chromatographic cleanup through a 10-g column of Florisil was carried out, drazoxolone being selectively eluted with chloroform.

L. Dyrene

Burke and Holswade[75] reported a retention time for this triazine fungicide of 2.29 relative to aldrin on a 15% QF-1/10% DC-200 (1:1) column at 200°C with electron capture detection. 40–50 ng Dyrene caused one-half full scale deflection. Using the same column at 210 °C but with a microcoulometric detector, the same workers reported five peaks for Dyrene with relative retentions of 1.33 (major), 1.77, 2.08, 2.37, and 2.67 and a sensitivity of 25 μg.

Wales and Mendoza[100] obtained the recovery of 5–20 ppm Dyrene from plant samples by acetonitrile extraction and hexane partitioning with analysis by GLC-ECD on SE-30/QF-1 or OV-17/SE-30/QF-1 mixed phases[101] at about 210°C. The sensitivity of the detector was $\frac{1}{2}$ f.s.d. to 5 ng Dyrene. Confirmation was obtained by reaction of the extracts with methanolic NaOH prior to GLC to produce two major products with longer retention times than Dyrene. Captan, if present, would also be recovered by this procedure and would be separated from Dyrene on the SE-30/QF-1 column. The methanolic NaOH treatment would destroy captan.

M. HCB

Besides those mentioned at the start of this article, several other multiresidue studies have included the fungicide HCB. Recovery of 92% at the 0.025-ppm level was obtained from dry poultry food and grain by the TLC cleanup method of Heatherington and Parouchais[102] prior to GLC. Development of samples on aluminum oxide G layers was with acetonitrile–tetrahydrofuran (1:1) in an unsaturated tank for 10 cm followed by air drying and re-development with acetonitrile for 12 cm. The alkaline precolumn procedure of Miller and Wells[103] was used to destroy certain pesticides prior to chromatography on a separate analytical GLC column, while other pesticides were converted to chromatographable derivatives with altered retention times and still others, including HCB and TCNB, were unchanged. This procedure eliminated many background peaks and offered evidence useful in confirming residue identity. HCB had a retention of 0.49 relative to aldrin on a 10% DC-200 column at 210°C[104], and 0.15, 0.25, and 0.12 relative to dieldrin on 1.3% columns of SE-52 (160°C), Apiezon L (190°C), and XE-60 (200°C), respectively, each containing 0.1–0.2% Epikote resin 1001 (ref. 105). The chromatography of HCB on a 5% QF-1 column is illustrated in Fig. 1.

The TLC of HCB has been reported[106] in the following systems: alumina layers/*n*-heptane solvent (R_{DDD} = 2.7), alumina/2% acetone in heptane (1.7), alumina impregnated with 25% N,N-dimethylformamide (DMF)/isooctane (5.7), and silica gel G-HR/1% acetone in heptane (2.5).

Problems encountered in the analysis of HCB residues in the ppb range in cereals were studied by Taylor and Keenan[107]. Extraction by refluxing with hexane, separation from interfering grain lipids by steam distillation, separation from α-BHC insecticide on a 5% Reoplex GLC column, and an alkaline degradation confirmatory

procedure were offered as partial solutions to some of the difficulties. α-BHC and HCB were not separated on some of the usual pesticide GLC columns but had $R_{dieldrin}$ values of 12 and 29, respectively, on a 5-ft. Reoplex column at 200°C.

Smyth[108] detected HCB in dairy products, meat fat, and eggs with a lower detection limit of 0.002 ppm (1-g fat sample) and recoveries in excess of 80%. After hexane extraction of fat, cleanup was on a deactivated Florisil column eluted with dichloromethane–hexane (2:8) and GLC-ECD on a 1% DC-200/1% QF-1 column supported on Varaport 30. At 185°C, HCB had an R_{aldrin} value of 0.40 (0.47-min elution time on a 6-ft. × 2-mm column, 18 ml/min nitrogen flow-rate) and was separated from α-BHC and other common chlorinated insecticides. Florisil deactivation at a level (*ca.* 0.5–1.5% water) which recovered 80% of added dieldrin provided adequate cleanup of 1-g fat samples.

DiMuccio *et al.*[109] designated GLC phases for the separation of HCB from BHC isomers and other chlorinated pesticides. Table 6 shows retention times on the recommended columns.

Weber *et al.*[110] used phenyl ether derivative formation (phenol/KOH reaction) to eliminate interference of contaminants in analyses of meat brei for HCB. Retentions relative to lindane on XE-60, SE-30, and DC-200 GLC columns were 2.2, 5.5, and 5.1 min, respectively, for the ether derivative.

Curley *et al.*[111] screened adipose tissue from people of Japan and detected < 0.003–0.77 ppm HCB among other chlorinated pesticides in 241 samples. GLC-ECD on columns of 1.5% OV-17/1.95% QF-1 or 5% OV-210 were used to separate and detect low picogram quantities after extraction and cleanup by the traditional Mills procedure, which is known to provide low recovery of HCB. Confirmation of this fungicide was made by Coulson conductivity detection, TLC, and combined

TABLE 6

RETENTION TIMES RELATIVE TO ALDRIN OF CHLORINATED PESTICIDES[109]

Compound	*DC-200/QF-1/XE-60**	*OV-61/QF-1/XE-60***
HCB	0.41	0.40
α-BHC	0.53	0.54
γ-BHC	0.70	0.73
Heptachlor	0.83	0.82
Aldrin	1.00 (8.9 min)***	1.00 (7.9 min)§
β-BHC	1.20	1.15
δ-BHC	1.29	1.31
Heptachlor Epoxide	1.60	1.74
p,p'-DDE	2.16	2.42
Dieldrin	2.46	2.74
o,p'-DDT	2.65	3.39
p,p'-DDT	3.78	4.36

* 1:1:1 mixture of three previously coated packings: 10% DC-200 on HP Chromosorb W, 7.5% QF-1 on Chromosorb W HP, and 3% XE-60 on silanized Anakrom AS; all percentages by weight, supports 80–100 mesh.

** 1:1:0.5 mixture of 3% OV-61 on 80–100 mesh silanized Gas-Chrom P plus QF-1 and XE-60 as above.

*** 2-m × 4-mm column, 190 °C, argon–10% methane carrier gas, 40 ml/min, ECD in pulsed mode.

§ As above, but 2-m × 3-mm column.

GLC–mass spectroscopy. The mass spectrum indicated all fragments caused by successive loss of chlorine from the parent ($C_6Cl_6^+$) to $C_6Cl_1^+$. TLC on 2-mm preparative silica gel fluorescent layers with light petroleum–diethyl ether–acetic acid (90:10:1) provided a quenched HCB spot with an R_F value of about 0.66 under UV light.

Wollenberg and Drossel[112] determined residues of HCB in meat products by its conversion into a mixture of ether derivatives by treatment under mild conditions (60°C) with potassium phenoxide solution in dimethylsulfoxide. The derivatives were separated by GLC, and the peak from the chief product [pentachloro(phenoxy)-benzene] was used to provide quantitation of HCB content.

Confusion between elemental sulfur and HCB on thin-layer chromatograms may be decreased by UV irradiation of the layer prior to application of the $AgNO_3$ detection spray[113].

Holdrinet[114] determined and confirmed HCB in fatty samples at low ppm levels in the presence of other residual haloginated pesticides and PCBs. Following initial hexane extraction and Florisil cleanup, organochlorine pesticides, PCBs, and HCB were successively eluted from a Fisher 5-690 charcoal column with acetone–diethyl ether (1:3), benzene, and toluene. HCB in the third fraction was determinated by GLC-ECD with a 4% SE-30/6% QF-1 column at 180°C. The HCB fraction was then subjected to caustic alkali at high temperature and the hydrolyzed product methylated to yield pentachlorophenol methyl ether derivative for confirmation.

N. Hexachlorophene

Although gas chromatography has been carried out directly[115,116] on hexachlorophene, most residue analyses have involved formation of methyl derivatives. The GLC-ECD analysis of hexachlorophene in cucumbers, tomatoes, corn, and milk at 0.02- to 0.2-ppm levels was reported by Gutenmann and Lisk[117]. After extraction with acetone, filtration, and cleanup by chloroform partitioning and batch treatment with Celite–H_2SO_4, the compound was methylated and chromatographed on a 2-ft. × 6-mm 10% DC-200 column at 200°C. One nanogram of methylated fungicide gave a peak with one-half full scale recorder response and a retention time of 16.5 min (75 ml/min carrier flow-rate). Ferry and Queen[118] also used GLC-ECD for the analysis of hexachlorophene in blood. Extraction was performed with diethyl ether, followed by methylation, addition of hexane to dissolve the methyl ether, and cleanup of the hexane phase by extraction with H_2SO_4 and saturated sodium sulfate. Concentrates were chromatographed on a 0.5-m × 2.5-mm 5% QF-1 column at 185°C with a carrier flow-rate of 40 ml/min. Retention of the methyl ether was 4.5 min, and linear GLC response was obtained over the range 0.005–0.200 ng.

Formation of trimethylsilyl (TMS) derivatives[119] and acetylation[120] have also been suggested for GLC analysis of hexachlorophene. This latter approach was chosen by Greenwood *et al.*[121] for determination of traces of the fungicide in blood. Whole blood (3 ml) was partitioned with ethyl acetate (10 ml), the extract concentrated, reacted with a mixture of equal volumes of acetic anhydride and pyridine (0.1 ml), and determination made by GLC with a ^{63}Ni ECD. A 3% OV-17 column at 265°C was employed and detection was made at a blood level of 330 pg of hexachlorophene per ml.

French *et al.*[122] studied variations in separability and sequence by TLC on different silica gel precoated plates for the mixture hexachlorophene, trichlorocarbanilide, and tribromosalicylanilide. With benzene–diethyl ether (8:2) as solvent, R_F values of hexachlorophene ranged from 0.07–0.36, while the range was 0.02–0.14 with hexane–ethyl acetate (7:3). The elution order for the three compounds was altered on different brands of layers, emphasizing the importance of using chromatographic conditions as similar as possible if published work is to be reproduced.

Carr[123] analyzed hexachlorophene by HSLC with a 50-cm × 0.2-cm MicroPak SI-10 small-particle (< 10 μm), porous silica gel column connected in series with a variable wavelength UV detector set at 296.5 nm. Hexachlorophene was eluted in *ca.* 1 min with hexane–dichloromethane–isopropanol–glacial acetic acid (89:8:1:2), obtaining a minimum detectable quantity of 10 ng. Using this optimum wavelength, a similar sensitivity as reported by Porcaro and Subiak[124] was achieved without formation of the higher absorbing di-*p*-methoxybenzoate ester and the resulting greater sample manipulation and analysis time required with the fixed-wavelength 254-nm detector used by the latter workers.

O. Parinol

Day *et al.*[125] described methods for determination of parinol in formulations, soils, and plant tissues. A GLC column of 1.5% OV-17 at 230°C was found to be generally applicable. An FID was suitable for formulation analysis and an ECD for trace analysis. Parinol eluted from a 4-ft. × 3-mm column in *ca.* 6 min with a flow-rate of 40 ml/min. Residues as low as 0.01–0.02 ppm in various crop and soil samples were determined by blending the sample with acetone, partition into hexane, extraction of the hexane solution with 0.5 *N* HCl, addition of base, and extraction with chloroform. The chloroform solution was evaporated to dryness and dissolved in a small volume of benzene for GLC as above.

If residues were present at levels greater than 0.1 ppm, a TLC procedure was successfully employed. The benzene solution of the chloroform extract was spotted on a silica gel layer and developed in diethyl ether–hexane–methanol (80:18:2) in an unsaturated chamber. The developed plate was sprayed with 10% H_2SO_4 in diethyl ether and heated for 5 min at 110°C. A red parinol spot appeared which became yellow on exposure to cool air. The limit of detection was about 0.2 μg. Quantitation was carried out by visual comparison of samples and standards on the same plate, or by removal of the spot from the plate, elution from the silica gel, evaporation of the solvent, development of the red color in H_2SO_4, and colorimetric analysis.

P. PCNB and TCNB

Klein and Gajan[126] determined PCNB and TCNB residues on lettuce, cabbage, and string beans by chloride microcoulometric GLC at 0.1–5 ppm using a 20% DC silicone grease column at 220 °C. Both PCNB and TCNB were separated and measured individually. Burke and Holswade[104] reported a retention time of 0.54 min relative to aldrin for PCNB and 0.36 for TCNB on a column of 10% DC-200 at 210°C with a microcoulometric detector. Sensitivities were 0.75 and 1 μg, respectively. Gorbach and Wagner[127] analyzed PCNB in potatoes by microcoulometric GLC on a silicone grease column at 170 °C and identified one of two metabolites as

pentachloroanaline. Methratta *et al.*[128] determined PCNB in vegetables, fruits, seeds, and soil at 0.01–0.3 ppm by GLC-ECD on a 4-ft. × ¼-in. 2% SE-30 column at 170°C. Samples were extracted with hexane and interferences removed on a silicic acid (10% moisture) column eluted with hexane. PCNB eluted in about 94 sec with a nitrogen flow-rate of 160 ml/min and the sensitivity was 150 pg for full scale response with a 0.3% noise level. Caseley[129] determined PCNB and TCNB residues in soils, after mechanical extraction with acetone and partition with hexane, by GLC-ECD on a 5-ft. × 1/8-in. column of 5% SE-30 at 175°C. Retention times were 3.0 and 1.5 min, respectively, at a nitrogen carrier flow-rate of 70 ml/min.

The GLC procedure of Methratta *et al.* (above) was adopted by Kuchar *et al.*[130] to study the metabolism of PCNB in beagle dogs, rats, and plants. Extraction was made with acetonitrile or hexane, depending on the sample. The metabolic products of PCNB were found to be pentachloroaniline (retention 1.4 relative to PCNB on 2% SE-30) and methyl pentachlorophenyl sulfide (2.0) in all three instances.

Photoreduction of the fungicides PCNB, PCP, and PCB by 254 nm UV light and sunlight was studied[131] using programmed-temperature thermal conductivity or chloride microcoulometric GLC with 20% DC-11 or 10% SE-30 columns. Preparative GLC was accomplished with an FID chromatograph and 5% DC-11 column. Programming between 110–220 °C provided separation (in order) of 1,2,4,5-tetrachlorobenzene (first eluted), PCB, 2,3,4,6-tetrachloronitrobenzene, 2,3,4,5-tetrachloronitrobenzene, and PCNB.

Residues (0.01–5 ppm) of PCNB in tomatoes, lettuce, and bananas were determined by Baker and Flaherty[132]. After extraction with hexane, PCNB was separated from interfering co-extractives by partition with DMF followed by chromatography on a deactivated alumina (5% water) column eluted with hexane and was quantitatively determined by GLC-ECD on a 5% EGSS-X column at 170 or 200 °C (retention PCNB = 0.19 or 0.25, relative to dieldrin). The limit of detection was 5 pg at a signal-to-noise ratio of 3:1, and response was linear from 0.1–1.0 ng. GC columns containing 1.3% SE-52/0.15% Epikote 1001 at 200 °C (relative retention = 0.25) and 1% Apiezon L/0.15% Epikote 1001 at 196 °C (0.26) were suitable for confirmation. A chemical confirmatory test for PCNB was also described, namely reduction with lithium aluminium hydride in diethyl ether to form pentachloroaniline (PCA), the formation of which was confirmed by GLC before and after shaking the reduced extract with H_2SO_4 to remove PCA. Any PCA present in untreated samples as a natural metabolite of PCNB was removed by treatment with H_2SO_4 prior to reduction. Lettuce samples known to have been treated with thiram and zineb gave GLC peaks which eluted in the vicinity of PCA and interfered with the PCA determination. These peaks, which were attributed to sulfur-containing degradation products of the fungicides, were removed from the organic extract by elution through a silver nitrate–alumina column prior to carrying out the confirmatory test.

Griffith and Blanke[133] included PCNB, captan, and folpet among 31 compounds in their improved microcoulometric method for organochlorines in blood. Results are shown in Table 7.

Q. PCP (see also Section 7H)

A rapid method[134] for determination of PCP in 2 ml human blood serum was

TABLE 7

MICROCOULOMETRY OF FUNGICIDES IN BLOOD[133]

Compound	*RRT**		*Response***		*Recovery****	
	A§*	*B*§§*	*ECD*§§§*	*MC*†*	*ng*	*%*
PCNB	0.70	0.91	1.5	2, 1	60	91
Folpet	1.85	2.53	20	20, 40	200	55
Captan	1.88	2.59	20	20, 40	200	55

* Retention time relative to aldrin, which has a retention of 3.6 min in system A and 4.7 min in B.

** Ng for one-half full scale deflection.

*** Recovery of fungicides added to 2 ml of whole blood at the middle level reported using a modified H_2SO_4 extraction method.

§ Column A: 6-ft. × 4-mm glass column packed with 4% SE-30/6% QF-1 on 80–100 mesh Supelcoport, 205 °C, nitrogen carrier gas flow-rate 120 ml/min.

§§ Column B: 6-ft. × 4-mm glass column packed with 5% OV-210 on 80–100 mesh Gas-Chrom Q, programmed at 2°C/min from 210–234 °C, initial hold 1 min, final hold 4 min, nitrogen carrier gas flow-rate 90 ml/min.

§§§ Column A.

† Response for chloride microcoulometric detector on columns A, B.

based on its conversion to a methyl ether with diazomethane after a 2-h extraction of the acidified sample with benzene. GLC-ECD on 4% SE-30/6% QF-1 or 5% OV-210 columns at 200°C was utilized for quantitation, comparing sample peaks against peaks from standards similarly methylated. Because of the widespread prevalence of PCP, a reagent blank must be carried through the entire procedure along with the samples. The method had a lower detection limit of 10 ppb with a detector sensitivity of 50 pg or less PCP methyl ester. A similar method for analysis of blood plasma or urine was reported by Rivers[135].

For determination of PCP residues in 5 ml human urine[136], the sample was made alkaline and extracted with hexane to remove basic and neutral interferences. The alkaline urine was acidified and re-extracted, PCP derivatized with diazomethane, and the alkylated PCP determined by GLC-ECD. Confirmation was achieved by *p*-value[137] determinations, GLC retention times on several columns, or preparation of additional alkyl ether derivatives of PCP[138]. Retention times of methylated PCP relative to aldrin on several recommended columns were as follows: 1.5% OV-17/1.95% QF-1 (200 °C), 0.47; 4% SE-30/6% QF-1 (200 °C), 0.63; and 5% OV-210 (180 °C), 0.56. Forty picograms of PCP ether resulted in a quantifiable peak, this amount corresponding to a level of 2 ppb PCP in the original urine sample.

A method for the analysis of PCP residues on nuts and stone fruits also employing methylation and electron capture detection was reported by Kilgore and Cheng[139]. Acidified samples were extracted with benzene, a sulfuric acid washing procedure was used for cleanup when required, and analysis was on a 5% DC-11 column at 180°C. Sensitivity was reported as 0.01 ppm. Bevenue *et al.*[140] used a similar method for determination of PCP in urine. Columns of 5% QF-1 and 10% DC-200 at 145°C and an ECD provided a linear range of 30–400 pg PCP. Other early analytical methods for PCP were reviewed by Bevenue and Beckman[141]. Bevenue *et al.*[142] later reported another similar method for PCP in human blood. Simultaneous

application of acid pH, mild heat, and agitation of the sample with benzene isolated the fungicide from 1–5 ml of sample, followed by methylation with diazomethane and GLC–ECD on 5% QF-1 and 3.3% DC-200 columns. Detectability limits were in the low ppb range.

Barthel *et al.*[143] determined PCP in blood, urine, tissue, and clothing as part of an investigation of illness and fatalities in a nursery treated with a mildew preventive containing PCP. Samples were extracted with diethyl ether, the ether solution extracted with 5% NaOH, the basic solution acidified and extracted with benzene, and this solution analyzed by GLC on a 3% DEGS column containing 2% syrupy H_3PO_4. This column at 150 °C with a ^{63}Ni ECD at 280°C allowed detection of PCP at the 0.02-ng level without derivatization. H_3PO_4 reduced the polarity of PCP and elution from the 4-ft. column was obtained in *ca.* 2 min.

Stark[144] used GLC-ECD for determinations of PCP in soil, water, and fish at 0.05- to 2-ppb levels after extraction, partition, and methylation. TMS derivatives were prepared for GLC confirmation. Higginbotham *et al.*[215] analyzed fats, oils, and fatty acids for PCP and 2,3,4,6-tetrachlorophenol by GLC-ECD on a 7-ft. 10% DEGS/2% H_3PO_4 column at 170°C. PCP eluted in *ca.* 25 min and the phenol in 10 min. Extraction of acidified samples was made with light petroleum followed by partition with aqueous alkali and chloroform to separate the phenols. After further treatment with H_2SO_4, GLC was carried out. Recoveries were reportedly low and variable and detection only at the 0.5-ppm level was claimed. TLC on Gelman ITLC Type SG sheets with light petroleum–heptane (1:1) solvent and detection under 350-nm UV light after spraying with Rhodamine B reagent yielded orange-pink spots (0.5–5 μg) at R_F 0.4–0.5 for both phenols in addition to 2,4,5-trichlorophenol. Phenols were further studied as impurities in PCP formulations by GLC-mass spectrometry on a temperature-programmed 4% SE-30 column after methylation[145].

The acetate derivative of PCP was recommended by Chau and Coburn[146] for the determination of PCP in natural and waste water at levels as low as 0.01 ppb/l. PCP was extracted with benzene and from the benzene into potassium carbonate solution. The addition of acetic anhydride to the aqueous alkali extract produced the acetate of PCP, which was extracted by hexane and analyzed by GLC-ECD on OV-17/QF-1 or OV-101/OV-210 GLC columns. Advantages of the acetyl derivative over the usual methyl ether derivative were found to be simplification of the procedure because of derivative formation in aqueous solution and a larger linear range of ECD (^{63}Ni) response. Seventeen other phenols were found not to interfere in the analytical procedure.

PCP was detected on thin-layer chromatograms along with other chlorinated phenols after fluorigenic labeling with dansyl chloride[147]. The dansyl derivative of PCP had an R_F value of 0.83 on a silica gel layer developed with benzene–chloroform (1:1). The nature of the derivative was investigated by scanning fluorescence *in situ* with a Zeiss PMQ II chromatogram spectrometer. Neither the sensitivity of the method nor calibration curves were presented, although it seems likely that densitometric quantitation of PCP residues in the low nanogram range could be made using this technique.

The cleanup of the fungicides PCP, 2,4,6-trichlorophenol, and 2,3,4,6-tetrachlorophenol and phenoxy acid herbicides by batch and column ion-exchange procedures was described by Renberg[148]. The acidic residues were bound under alkaline

conditions to a strong base anion-exchange resin and removed subsequently under acid conditions. Gas chromatography of methylated derivatives of the fungicides was carried out, after ion-exchange cleanup, on columns of 1% OV-17 (160°C) and 8% QF-1/4% SF-96 (150°C). PCP was determined at levels as low as 0.35 ppm in contaminated water and fish tissue.

Colvin *et al.*[149] added PCP as the internal standard for the formulation analysis of carbaryl insecticide by high-pressure liquid chromatography. With a 2-ft. × 1/8-in. Carbowax/Porasil column and 20% chloroform in isooctane as solvent at a flow-rate of 1.5 ml/min, 4 μg PCP was eluted in 1.5 min and gave 60% f.s.d. with a UV photometric detector.

R. Triarimol

Frank *et al.*[150] analyzed formulations of this fungicide by GLC-FID after the sample had been dissolved in or extracted with chloroform. Triarimol eluted in *ca.* 4 min from a 4-ft. × 3-mm column of 2% OV-17 with a carrier gas flow-rate of 35 ml/min. Dibenzyl phthalate was a suitable internal standard. GLC columns packed with UC-W98 and JXR liquid phases were also successfully used. Pesticides commonly occurring with triarimol, including the fungicides captan and maneb, did not interfere with the analysis.

7. MISCELLANEOUS FUNGICIDES

A. BAS-3191

The metabolism of this new systemic anilide fungicide was studied employing silica gel TLC and alumina column chromatography to purify and separate the parent compound and its metabolites prior to determination and identification by spectroscopy. Benzene–acetic acid (9:1) and chloroform–acetone–acetic acid (15:2:3) were solvents for TLC, and chloroform was used to fractionate compounds on the column[151].

B. Benomyl

The standard analytical method for benomyl residues involves determination at 0.1 ppm (25-g sample) by either direct fluorimetric measurement or by colorimetric analysis following bromination, after conversion to 2-aminobenzimidazole. Benomyl is quantitatively converted to methyl 2-benzimidazolecarbamate (MBC) and then to 2-aminobenzimidazole (2-AB) by a two-stage acid–base hydrolysis procedure after extraction from the sample substrate with ethyl acetate. The extract is purified by liquid–liquid partitioning steps. In addition to this procedure[152], which measures benomyl, its principal degradation product MBC, and a minor component in plants, 2-AB, as a composite value, others involving chromatography have been developed as outlined below.

Rouchaud and Decallonne[153] determined benomyl and MBC hydrolysis prod-

uct in plants and soil by extracting residues with benzene, partitioning into 0.1 *N* HCl, washing the acidic layer with chloroform and then neutralizing. At this point the residues were completely in the form of MBC (present initially or formed during acid hydrolysis), which was partitioned into ethyl acetate, trifluoroacetylated, and measured by GLC-ECD on a 1.5-m × 2.2-mm 5% SE-30 column at 140°C. The MBC-TFA derivative had a retention time of 3.1 min, with a carrier flow-rate of 40 ml/min. Parathion, used as an internal standard, eluted in 7.9 min. The limit of sensitivity was 0.02 ppm.

Technical samples of benomyl were separated into two toxic components on silica gel layers developed with ethyl acetate[154]. The breakdown product was identified as MBC. Peterson and Edgington[155] used bioautographic TLC to estimate quantitatively benomyl and MBC. A silica gel Chromagram sheet was sprayed with a mixture of agar and *Penicillium* spores, and the diameter of the zones of inhibited growth on the layer was related to the amount of fungitoxic chemicals present. Development with acetone separated benomyl ($R_F \approx 0.3$) and MBC (≈ 0.5). Amounts equivalent to as little as 0.03 μg benomyl were spotted and two spots were readily detected. The diameter of both spots increased linearly with log concentration (0.03–0.4 μg benomyl equivalent) for both spots. Homans and Fuchs[156] likewise used bioautography on thin-layer chromatograms to detect 1-μg amounts of benomyl after development with diethyl ether or ethyl acetate and location of the pesticide by its UV absorption on Merck silica gel F_{254} aluminum-backed layers. The spray solution was a fungus organism such as *Cladosporium cucumerinum* in a suitable medium.

Mallet *et al.*[157] studied the fluorescence of benomyl and the fumigants fuberidazole and Quinomethionate on silica gel thin layers. Fluorescence spectra were determined *in situ* and the effects of heating the chromatogram at 200°C for 45 min were noted. Heat treatment shifted the emission and excitation maxima and in some cases increased the number of peaks. These changes are useful evidence along with R_F values in confirming residue identity. Limits of detection were also determined after development of layers with hexane–acetone (9:1). Heating may increase or decrease fluorescence intensity. Results are shown in Table 8.

Vogel *et al.*[158] extracted benomyl residues from disintegrated fruits and vegetables with ethyl acetate, hydrolyzed benomyl and/or MBC to 2-AB, and chromatographed this on silica gel layers with detection by exposure to bromine vapor. Yellow-brown spots with an R_F value of 0.4 (hexane–ethyl acetate–methanol, 1:1:1)

TABLE 8

FLUORESCENCE SPECTRAL DATA AND VISUAL LIMITS OF DETECTION ON SILICA GEL THIN LAYERS[157]

Fungicide	*Wavelength**				*Limit of detection (μg)*	
	Natural		*After heat treatment*		*No heat*	*Heat*
	EX	*EM*	*EX*	*EM*		
Benomyl	298	422	362	464	0.06	0.02
Fuberidazole	328	402	323, 373	447	1.00	0.005
Quinomethionate	363	418	335, 360	465, 478	0.004	0.04

* EX = excitation, EM = emission.

were formed with a detectability of 0.2 ppm. Tjan and Burgers[159] determined benomyl and thiabendazole in fruits by ethyl acetate extraction, cleanup by partition with 0.1 *N* HCl, neutralization and extraction back into ethyl acetate, and TLC of concentrates on silica gel G or GF_{254} plates with chloroform–acetone (8:2) solvent. The separated fungicides [R_F 0.35 for thiabendazole, 0.18 and 0.70 for benomyl (two spots) on silica gel GF] were detected as pink-blue spots under 254-nm UV light on fluorescent layers, or more specifically by an enzyme-inhibition method (honey bee extract/2-naphthyl acetate–Fast Blue B) using 0.50-mm non-fluorescent layers. Recoveries at 1–2 ppm levels from a variety of fruit were 75–83% for thiabendazole but only 40–43% for benomyl, probably because of loss of hydrolysis products of the latter through the procedure.

White and Kilgore[160] used a TLC-UV spectrophotometric method to assay various food crops for benomyl and MBC at 0.05-ppm levels. The compounds were extracted with benzene, partitioned into 0.1 *N* HCl, the acidic layer was washed with chloroform, neutralized to pH 8 with NaOH, and the single product MBC present at this point was partitioned into ethyl acetate, concentrated, and chromatographed. Plastic sheets precoated with polyamide II containing fluorescent indicator were prewashed with chloroform–ethyl acetate–acetic acid (190:10:4) solvent, dried, spotted, and developed with the same solvent. MBC spots (R_F 0.7) were detected under shortwave UV light, excised from the plate with a Brinkmann vacuum spot collector, and the compound was eluted with methanol prior to spectrophotometric analysis at 287 nm. White *et al.*[161] used this analytical procedure plus TLC on silica gel F_{254} precoated glass plates with benzene–methanol (9:1) as developing solvent to identify thermal and base-catalyzed hydrolysis products of benomyl. Benomyl standard exhibited an R_F value of 0.48 when detected under 254-nm UV light. Baude *et al.* separated radiolabeled benomyl and plant[162] and soil[163] metabolites by silica gel TLC and quantitated by radioscanning or radioautography and liquid scintillation counting. Solvents employed were ethyl acetate–methanol–NH_4OH (100:25:0.5–1) or ethyl acetate–dioxane–methanol–NH_4OH (320:40:10:1). This method involved the conversion of benomyl to the stable derivative 3-butylureidobenzimidazole while MBC hydrolyzed to 2-AB.

Kirkland[164] used HSLC to determine residues of benomyl and/or MBC, and the hydroxylated metabolites methyl 5-hydroxy-2-benzimidazolecarbamate (5-HBC, a major metabolite in animal urine which was originally identified by TLC[165]) and methyl 4-hydroxy-2-benzimidazolecarbamate (4-HBC, a minor metabolite in animal urine) in cow milk, tissues, urine, and feces. The sample was hydrolyzed in acid to convert benomyl to MBC and to free the metabolites from conjugates. The freed materials were extracted into ethyl acetate, the extract was cleaned-up by solvent partitioning, and determined on a 100-cm × 2.1-mm column of Zipax SCX strong-acid cation-exchange packing with a carrier phase of 0.15 *N* sodium acetate–0.15 *N* acetic acid (7:3) at 60°C. With a carrier flow-rate of 0.5 ml/min, 4-HBC eluted in 7.2 min and 5-HBC in 8.6 min. Fifteen minutes after sample injection the flow-rate was increased to 1.5 ml/min, and MBC eluted 22 min from the start. Recoveries were demonstrated at 0.01- to 0.2-ppm levels (25-g samples), and 28 other pesticides with tolerances in milk and meat caused no interference with this procedure. These included the fungicides thiabendazole and chloroneb. The fate of benomyl was studied in other animals[166] and 5-HBC was indicated as the major metabolite in feces

and urine. TLC on silica gel layers developed twice with ethyl acetate–methanol–glacial acetic acid (100:100:4) followed by methanol–water (2:1) (same direction) or once with ethyl acetate–dioxane–methanol–NH_4OH (160:20:5:0.5) was used for separation of metabolites.

Another method employing Zipax SCX cation exchanger after extraction and liquid–liquid partitioning cleanup was reported by Kirkland *et al.*[167] for benomyl residues (sensitivity, 0.05 ppm) in soils and plant tissues. With a 254-nm UV detector and elution at 60°C and 300 p.s.i.g. using 0.025 *N* tetramethylammonium nitrate–0.025 *N* HNO_3 solvent, peaks with retention times of *ca.* 18 and 22 min for MBC and 2-AB, respectively, were detected.

Another fluorimetric procedure[168] for benomyl, MBC, and the fungicide thiabendazole included cleanup on a magnesium oxide–Celite–alumina column. Thiabendazole was eluted with ethyl acetate followed by benomyl/MBC with ethanol–ethyl acetate (1:1).

C. Binapacryl

Baker and Hoodless[169] determined residues of binapacryl (0.3–0.5 ppm) in selected fruits by extraction with hexane–diethyl ether–DMF (4:1:2), cleanup on an activated silica gel column eluted with hexane–diethyl ether (4:1), and GLC-ECD determination on 3% XE-60 (200°C) or 15% DC-200 (204°C) columns. A confirmatory chemical test was described in which binapacryl was hydrolyzed with methanolic KOH to give the free phenol (DNBP, dinoseb), and the phenol was then methylated with diazomethane to give the corresponding ether which was detected by GLC. The ether had a retention time of 0.29 relative to binapacryl on the XE-60 column. Dinoseb herbicide, a potential breakdown product of binapacryl on the fruit, would not be detected under the GLC conditions used. Dinoseb acetate or dinobuton fungicide, if present, would invalidate the confirmatory test since both of these would form the same ether [2-(1-methyl-*n*-propyl)-4,6-dinitroanisole].

Buxton and Mohr[170] mentioned the GLC-ECD determination of binapacryl residues on cottonseed after extracts were purified by partitioning from hexane into acetonitrile, but no details were given.

D. Biphenyl (see also Section 7L)

Morries[171] determined biphenyl and *o*-phenylphenol in the peel of citrus fruits by GLC-FID following homogenization with diethyl ether and filtration of the extract. A polar column consisting of 15% ethanediol adipate polyester on Chromosorb and fluorene as internal standard were employed. Detection at 1 ppm and 5 ppm, respectively, was made without significant interferences. A GLC method reported by Beernaert[172] employed steam distillation to free biphenyl and *o*-phenylphenol from citrus fruit, extraction of the distillated phase with light petroleum, and GC on a 1% SE-30 column with a temperature program from 50 to 300°C. Benzophenone was used as internal standard for recovery studies at 40 and 20 ppm, respectively. The FID was again employed successfully due to the high levels studied as dictated by the high tolerance levels of residues established in various countries for these compounds. Hites[173] examined river waters by computerized GLC–high-resolution

mass spectrometry and found several plasticizers, trichlorobenzene, butyl benzoate, and biphenyl at levels of 0.1–30 ppb. GLC conditions included a 150-cm × 0.32-cm column of 0.05% OV-17 on glass beads with a temperature program from 70 to 250°C at 12°C/min. Hahn and Thier[174] determined biphenyl and *o*-phenylphenol in citrus fruits as their bromo-derivatives. The residues were extracted and subsequently reacted with bromine–iodine solution prior to GLC-ECD on a 1.5-m glass column packed with 1.5% QF-1/1% DC-200 (or 2.5% XE-60) on Chromosorb G AW DMCS operated at 200°C. The method was suitable for routine analysis at the 2- and 4-ppm levels, respectively (*ca.* 2 ng absolute sensitivity for each fungicide).

The TLC and HSLC of the fungicides biphenyl and *o*-hydroxybiphenyl and other hydroxybiphenyl metabolites was reported by Cassidy *et al.*[175]. Dansylated hydroxybiphenyls were separated on silica gel thin layers with chloroform–benzene (1:1) mobile phase in the order *o,o'*-(most sorbed), *p,p'*-, *o*-, and *p*-hydroxybiphenyl. HSLC on 15- or 40-cm columns of silica gel (7–18 μ) packed by a balanced-density slurry technique was used to separate the *o*- and *p*-dansyl derivatives (hexane–chloroform, 9:1, solvent) and the *o,o'*- and *p,p'*-derivatives (hexane–chloroform, 7:3). Both UV and fluorescence detectors provided linear calibration curves from 1 to 300 ng. The fluorescence detector was more sensitive (*ca.* 0.1 ng at 3:1 signal-to-noise ratio) but resulted in a loss of column efficiency (band broadening). An earlier report[176] demonstrated reaction conditions for the dansylation reaction of hydroxybiphenyls and *in situ* thin-layer quantitation between 5 and 500 ng in rat urine. TLC was carried out on silica gel layers with two solvent systems (Table 9). After development, the dried plates were sprayed with triethanolamine and evaluated with a Zeiss PMQ II Spectrometer, and identities of residues confirmed by mass spectrometry.

A thin-layer fluorescence method for detection of biphenyl and *o*-phenylphenol in foods was described[177]. After diethyl ether extraction, the compounds were separated on silica gel with hexane–ethanol–chloroform (97:2:1) and detected in 254-nm UV light. Respective R_F values were 0.70 and 0.25. Corneliussen[178] determined biphenyl in citrus fruits (10–100 ppm) by separation of residues with a steam liquid–liquid extraction and cleanup of the *n*-heptane extract by silica gel preparative TLC. The plate was developed with heptane and biphenyl detected under UV light as a bright blue spot on the yellow background of the fluorescent layer. Spots were removed from the layer and extracted with methanol for quantitative spectrophotometry at 248 nm.

A Varian HSLC detector was modified to improve stability and sensitivity by

TABLE 9

R_F VALUES FOR HYDROXYBIPHENYL-DANSYL DERIVATIVES

Solvents: I = benzene–chloroform (1:1); II = acetone–hexane (3:7).

Dansyl derivative	R_F	
	I	*II*
4-Hydroxybiphenyl*	0.58	0.62
2-Hydroxybiphenyl	0.47	0.62
4,4'-Dihydroxybiphenyl	0.20	0.48
2,2'-Dihydroxybiphenyl	0.10	0.45

* Equivalent to *p*-hydroxybiphenyl.

Callmer and Nilsson[179], who demonstrated linear calibration curves from 0.4 to 1000 ng biphenyl when chromatography on a 2.4-mm × 400-mm column packed with 0.88% 1,2,3-tricyanoethoxypropane on Corasil I support with isooctane mobile phase was used. Biphenyl eluted in 2 min at a flow-rate of 80 ml/h. Reeder[180] developed HSLC methods for the quantitation of biphenyl, thiabendazole, and *o*-phenylphenol in citrus products using 20-μ silica gel and three different solvents. These fungicides were detected by UV absorption with limits of less than 1 ppm.

E. Carboxin

Sisken and Newell[181] determined residues of carboxin (Vitavax) and its sulfoxide in seeds and seed oils by methanol extraction, partition cleanup of the extract, reductive hydrolysis to liberate aniline, distillation of aniline, and N-selective microcoulometric GLC. An 18-ft. 4% Carbowax 20M column, temperature programmed from 100 to 200 °C, eluted with hydrogen carrier gas (40–55 ml/min depending on column temperature), gave a retention time of about 15 min for the aniline peak from carboxin and its sulfoxide. The method was sensitive to $<$ 0.2 ppm carboxin (60 ng aniline).

Chin *et al.*[182] used TLC on silica gel Chromagram sheets to study the degradation of carboxin in water and soil and its metabolism by barley and wheat plants. Five different TLC solvents were studied, and R_F values evaluated by spraying a 0.05% fluorescein methanolic solution and observation under 254-nm UV light or by performing radioautography. The solvents with R_F values of carboxin were methanol (0.6), 20% methanol–acetone (0.9), chloroform (0.8), benzene (0.1), and acetone (0.9). Tripathi and Bhaktavatsalam[183] detected carboxin and oxycarboxin on silica gel G layers with silver nitrate–bromophenol blue and potassium permanganate–sulfuric acid chromogenic reagents. The former reagent detected only carboxin as a blue spot at 2-μg levels while the latter detected 3-μg amounts of both fungicides as white spots on a pink background. R_F values were 0.8 and 0.4 for carboxin and oxycarboxin, respectively, when development was with chloroform. This same solvent was reported earlier by Allam and Sinclair[184], who detected the fungicide spots under UV light on a layer having a fluorescent indicator.

F. Dinocap

Karathane pesticide is a mixture of dinitrooctylphenyl crotonates, dinitrooctyl phenols, and mononitrooctyl phenols, of which dinocap is one component. Karathane formulations have been examined by various workers using GLC. Clifford *et al.*[185] and Clifford and Watkins[186] used columns containing mixed phases of diethylene glycol adipate polyester plus H_3PO_4. Boggs[187] reported a double peak for dinocap on a 10% DC-200 column used for the GLC-ECD of a series of dinitro herbicide methyl ethers. Kurtz and Baum[188] and Kurtz *et al.*[189] used columns packed with 3% QF-1 silicone oil on 60–80 mesh Gas-Chrom Q programmed from 100 to 230°C and an FID.

Dinocap was determined in formulations with TLC by Chiba and Yatabe[190]. Silica gel layers were developed with hexane–acetone (8:2), the dinocap spot was eluted with benzene and determined photometrically at 430 nm.

Chromatographic determination of dinocap residues has not been reported.

G. Dithianon

Eisenbeiss and Sieper[191] demonstrated the HSLC of this fungicide on Perisorb A porous-layer bead adsorbent with a Zeiss PMQ II 254-nm detector modified for column liquid chromatography and heptane–ethyl acetate (96.5:3.5) as carrier liquid. Five nanograms were the minimum detectable amount and linear calibration curves between 50 and 2000 ng were illustrated. Apples were analyzed after extraction and cleanup at 0.02- and 0.1-ppm levels with 80% recoveries.

H. DNOC

This phenolic compound, which acts as a fungicide, insecticide, and herbicide (ammonium salt), has been determined in urine along with PCP, several phenoxy acid herbicides, and a series of halo- and nitrophenol metabolites of organophosphate pesticides by a GLC-ECD method[192]. After extraction with diethyl ether, the phenols were ethylated with diazoethane, and the ethers chromatographed on a 2-g silica gel column (2% water). PCP was eluted in the first fraction with 8 ml of 20% benzene–hexane, and DNOC in the third through fifth fractions (10 ml each of 60% benzene–hexane, 80% benzene–hexane, and straight benzene). Chromatographic analysis at the 0.01- to 0.1-ppm level was made on a 4% SE-30/6% OV-210 column at 175°C, with a sensitivity of 0.01–0.3 ng for the individual phenols.

Phenolic pesticides are either converted to corresponding ethers or tailing is avoided by gas chromatographing the free phenols on low-loaded polar phases in the presence of H_3PO_4 at relatively low column temperatures. DNOC had the following retention times (min) on 80-cm × 3-mm columns packed with 3% of each following phase plus 1% H_3PO_4 at 200°C and a carrier flow-rate of 1.0 kp/cm^2: neopentyl glycol succinate (0.82), cyclohexanedimethanol succinate (1.04), butanediol succinate (0.93)[193].

Free DNOC had an R_F value of 0.83 on a thin layer of cellulose impregnated with mineral oil–acetic acid–diethyl ether (5:2:93) and developed with methanol–acetic acid–water (73:2:25). The methyl ether had an R_F value of 0.87 on mineral oil-impregnated cellulose developed with methanol–acetonitrile–water (30:25:45). Detection at 0.05 μg (free) and 0.1 μg (ether) was made by spraying with stannous chloride–dimethylaminobenzaldehyde reagent to produce yellow-orange fluorescent spots[194]. Guardigli *et al.*[195] identified and quantitated DNOC by TLC in various crops at a sensitivity below 0.05 ppm (0.5-μg detection). After extraction and cleanup by alkaline hydrolysis and liquid–liquid partition, residues were converted to nitro derivatives by reaction with 2% $NaNO_2$ in concentrated H_3PO_4. The derivative was developed on silica gel with benzene–acetic acid (85:15) and detected by reduction of the nitro group to the corresponding amine followed by diazotization and coupling with Bratton–Marshall reagent.

I. Ethirimol and dimethirimol

Bratt *et al.*[196] elucidated the metabolism of the systemic pyrimidine fungicide dimethirimol by rats and dogs with the aid of radio-TLC. Silica gel GF layers were developed with the listed solvents and compounds detected by viewing under 254-nm

UV light: (a) water-saturated butanol (R_F dimethirimol = 0.54), (b) *n*-butanol–acetic acid–water (4:1:5) (0.48), (c) *n*-butanol saturated with 3 *N* ammonia (0.72), (d) chloroform–methanol (9:1) (0.71). Two-dimensional chromatography with solvents (d) and (c) in turn separated nineteen urinary metabolites and solvents (a) and (b) resolved thirteen metabolites.

Bagness and Sharples[197] determined ethirimol and dimethirimol in technical and formulated materials by GLC-FID and quantitative TLC. For GLC, the fungicides were converted into volatile trimethylsilyl ethers and chromatographed on a 5-ft. × 4-mm column of 10% E-301 on silanized 100–200 mesh Celite. The retention of ethirimol relative to *n*-nonadecane internal standard was 0.53 at 220°C while that of dimethirimol relative to *n*-octadecane was 0.47 at 200°C. TLC was carried out on silica gel GF_{254} layers developed with chloroform–acetone–acetic acid (75:10:15) for ethirimol and methanol–dichloromethane (1:9) for dimethirimol. The compounds were located as quenched zones under UV light, adsorbent bands were removed by the "vacuum-cleaner" technique, and methanol extracts were analyzed by UV absorption spectroscopy at 297 and 303 nm, respectively.

J. Fentin acetate and fentin hydroxide

These compounds, which are active ingredients in the fungicides Brestan and Du-Ter, and their degradation products have been analyzed after photolysis *in vitro* and in soils by Cenci and Cremonini[198]. Samples were cold extracted with 95% ethanol–ethyl acetate (1:1) and extracts chromatographed on silica gel H layers with *n*-butanol–ethanol–water (4:2:1). Both compounds yielded three spots with R_F values of approximately 0.0, 0.45, and 0.88. The former two were detected with 0.5% aqueous catechol violet and the third with aqueous vanadophosphoric acid at 0.05-μg levels.

Tin was determined in organic material, after application of tin-containing fungicides to plants, by wet ashing, extraction with cupferron into chloroform, oxidation of the cupferron complex with HNO_3–$HClO_4$, and radial PC with 3 *N* HCl–saturated *n*-butanol as solvent to separate tin from other metals. The tin-containing chromatographic zone was wet ashed and the phenylfluorone complex photometrically estimated at 546 nm in acid solution[199].

K. o-Phenylphenol (see also Sections 7D and 7L)

Davenport[200] reported a TLC method for determination of *o*-phenylphenol residues in acetonitrile extracts of fruits and vegetables at 0.1 to 200 ppm which was satisfactorily validated at 0.5- to 10-ppm levels by interlaboratory studies. Extraction and Florisil column cleanup were carried out as described by Porter *et al.*[201] for carbaryl residues in fruits and vegetables. The cleaned-up extract was evaporated, applied to an aluminum-backed EM silica gel layer, and developed along with 0.05- to 0.5-μg standard spots using benzene as solvent. The layer was sprayed with 1 *N* alcoholic KOH followed by *p*-nitrobenzenediazonium fluoborate chromogenic reagent to produce a pink spot with R_F 0.5–0.8 for the fungicide. Semiquantitation was based on visual comparison of sample and standard spot sizes and intensities. Folpet residues, if also present, would interfere with the *o*-phenylphenol spot, in which case

development was carried out with hexane–ethyl acetate (2:1) to separate the two fungicides. Other fungicides detected by the chromogenic reagent include captan (salmon-orange, 25 μg detected), folpet (yellow-orange, 25 μg), and DNOC (yellow, > 100 μg).

L. Thiabendazole (see also Section 7B)

In a method by Hey[202] for analysis of citrus fruits and bananas, samples were reflux-extracted for 3 h with dichloromethane and concentrates cleaned-up on silica gel GF_{254} plates with chloroform and benzene–acetic acid–acetone–water as solvents. Spots located under UV light were removed, extracted with methanol, and determined by UV spectrophotometry or GLC of silyl or methyl derivatives on a 5% SE-30 column at 200 °C with flame ionization or nitrogen-specific detectors. A rapid TLC screening method reported by Reinhard[203] detected the fungicides thiabendazole, biphenyl, *o*-phenylphenol, and diphenylamine in peels of citrus fruits at 1- to 2-μg levels. Extraction was made in turn with alkaline and then acidic dichloromethane, followed by silica gel TLC and detection under UV light or by spraying with Dragendorff's reagent for thiabendazole, or with LeRosen's reagent and spraying with HNO_3 for the others.

A TLC-spectrophotofluorimetric method was reported by Norman *et al.*[204] for quantitation of thiabendazole on and in citrus fruits from 0.2 to 6 ppm. TLC ways carried out on fluorescent, aluminum-backed alumina layers developed with chloroform–methanol (98:2) after extraction of residues with ethyl acetate and partition cleanup, if required. Detection down to 0.2 μg was made under short-wave UV light, and the purple, absorbing spot was eluted by cutting out the spot area and immersing in methanol–0.1 *N* HCl (99:1) for 30 min.

Kroeller used TLC to assay tobacco smoke condensate[205] and orange peels[206] for thiabendazole. In the former method, cleanup by chloroform extraction and steam distillation preceded silica gel TLC with benzene–acetic acid–acetone–water (10:4:1:0.4) solvent. Residues were extracted with dichloromethane from orange peel, cleanup was by washing with 0.1 *N* HCl and then 0.1 *N* NaOH, and chromatography was on alumina layers with benzene–acetone–water solvent. In each case the fungicide was detected under UV light, eluted, reacted with *p*-phenylenediamine, and the resulting blue color evaluated by spectroscopy. The limit of detection was 0.1 ppm in orange peels.

M. Thiophanate (see also Section 8)

The translocation of the fungicides MBC (degradation product of benomyl) and thiophanate-methyl as affected by plant nutrition was studied by Al-Adil *et al.*[207]. TLC was carried out on polyamide 11-F sheets with chloroform–ethyl acetate–acetic acid (190:10:4) solvent for MBC and silica gel F sheets with chloroform–methanol (9:1) for thiophanate-methyl. Spots were located under UV light and mapped by autoradiography. Soeda *et al.*[208] performed TLC of thiophanate-methyl and its metabolites on Eastman silica gel Chromagram sheets developed in unsaturated tanks with ethyl acetate–hexane–acetic acid (20:80:2) as solvent. The compounds were detected under 254-nm UV light, thiophanate-methyl having an R_F value of 0.3.

Other solvents employed for metabolite separations were ethyl acetate–hexane (saturated tank) and ethyl acetate–chloroform–acetic acid (10:90:2, unsaturated tank).

N. Tutane

Day *et al.*[209] analyzed *sec.*-butylamine residues in certain fruit samples at 2.0 ppm by steam distillation of the amine from the tissue, removal of interferences by a carbon tetrachloride wash, reaction with 1-fluoro-2,4-dinitrobenzene, and GLC-ECD analysis on a 6-ft. × ¼-in. 2% DEGS column at 188°C. With a 80 ml/min carrier gas flow-rate, the derivative (N-*sec.*-butyl-2,4-dinitroaniline) had a retention time of 8 min. In some cases, cleanup by TLC prior to GLC was required. For this, silica gel G layers and hexane–diethyl ether (7:3) solvent were employed. The area of adsorbent containing the DNP derivative, as indicated by a guide zone of pure standard developed on the same plate, was removed, the derivative was eluted with chloroform, and the solvent was exchanged for benzene prior to GLC analysis.

8. MIXED FUNGICIDES

The following systemic fungicides and metabolites were separated and detected by Von Stryk[210] on Eastman Chromagram silica gel sheets containing fluorescent indicator: benomyl, thiophanate, thiophanate-methyl, MBC, benzimidazole, and 2-aminobenzimidazole. Solvent systems for the two-dimensional separation were benzene–methanol (9:1) followed by ethyl acetate–chloroform (6:4). One-dimensional development with the first of these solvents separated all compounds except MBC and benzimidazole, while the second resolved all but the thiophanate isomers. All compounds were visible under 254-nm UV light as dark spots on a pink background,

TABLE 10

TLC OF SYSTEMIC FUNGICIDES[211]

Fungicide	*Detection method*	*Detection limit (μg)*	*Spot position***
Dimethirimol	color*	0.6	CBBA
Ethirimol	color*	0.6	BBAA
Thiabendazole	UV	1.0	BBAB
Benomyl	UV	0.8	DCCC
MBC	UV	NR***	CBAA
Carboxin	UV	0.5	DCBC
Oxycarboxin	UV	0.5	BCAA

* Potassium iodobismuthate spray followed by exposure to bromine vapor.

** Spot locations for chromatographic systems 1–4, in order, where A = R_F 0.0, B = 0.25–0.50, C = 0.50–0.75, D = 0.75–1.0. Systems 1–3: silica gel 60 F_{254} adsorbent developed with diethyl ether–glacial acetic acid–methanol (100:5:2), acetone, and light petroleum (60–80°C)–acetone (3:1, double development), respectively. System 4: aluminum oxide F_{254} neutral (Type E) adsorbent developed with diethyl ether–methanol (40:1).

*** None reported.

and MBC was selectively detected as a blue spot at a level as low as 25 ng by N-2,6-trichloro-*p*-benzoquinoneimine reagent. However, this latter result was not reproduced by Baker *et al.*[211], who studied the TLC of a group of systemic fungicides and MBC. The results of these latter workers are shown in Table 10. All compounds could be separated by use of these four systems. These TLC separations were combined with a bioassay technique to detect and identify thiabendazole, benomyl, MBC, and thiophanate-methyl on citrus fruit skin at 0.5- to 2-ppm levels[212]. Minimum amounts of the fungicides listed in Table 10 and thiophanate-methyl detectable as standards were 0.05–10 μg, using a fungal growth inhibition procedure.

Fishbein[213] studied the TLC of some sixty isomeric halo- and nitro-derivatives of aniline and benzene including several fungicides. Silica gel DF-5 chromatoplates activated at 75°C were developed by the ascending method with (A) 2.5% ethyl alcohol in benzene, (B) 20% ethyl acetate in benzene, and (C) 2.5% acetone in benzene. After evaporating the solvent, spots were located under 254-nm UV light and by DDQ, TCNE, and Gibb's reagents. $R_F \times 100$ values of the fungicides studied were as follows:

	A	*B*	*C*
DCNA	41	67	66
PCNB	70	66	71
TCNB	63	56	65
o-Dichlorobenzene	64	65	63
m-Dichlorobenzene	63	60	63
p-Dichlorobenzene	63	62	65

9. APPENDIX

NAMES, CHEMICAL NAMES, AND ALTERNATE NAMES OF FUNGICIDES[214]

*Name**	*Chemical name*	*Alternate name**
BAS-3191	2,5-dimethyl-3-furancarboxylic acid anilide	
benomyl	methyl 1-(butylcarbamoyl)-2-benzimidazole-carbamate	Benlate
binapacryl	2-*sec*.-butyl-4,6-dinitrophenyl-3-methyl-2-butenoate or 2-(1-methyl-*n*-propyl)-4,6-dinitrophenyl-2-methylcrotonate	Morocide
biphenyl	diphenyl, phenylbenzene	
Brestan	triphenyltin acetate	fentin acetate, Cercostan
Bulbosan	1,3,5-trichloro-2,4,6-trinitrobenzene	
captafol	N-(1,1,2,2-tetrachloroethylthio)-3α,4,7,7α-tetrahydrophthalimide	Difolatan

* Trade names are capitalized while common names are not, except for abbreviations such as PCNB.

Name	*Chemical name*	*Alternate name*
captan	N-(trichloromethylthio)-3α,4,7,7α-tetrahydrophthalimide	Merpan, Orthocide 406
captax	2-mercaptobenzothiazole	
carboxin	5,6-dihydro-2-methyl-1,4-oxathiin-3-carboxanilide	Vitavax
Cela W 524	N,N'-bis(1-formamido-2,2,2-trichloroethyl)piperazine	triforine
Cerenox	quinoneoximebenzoyl hydrazone	
Ceresan	methoxyethylmercuric chloride	
chloranil	2,3,5,6-tetrachloro-1,4-benzoquinone	Spergon
chloroneb	1,4-dichloro-2,5-dimethoxybenzene	Demosan
chloronitropropane	1-chloro-2-nitropropane	Korax, Lanstan
chlorothalonil	2,4,5,6-tetrachloroisophthalonitrile	Bravo, Daconil 2787
dazomet	tetrahydro-3,5-dimethyl-2H-thiadiazine-2-thione	Mylone
DCNA	2,6-dichloro-4-nitroaniline	Botran, dichloran
dichlofluanid	N,N-dimethyl-N'-phenyl-(N'-fluorodichloromethylthio)sulfamide	Euparen, Elvaron
dichlone	2,3-dichloro-1,4-naphthoquinone	Phygon
dichlozoline	3-(3,5-dichlorophenyl)-5,5-dimethyloxazolidinedione-2,4	Sclex
dimethirimol	2-dimethylamino-4-hydroxy-5-*n*-butyl-6-methylpyrimidine	Milcurb
dinobuton	2-(1-methyl-2-propyl)-4,6-dinitrophenyl isopropylcarbonate	
dinocap	2,4-dinitro-6-(1-methylheptyl)phenyl crotonate	Karathane, Arathane
Dithane M-45	a coordination product of Zn^{2+} and manganese ethylenebisdithiocarbamate, related to maneb and zineb	
dithianon	1,4-dithiaanthroquinone-dicarbonitrile-2,3	Thynon
DNOC	4,6-dinitro-*o*-cresol (2-methyl-4,6-dinitrophenol)	
dodine	*n*-dodecylguanidine acetate	Cyprex
drazoxolone	4-(2-chlorophenylhydrazono)-3-methyl-5-isoxazolone	
Du-Ter	triphenyltin hydroxide	fentin hydroxide, TPTH
Dyrene	2,4-dichloro-6-(*o*-chloroanilino)-*s*-triazine	anilazine
ethirimol	2-ethylamino-4-hydroxy-5-*n*-butyl-6-methylpyrimidine	
ferbam	ferric dimethyldithiocarbamate	

Name	*Chemical name*	*Alternate name*
folpet	N-(trichloromethylthio)phthalimide	Phaltan
fuberidazole	2-(2′-furyl)-benzimidazole	Voronit
Germisan	phenylmercury pyrocatechol	
glyodin	2-heptadecyl-2-imidazoline acetate	
HCB	hexachlorobenzene	
hexachlorophene	2,2′-methylene bis(3,4,6-trichlorophenol)	Nabac
maneb	manganese ethylenebisdithiocarbamate	
Memmi	N-methylmercuri-1,2,3,6-tetrahydro-3,6-methano-3,4,5,6,7,7-hexachlorophthalamide	
Merthiolate	sodium ethylmercury thiosalicylate	Thimerosal, Elcide
nabam	sodium ethylenebisdithiocarbamate	
Panogen	cyano(methyl mercuri)guanidine	Morsodren, Panodrin A-13
parinol	α,α-bis(*p*-chlorophenyl)-3-pyridinemethanol	Parnon
PCNB	pentachloronitrobenzene	quintozene, terrachlor
PCP	pentachlorophenol	
o-Phenylphenol	2-phenylphenol	
Polyram	a mixture of ethylenebis(dithiocarbamate)-zinc and [dithiobis(thiocarbonyl)imino-ethylene]bis(dithiocarbamato)zinc	
Quinex	phenylmercury oxyquinolate	Ortho LM
Quinomethionate	6-methyl-2,3-quinoxalinedithiol cyclic *S,S*-dithiocarbonate	Morestan, oxythioquinox
TCNB	1,2,4,5-tetrachloro-3-nitrobenzene	Fusarex, Folosan, tecnazene
tetraiodoethylene	1,1,2,2-tetraiodoethylene	
thiabendazole	2-(4′-thiazolyl)-benzimidazole	
thiophanate	1,2-bis(3-ethoxycarbonyl-2-thioureido)-benzene	Topsin
thiophanate-methyl	1,2-bis(3-methoxycarbonyl-2-thioureido)-benzene	
thiram	bis(dimethylthiocarbamoyl) disulfide	
triarimol	α-(2,4-dichlorophenyl)-α-phenyl-5-pyrimidinemethanol	
trichlorophenol	2,4,5-trichlorophenol and 2,4,6-trichlorophenol	Dowcides 2 and 2S
Tutane	*sec.*-butylamine or 2-aminobutane	
Urbazit	methylarsinebisdimethyldithiocarbamate	
Vapam	sodium N-methyldithiocarbamate	metham
Zineb	zinc ethylenebisdithiocarbamate	
Ziram	zinc dimethyldithiocarbamate	

10. SUMMARY

The separation and analysis of a wide range of fungicides by gas, liquid, column, paper, and thin-layer chromatography have been reviewed. Major attention has been given to methods for the identification and quantitation of individual and multiresidues of fungicides in environmental and agricultural samples.

REFERENCES

1 G. Zweig and J. Sherma (Editors), *Analytical Methods for Pesticides and Plant Growth Regulators, Vol. VI, Gas Chromatographic Analysis*, Academic Press, New York, 1972.
2 J. Sherma and G. Zweig (Editors), *Analytical Methods for Pesticides and Plant Growth Regulators, Vol. VII, Thin-Layer and Liquid Chromatography and Pesticides of International Importance*, Academic Press, New York, 1973.
3 J. Sherma, *Crit. Rev. Anal. Chem.*, 3 (1973) 299.
4 W. P. Cochrane and R. Purkayastha, *Toxicol. Environ. Chem. Rev.*, 1 (1973) 137.
5 I. H. Williams, *Residue Rev.*, 38 (1971) 1.
6 L. Fishbein and W. L. Zielinski, Jr., *Chromatographia*, 2 (1969) 38.
7 L. Fishbein, *Chromatogr. Rev.*, 12 (1970) 177.
8 B. Malone, *Residue Rev.*, 38 (1971) 21.
9 L. Fishbein, *Chromatogr. Rev.*, 13 (1970) 83.
10 D. Natusch and T. Thorpe, *Anal. Chem.*, 45 (1973) 1184A.
11 M. Krejci and M. Dressler, *Chromatogr. Rev.*, 13 (1970) 1.
12 W. P. Cochrane and R. G. Greenhalgh, *Ann. Symp. Recent Adv. Anal. Chem. Pollut., 3rd, Athens, Ga., May, 1973.*
13 W. P. Cochrane, B. P. Wilson and R. Greenhalgh, *J. Chromatogr.*, 75 (1973) 207.
14 R. Greenhalgh and W. P. Cochrane, *J. Chromatogr.*, 70 (1972) 37.
15 W. P. Cochrane and B. P. Wilson, *J. Chromatogr.*, 63 (1971) 364.
16 R. Purkayastha and W. P. Cochrane, *J. Agr. Food Chem.*, 21 (1973) 93.
17 H. A. McLeod, C. Mendoza, P. J. Wales and W. P. McKinley, *J. Ass. Offic. Anal. Chem.*, 50 (1967) 1216.
18 H. A. McLeod and W. R. Ritcey (Editors), *Analytical Methods for Pesticide Residues in Foods*, Health Protection Branch, Department of National Health and Welfare, Ottawa, Canada, revised ed., 1973.
19 *Pesticide Analytical Manual*, Vol. 1, Food and Drug Administration, Rockville, Md., revised ed., September, 1972.
20 P. A. Mills, B. A. Bong, L. R. Kamps and J. A. Burke, *J. Ass. Offic. Anal. Chem.*, 55 (1972) 39.
21 H. A. McLeod and P. J. Wales, *J. Agr. Food Chem.*, 20 (1972) 624.
22 A. V. Holden and K. Marsden, *J. Chromatogr.*, 44 (1969) 481.
23 A. V. Holden, *Pestic. Sci.*, 4 (1973) 399.
24 V. Zitko and P. M. K. Choi, *Tech. Rep.*, No. 272, Fisheries Research Board of Canada, Biological Station, St. Andrews, N.B., 1971.
25 H. V. Morley and K. A. McCully, in F. Korte (Editor), *Methodicum Chimicum*, Vol. 1, George Thieme Verlag, Stuttgart, 1973, Ch. 11.1, pp. 850–904.
26 J. O'G. Tatton and J. Wagstaffe, *J. Chromatogr.*, 44 (1969) 284.
27 S. Gherardi, G. Dall'Aglio and G. Carpi, *Ind. Conserve*, 48 (1973) 9.
28 G. Westöö, *Acta Chem. Scand.*, 20 (1966) 2131; 21 (1967) 1790; 22 (1968) 2277.
29 F. Geike and I. Schuphan, *J. Chromatogr.*, 72 (1972) 153.
30 C. A. Bache and D. J. Lisk, *Anal. Chem.*, 43 (1971) 950.
31 C. A. Bache, W. H. Guntenmann, L. E. St. John, Jr., R. D. Sweet, H. H. Hatfield and D. J. Lisk, *J. Agr. Food Chem.*, 21 (1973) 607.
32 J. A. Ealy, W. D. Shults and J. A. Dean, *Anal. Chim. Acta*, 64 (1973) 235.
33 P. Zarnegar and P. Mushak, *Anal. Chim. Acta*, 69 (1974) 389.
34 G. E. Keppel, *J. Ass. Offic. Anal. Chem.*, 52 (1969) 162; 54 (1971) 528.
35 S. F. Howard and G. Yip, *J. Ass. Offic. Anal. Chem.*, 54 (1971) 1371.

36 G. Yip, J. H. Onley and S. F. Howard, *J. Ass. Offic. Anal. Chem.*, 54 (1971) 1373.
37 F. Ghezzo and L. Margos, *Pestic. Sci.*, 2 (1971) 249.
38 H. A. McLeod and K. A. McCully, *J. Ass. Offic. Anal. Chem.*, 52 (1969) 1226.
39 W. L. Zielinski, Jr. and L. Fishbein, *J. Chromatogr.*, 23 (1966) 302.
40 W. K. Lowen, *Anal. Chem.*, 23 (1951) 1846.
41 J. W. Hylin, *Bull. Environ. Contam. Toxicol.*, 1 (1966) 76.
42 W. P. McKinley and S. A. Magarvey, *J. Ass. Offic. Agr. Chem.*, 43 (1960) 717.
43 H. C. Weltzien, *Naturwissenschaften*, 45 (1958) 228.
44 M. Sh. Vekshtein and M. A. Klisenko, *Vopr. Pitan.*, 29 (1970) 56.
45 E. S. Kosmatyi, L. I. Bublik and G. V. Gavrilova, *Fiziol. Biokhim. Kul't. Rast.*, 4 (1972) 317; L. I. Bublik, G. V. Gavrilova and E. S. Kosmatyi, *C.A.*, 78 (1973) 93425r.
46 N. G. Porter, *J. Chromatogr.*, 28 (1967) 469.
47 S. C. Vyas and R. K. Tripathi, *Indian Phytopathol.*, 25 (1972) 513.
48 B. G. Tweedy, *J. Agr. Food Chem.*, 21 (1973) 323.
49 W. R. Benson, R. D. Ross, J. T. Chen, R. P. Barron and D. Mastbrook, *J.Ass. Offic. Anal. Chem.*, 55 (1972) 44.
50 J. H. Onley and G. Yip, *J. Ass. Offic. Anal. Chem.*, 54 (1971) 165.
51 G. Yip, J. H. Onley and S. F. Howard, *J. Ass. Offic. Anal. Chem.*, 54 (1971) 1373.
52 L. Giuffrida, *J. Ass. Offic. Anal. Chem.*, 48 (1965) 354.
53 W. R. Bontoyan, J. B. Looker, T. E. Kaiser, P. Giang and B. M. Olive, *J. Ass. Offic. Anal. Chem.*, 55 (1972) 923; W. R. Bontoyan and J. B. Looker, *J. Agr. Food Chem.*, 21 (1973) 338.
54 W. H. Newsome, *J. Agr. Food Chem.*, 20 (1972) 967.
55 C. H. Blazquez, *J. Agr. Food Chem.*, 21 (1973) 330.
56 J. H. Onley, R. W. Storherr, R. R. Watts and N. F. Ives, *ACS Natl. Meet., 164th, New York, N.Y., Aug. 27th, 1972*, Abstr. PEST 45.
57 R. F. Cook and B. C. Leppert, *ACS Natl. Meet., 164th, New York, N.Y., Aug. 27th, 1972*, Abstr. PEST 50.
58 L. D. Haines and J. L. Adler, *J. Ass. Offic. Anal. Chem.*, 56 (1973) 333.
59 P. A. Cruickshank and H. C. Jarrow, *J. Agr. Food Chem.*, 21 (1973) 333.
60 W. A. Steller, *J. Agr. Food Chem.*, 8 (1960) 460.
61 *Pesticide Analytical Manual*, Vol. II, Food and Drug Administration, Rockville, Md., revised ed., July 1970.
62 W. W. Kilgore and E. R. White, *J. Chromatogr. Sci.*, 8 (1970) 166.
63 O. Hutzinger, W. D. Jamieson and S. Safe, *J. Ass. Offic. Anal. Chem.*, 54 (1971) 178.
64 J. Crossley, in G. Zweig and J. Sherma (Editors), *Analytical Methods for Pesticides and Plant Growth Regulators, Vol. VI, Gas Chromatographic Analysis*, Academic Press, New York, 1972, Ch. 61.
65 J. Crossley, in G. Zweig and J. Sherma (Editors), *Analytical Methods for Pesticides and Plant Growth Regulators, Vol. VI, Gas Chromatographic Analysis*, Academic Press, New York, 1972, Ch. 66.
66 I. H. Pomerantz, L. J. Miller and G. Kava, *J. Ass. Offic. Anal. Chem.*, 53 (1970) 152.
67 W. W. Kilgore, W. Winterlin and R. White, *J. Agr. Food Chem.*, 15 (1967) 1035.
68 I. H. Pomerantz and R. Ross, *J. Ass. Offic. Anal. Chem.*, 51 (1968) 1058.
69 R. W. Storherr, P. Ott and R. R. Watts, *J. Ass. Offic. Anal. Chem.*, 54 (1971) 513.
70 P. B. Baker and B. Flaherty, *Analyst (London)*, 97 (1972) 713.
71 A. Bevenue and J. N. Ogata, *J. Chromatogr.*, 36 (1968) 529.
72 T. E. Archer and J. B. Corbin, *Bull. Environ. Contam. Toxicol.*, 4 (1969) 55.
73 L. Fishbein, J. Fawkes and P. Jones, *J. Chromatogr.*, 23 (1966) 476.
74 V. v. Bruchhausen and Ch. Dramdarevski, *Pestic. Sci.*, 2 (1971) 219; see also V. v. Bruchhausen and M. Stiansi, *Pestic. Sci.*, 4 (1973) 767 for later work on the same subject.
75 J. A. Burke and W. Holswade, *J. Ass. Offic. Anal. Chem.*, 49 (1966) 374.
76 J. P. Barrette and R. Payfer, *J. Ass. Offic. Agr. Chem.*, 47 (1964) 259.
77 H. L. Pease and R. W. Reiser, in J. Sherma and G. Zweig (Editors), *Analytical Methods for Pesticides and Plant Growth Regulators*, Vol. VII, Academic Press, New York, 1973, Ch. 39, pp. 657–664.
78 W. K. Hock and H. D. Sisler, *J. Agr. Food Chem.*, 17 (1969) 123.
79 R. C. Rhodes, H. L. Pease and R. K. Brantley, *J. Agr. Food Chem.*, 19 (1971) 745.

80 R. C. Rhodes and H. L. Pease, *J. Agr. Food Chem.*, 19 (1971) 750.
81 J. Yaffe, R. F. Cook, T. E. Cullen and R. P. Stanovick, in G. Zweig (Editor), *Analytical Methods for Pesticides and Plant Growth Regulators*, Vol. V, Academic Press, New York, 1967, Ch. 15.
82 T. C. Cullen and R. P. Stanovick, *J. Agr. Food Chem.*, 13 (1965) 118.
83 J. M. Devine, B. Fletcher and G. Zweig, *J. Ass. Offic. Anal. Chem.*, 52 (1969) 1106.
84 H. Beckman and A. Bevenue, *J. Food Sci.*, 27 (1962) 602.
85 K. W. Cheng and W. W. Kilgore, *J. Food Sci.*, 31 (1966) 259.
86 H. V. Brewerton, P. J. Clark and H. J. W. McGrath, *N. Z. J. Sci.*, 10 (1967) 124.
87 Upjohn Co., in G. Zweig and J. Sherma (Editors), *Analytical Methods for Pesticides and Plant Growth Regulators, Vol. VI, Gas Chromatographic Analysis*, Academic Press, New York, 1972, Ch. 65.
88 C. L. Keswani and D. J. Weber, *J. Chromatogr.*, 30 (1967) 130.
89 F. G. von Stryk, *J. Chromatogr.*, 31 (1967) 574.
90 R. F. Moseman, *ACS Natl. Meet., 165th, Dallas, Texas, April 1973*, Pesticide Division.
91 N. K. van Alfen and T. Kosuge, *J. Agr. Food Chem.*, 22 (1974) 221.
92 J. F. Eades and K. D. Gardiner, *Chem. Ind. (London) 1967*, p. 1359.
93 W. W. Kilgore and E. R. White, *J. Agr. Food Chem.*, 15 (1967) 1118.
94 E. R. White, W. W. Kilgore and G. Mallett, *J. Agr. Food Chem.*, 17 (1969) 585.
95 J. D. MacNeil, R. W. Frei and O. Hutzinger, *Mikrochim. Acta*, 5 (1973) 641.
96 F. I. Patrashku, L. B. Sorokskaya and B. A. Rekhter, *C.A.*, 78 (1973) 80691z.
97 D. E. Pack, H. Lee, L. Bouco, S. Sumida, Y. Hisada and J. Miyamoto, *J. Ass. Offic. Anal. Chem.*, 56 (1973) 53.
98 R. Mestres, L. Bouco and C. Espinoza, *Trav. Soc. Pharm. Montpellier*, 33 (1973) 195.
99 S. H. Yuen, in J. Sherma and G. Zweig (Editors), *Analytical Methods for Pesticides and Plant Growth Regulation, Vol. VII, Thin Layer and Liquid Chromatography and Pesticides of International Importance*, Academic Press, New York, 1973, Ch. 40, p. 665.
100 P. J. Wales and C. E. Mendoza, *J. Ass. Offic. Anal. Chem.*, 53 (1970) 509.
101 C. E. Mendoza and K. A. McCully, *Anal. Chem.*, 40 (1968) 2225.
102 R. M. Heatherington and C. Parouchais, *J. Ass. Offic. Anal. Chem.*, 53 (1970) 146.
103 G. A. Miller and C. E. Wells, *J. Ass. Offic. Anal. Chem.*, 52 (1969) 548.
104 J. A. Burke and W. Holswade, *J. Ass. Offic. Agr. Chem.*, 47 (1964) 845.
105 J. H. Simmons and J. O'G. Tatton, *J. Chromatogr.*, 27 (1967) 253.
106 J. Sherma, in J. Sherma and G. Zweig (Editors), *Analytical Methods for Pesticides and Plant Growth Regulation, Vol. VII, Thin Layer and Liquid Chromatography and Pesticides of International Importance*, Academic Press, New York, 1973, Ch. 1.
107 I. S. Taylor and F. P. Keenan, *J. Ass. Offic. Anal. Chem.*, 53 (1970) 1293.
108 R. J. Smyth, *J. Ass. Offic. Anal. Chem.*, 55 (1972) 806.
109 A. DiMuccio, L. Boniforti and R. Monacelli, *J. Chromatogr.*, 71 (1972) 340.
110 R. Weber, H. Wollenberg and C. Drossel, *Arch. Lebensmittelhyg.*, 24 (1973) 11.
111 A. Curley, V. W. Burse, R. W. Jennings, E. C. Villanueva, L. Tomatis and K. Akazaki, *Nature (London)*, 243 (1973) 338.
112 H. Wollenberg and C. Drossel, *Arch. Lebensmittelhyg.*, 24 (1973) 11.
113 D. J. Hamilton, *Queensl. J. Agr. Anim. Sci.*, 30 (1973) 109.
114 M. V. H. Holdrinet, *J. Ass. Offic. Anal. Chem.*, 57 (1974) 580.
115 P. J. Porcaro, *Anal. Chem.*, 36 (1964) 1664.
116 R. C. Bachmann and M. R. Shetlar, *Biochem. Med.*, 2 (1969) 313.
117 W. H. Gutenmann and D. J. Lisk, *J. Ass. Offic. Anal. Chem.*, 53 (1970) 522.
118 D. G. Ferry and E. G. Queen, *J. Chromatogr.*, 76 (1973) 233.
119 P. J. Porcaro, P. Shubiak and M. Manowitz, *J. Pharm. Sci.*, 58 (1969) 21.
120 R. S. Browning, J. Grego and H. P. Warrington, *J. Pharm. Sci.*, 57 (1968) 2165.
121 N. D. Greenwood, C. Hetherington, W. J. Cunliffe, J. C. Edwards and B. Williamson, *J. Chromatogr.*, 89 (1974) 103.
122 W. N. French, F. Matsui and S. J. Smith, *J. Chromatogr.*, 86 (1973) 211.
123 C. David Carr, *Anal. Chem.*, 46 (1974) 743.
124 P. J. Porcaro and P. Subiak, *Anal. Chem.*, 44 (1972) 1865.
125 E. W. Day, Jr., O. D. Decker, J. R. Koons and F. J. Holzer, *J. Ass. Offic. Anal. Chem.*, 53 (1970) 747.

126 A. K. Klein and R. J. Gajan, *J. Ass. Offic. Agr. Chem.*, 44 (1961) 712.
127 S. Gorbach and U. Wagner, *J. Agr. Food Chem.*, 15 (1967) 654.
128 T. P. Methratta, R. W. Montagna and W. P. Griffith, *J. Agr. Food Chem.*, 15 (1967) 648.
129 J. C. Caseley, *Bull. Environ. Contam. Toxicol.*, 3 (1968) 180.
130 E. J. Kuchar, F. O. Geenty, W. P. Griffith and R. J. Thomas, *J. Agr. Food Chem.*, 17 (1969) 1237.
131 D. G. Crosby and N. Hamadmad, *J. Agr. Food Chem.*, 19 (1971) 1171.
132 P. B. Baker and B. Flaherty, *Analyst (London)*, 97 (1972) 378.
133 F. D. Griffith, Jr. and R. V. Blanke, *J., Ass. Offic. Anal. Chem.*, 57 (1974) 595.
134 J. F. Thompson (Editor), *Analysis of Pesticide Residues in Human and Environmental Samples*, EPA Perrine Primate Laboratory, Perrine, Fla. (now EPA Pesticides and Toxic Substances Effects Laboratory, Research Triangle Park, N.C.), revised ed., November, 1972, Sec. 5, A, (3), (b).
135 J. B. Rivers, *Bull. Environ. Contam. Toxicol.*, 8 (1972) 294.
136 J. F. Thompson (Editor), *Analysis of Pesticide Residues in Human and Environmental Samples*, EPA Perrine Laboratory, Perrine, Fla. (now EPA Pesticides and Toxic Substances Effects Laboratory, Research Triangle Park, N.C.), revised ed., November, 1972, Sec. 5, A, (4), (a).
137 M. C. Bowman and M. Beroza, *J. Ass. Offic. Anal. Chem.*, 48 (1965) 943.
138 M. F. Cranmer, J. J. Carroll and M. F. Copeland, *Bull. Environ. Contam. Toxicol.*, 4 (1969) 214.
139 W. W. Kilgore and K. W. Cheng, in G. Zweig (Editor), *Analytical Methods for Pesticides and Plant Growth Regulators, Vol. V, Additional Principles and Methods of Analysis*, Academic Press, New York, 1967, Ch. 16, p. 313.
140 A. Bevenue, J. R. Wilson, E. F. Potter, M. K. Song, H. Beckman and G. Mallett, *Bull. Environ. Contam. Toxicol.*, 1 (1966) 257.
141 A. Bevenue and H. Beckman, *Residue Rev.*, 19 (1967) 83.
142 A. Bevenue, M. L. Emerson, L. J. Casarette and W. L. Yauger, Jr., *J. Chromatogr.*, 38 (1968) 467.
143 W. F. Barthel, A. Curley, C. L. Thrasher, V. A. Sedlak and R. Armstrong, *J. Ass. Offic. Anal. Chem.*, 52 (1969) 294.
144 A. Stark, *J. Agr. Food Chem.*, 17 (1969) 871.
145 V. Kubelka and M. Popl, *Chem. Prům.*, 23 (1973) 304.
146 A. S. Y. Chau and J. A. Coburn, *J. Ass. Offic. Anal. Chem.*, 57 (1974) 389.
147 M. Frei-Häusler, R. W. Frei and O. Hutzinger, *J. Chromatogr.*, 84 (1973) 214.
148 L. Renberg, *Anal. Chem.*, 46 (1974) 459.
149 B. M. Colvin, B. S. Engdahl and A. R. Hanks, *J. Ass. Offic. Anal. Chem.*, 57 (1974) 648.
150 R. Frank, O. D. Decker and E. W. Day, Jr., *J. Ass. Offic. Anal. Chem.*, 56 (1974) 11.
151 P. R. Wallnöfer, M. Königer, S. Safe and O. Hutzinger, *J. Agr. Food Chem.*, 20 (1972) 20.
152 H. L. Pease, J. A. Gardiner and R. F. Holt, in J. Sherma and G. Zweig (Editors), *Analytical Methods for Pesticides and Plant Growth Regulators*, Vol. VII, Academic Press, New York, 1973, Ch. 38, pp. 647–655.
153 J. P. Rouchaud and J. R. Decallonne, *J. Agr. Food Chem.*, 22 (1974) 259.
154 G. P. Clemons and H. D. Sisler, *Phytopathology*, 59 (1969) 705.
155 C. A. Peterson and L. V. Edgington, *J. Agr. Food Chem.*, 17 (1969) 898.
156 A. L. Homans and A. Fuchs, *J. Chromatogr.*, 51 (1970) 327.
157 V. Mallet, D. Surette and G. L. Braun, *J. Chromatogr.*, 79 (1973) 217.
158 J. Vogel, C. Corvi and G. Veyrat, *Mit. Geb. Lebensmitteluners. Hyg.*, 63 (1972) 453.
159 G. H. Tjan and L. J. Burgers, *J. Ass. Offic. Anal. Chem.*, 56 (1973) 223.
160 E. R. White and W. W. Kilgore, *J. Agr. Food Chem.*, 20 (1972) 1230.
161 E. R. White, E. A. Bose, J. M. Ogawa, B. T. Manji and W. W. Kilgore, *J. Agr. Food Chem.*, 21 (1973) 616.
162 F. J. Baude, J. A. Gardiner and J. C. Y. Han, *J. Agr. Food Chem.*, 21 (1973) 1084.
163 F. J. Baude, H. L. Pease and R. F. Holt, *J. Agr. Food Chem.*, 22 (1974) 413.
164 J. J. Kirkland, *J. Agr. Food Chem.*, 21 (1973) 171.
165 J. A. Gardiner, R. K. Brantley and H. Sherman, *J. Agr. Food Chem.*, 16 (1968) 1050.
166 J. A. Gardiner, J. J. Kirkland, H. L. Klopping and H. Sherman, *J. Agr. Food Chem.*, 22 (1974) 419.
167 J. J. Kirkland, R. F. Holt and H. L. Pease, *J. Agr. Food Chem.*, 21 (1973) 368.
168 N. Aharonson and A. Ben-Aziz, *J. Ass. Offic. Anal. Chem.*, 56 (1973) 1330.
169 P. B. Baker and R. A. Hoodless, *Analyst (London)*, 98 (1973) 172.

170 R. W. Buxton and T. A. Mohr, in G. Zweig (Editor), *Analytical Methods for Pesticides and Plant Growth Regulators, Vol. V, Additional Principles and Methods of Analysis*, Academic Press, New York, 1967, Ch. 10.
171 P. Morries, *J. Ass. Publ. Anal.*, 11 (1973) 44.
172 H. Beernaert, *J. Chromatogr.*, 77 (1973) 331.
173 R. A. Hites, *J. Chromatogr. Sci.*, 11 (1973) 570.
174 H. Hahn and H.-P. Thier, *Lebensmittelchem. Gerichtl. Chem.*, 26 (1972) 185.
175 R. M. Cassidy, D. S. LeGay and R. W. Frei, *J. Chromatogr. Sci.*, 12 (1974) 85.
176 M. Frei-Häusler, R. W. Frei and O. Hutzinger, *J. Chromatogr.*, 79 (1973) 209.
177 J. Nordal, *Nor. Vet.-Tidsskr.*, 84 (1972) 91.
178 P. E. Corneliussen, *J. Ass. Offic. Anal. Chem.*, 50 (1969) 934.
179 K. Callmer and O. Nilsson, *Chromatographia*, 6 (1973) 517.
180 S. K. Reeder, *ACS Natl. Meet., 167th, Los Angeles, Calif., April 1–5, 1974*, Pesticide Chemistry Division, Abstr. 85.
181 H. R. Sisken and J. E. Newell, *J. Agr. Food Chem.*, 19 (1971) 738.
182 W.-T. Chin, G. M. Stone and A. E. Smith, *J. Agr. Food Chem.*, 18 (1970) 709 and 731.
183 R. K. Tripathi and B. Bhaktavatsalam, *J. Chromatogr.*, 87 (1973) 283.
184 A. I. Allam and J. B. Sinclair, *Phytopathology*, 59 (1969) 1548.
185 D. R. Clifford, D. A. M. Watkins and D. Woodcock, *Chem. Ind. (London)*, p. 1654, 1965.
186 D. R. Clifford and D. A. M. Watkins, *J. Gas Chromatogr.*, 6 (1968) 191.
187 H. M. Boggs, *J. Ass. Offic. Anal. Chem.*, 49 (1966) 772.
188 C. P. Kurtz and H. Baum, *J. Ass. Offic. Anal. Chem.*, 52 (1969) 872.
189 C. P. Kurtz, H. Baum and C. Swithenbank, *J. Ass. Offic. Anal. Chem.*, 53 (1970) 887.
190 K. Chiba and H. Yatabe, *Noyaku Seisan Gijutsu (Pestic. Tech.)*, 14 (1966) 14.
191 F. Eisenbeiss and H. Sieper, *J. Chromatogr.*, 83 (1973) 439.
192 T. M. Shafik, H. C. Sullivan and H. F. Enos, *J. Agr. Food Chem.*, 21 (1973) 295.
193 J. Hrivnak and Z. Sota, *J. Gas Chromatogr.*, 6 (1968) 9.
194 G. Yip and S. F. Howard, *J. Ass. Offic. Anal. Chem.*, 49 (1966) 1166.
195 A. Guardigli, W. Chow and M. S. Lefar, *J. Agr. Food Chem.*, 19 (1971) 1181.
196 H. Bratt, J. W. Daniel and I. H. Monks, *Food Cosmet. Toxicol.*, 10 (1972) 489.
197 J. E. Bagness and W. G. Sharples, *Analyst (London)*, 99 (1974) 225.
198 P. Cenci and B. Cremonini, *Ind. Sacc. Ital.*, 62 (1969) 313.
199 G. Boenig and H. Heigener, *Landwirtsch. Forsch.*, 25 (1972) 378.
200 J. E. Davenport, *J. Ass. Offic. Anal. Chem.*, 54 (1971) 975.
201 M. L. Porter, R. J. Gajan and J. A. Burke, *J. Ass. Offic. Anal. Chem.*, 52 (1969) 177.
202 H. Hey, *Z. Lebensm.-Unters.-Forsch.*, 149 (1972) 79.
203 C. Reinhard, *Chem. Mikrobiol. Technol. Lebensm.*, 2 (1973) 57.
204 S. M. Norman, D. C. Fouse and C. C. Craft, *J. Ass. Offic. Anal. Chem.*, 55 (1972) 1239; *J. Agr. Food Chem.*, 20 (1972) 1227.
205 E. Kroeller, *Deut. Lebensm.-Rundsch.*, 65 (1969) 85.
206 E. Kroeller, *Deut. Lebensm.-Rundsch.*, 67 (1971) 229.
207 K. M. Al-Adil, E. R. White, M. W. McChesney and W. W. Kilgore, *J. Agr. Food Chem.*, 22 (1974) 242.
208 Y. Soeda, S. Kosaka and T. Noguchi, *Agr. Biol. Chem.*, 36 (1972) 931.
209 E. W. Day, Jr., F. J. Holzer, J. B. Tepe and M. J. Kolbezen, *J. Ass. Offic. Anal. Chem.*, 51 (1968) 39.
210 F. G. von Stryk, *J. Chromatogr.*, 72 (1972) 410.
211 P. B. Baker, J. E. Farrow and R. A. Hoodless, *J. Chromatogr.*, 81 (1973) 174.
212 P. B. Baker and R. A. Hoodless, *J. Chromatogr.*, 87 (1973) 585.
213 L. Fishbein, *J. Chromatogr.*, 27 (1967) 368.
214 *Pesticide Dictionary*, Meister Publishing Co., Willoughby, Ohio, 1973.
215 G. R. Higginbotham, J. Ress and A. Rocke, *J. Ass. Offic. Anal. Chem.*, 53 (1970) 673.