Multiple Insecticide Residue Determination Using Column Chromatography, Chemical Conversion, and Gas-Liquid Chromatography

W. W. SANS

Florisil column fractionation separates compounds which are difficult to resolve gas chromatographically and permits detection of greater numbers of pesticides in one sample. Chemical conversion of parent insecticides to alternate gas chromatographically responsive products results in specific identification. Two-column gas chromatography using column liquid stationary phases of Dow 11 and QF-1 results in altered retention time and elution order. These procedures make it possible to identify and evaluate samples containing multiple pesticide residues.

One of the newer tools for pesticide residue evaluation is the gas-liquid chromatograph, which can detect mixtures of insecticides in a single sample (*1*). In some cases, however, insecticides, their metabolites, and artifacts have similar or identical retention times (*3*, *10*). It has been difficult to identify responses of DDE–dieldrin and *o,p'*-DDT–DDD in a sample, unless the insecticides are used separately (*2*, *7*). Determination of insecticide residues in soils and crops, however, becomes more complicated if the numerous responses resulting from varied pesticide treatments over many years are present. Consequently, identification based on retention time by a single column on a 20- to 30-minute chromatogram cannot be taken as absolute proof. This time lapse is generally chosen as convenient for a single analysis (*5*, *17*).

The separation of insecticides by liquid-solid fractionation—e.g., Florisil—has been suggested (*3*) and reported (*13*), but the primary use of this technique has been as a cleanup step. Multicolumn gas chromatography (*5*) and chemical conversion techniques (*8*, *10*, *15*, *16*) have also been reported. Their purpose and method differ from that reported here, in that they were used for increasing pesticide sensitivity (*8*, *10*) and in chemical coupling with preferred chromogens (*15*, *16*).

This paper describes a combination of these procedures with gas chromatography for identification of insecticide residues in samples having an unknown or incomplete history of treatment.

Reagents

Potassium hydroxide solution, 2% (w./v.) in 95% ethanol.

Chromic acid solution, 15 grams of CrO_3, 50 ml. of acetic acid, and 6 ml. of distilled water.

Petroleum ether, b.p. 30–60° C. Distill.

Benzene, reagent grade. Distill.

Chloroform, reagent grade. Distill.

Acetone, reagent grade. Distill.

Absolute alcohol.

Florisil, 60- to 100-mesh, 660° C., factory-treated (Floridin Co., Tallahassee, Fla.).

Sodium sulfate, granular, anhydrous reagent.

Sodium-lead alloy (dri-Na), 9% active sodium.

Apparatus

Glass columns for column chromatography: 15-mm. i.d. × 500 mm., fitted with a coarse sintered disk (Corning Glass Works, Corning, N.Y.).

Construct a constant liquid level device by attaching a 7-mm. o.d., 7-cm. long glass tube to a 24/40 ⅀ ground-glass joint. Fill a 250-ml. Erlenmeyer flask, equipped with a 24/40 ⅀ glass joint, with the appropriate solvent. Insert the adapter and invert into the top of the chromatographic column.

Quickfit and quartz test tubes 200 mm. long having MF 24/3/8 ⅀ ground-glass joints.

Wilkens Aerograph, Model 600C Hy-Fi gas chromatograph, and an additional oven, Model 550, with electron-capture detectors or a dual column oven unit.

Gas Chromatographic Parameters

COLUMN 1. Borosilicate glass column, 3.2-mm. o.d. × 2.0-mm. i.d. × 152 mm. long, packed with 0.86 gram of Chromosorb W, nonacid-washed, 60- to 80-mesh, 5% Dow 11 silicone–coated. Condition for 24 hours at 250° C. prior to use at 187° C., 60 cc. per minute nitrogen flow, attenuation 8, and detector at 30 volts (chromatograms, Figure 1, *a*, *b*, *c*, and *d*; Figure 2, *a*, *b*, *c*, and *d*; Figure 3, *a* and *b*; Figure 4, *a* and *b*).

COLUMN 2. Glass column as above, packed with 1.12 grams of Chromosorb W, HMDS-treated, 60- to 80-mesh 5% QF-1 silicone fluid–coated. Heat the QF-1 column substrate in a forced air oven at 105° C. for 18 hours

Entomology Laboratory, Canada Department of Agriculture, Chatham, Ontario, Canada.

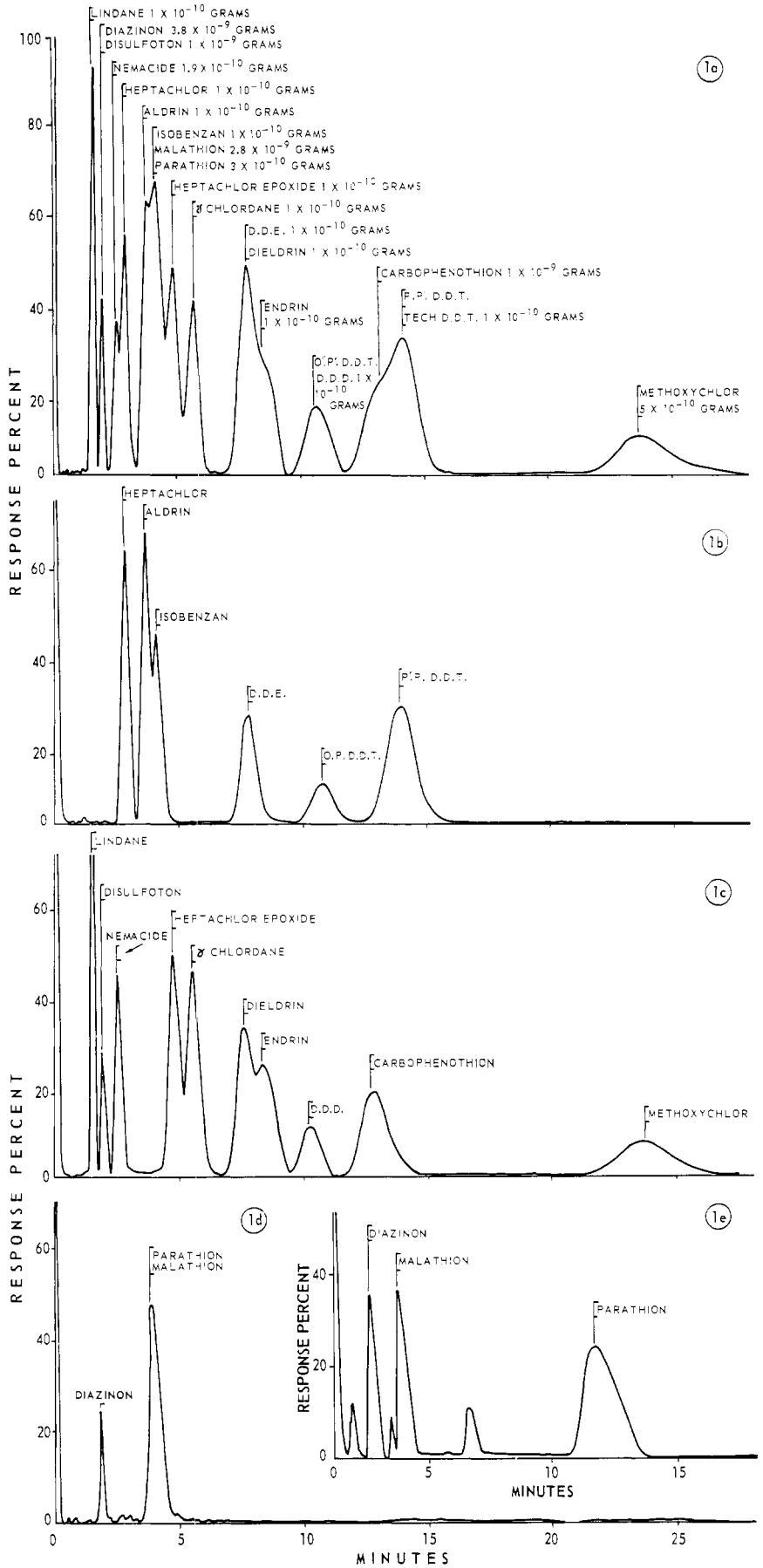

Figure 1. Chromatograms of insecticides before and after Florisil column fractionation

a. Composite sample of 18 insecticides
b. First fraction, 200 ml. of petroleum ether containing six insecticides
c. Second fraction, 200 ml. of 5 to 1 benzene–petroleum ether containing 10 insecticides
d. Third fraction, 200 ml. of chloroform containing three insecticides
e. Resolution of diazinon, malathion, and parathion

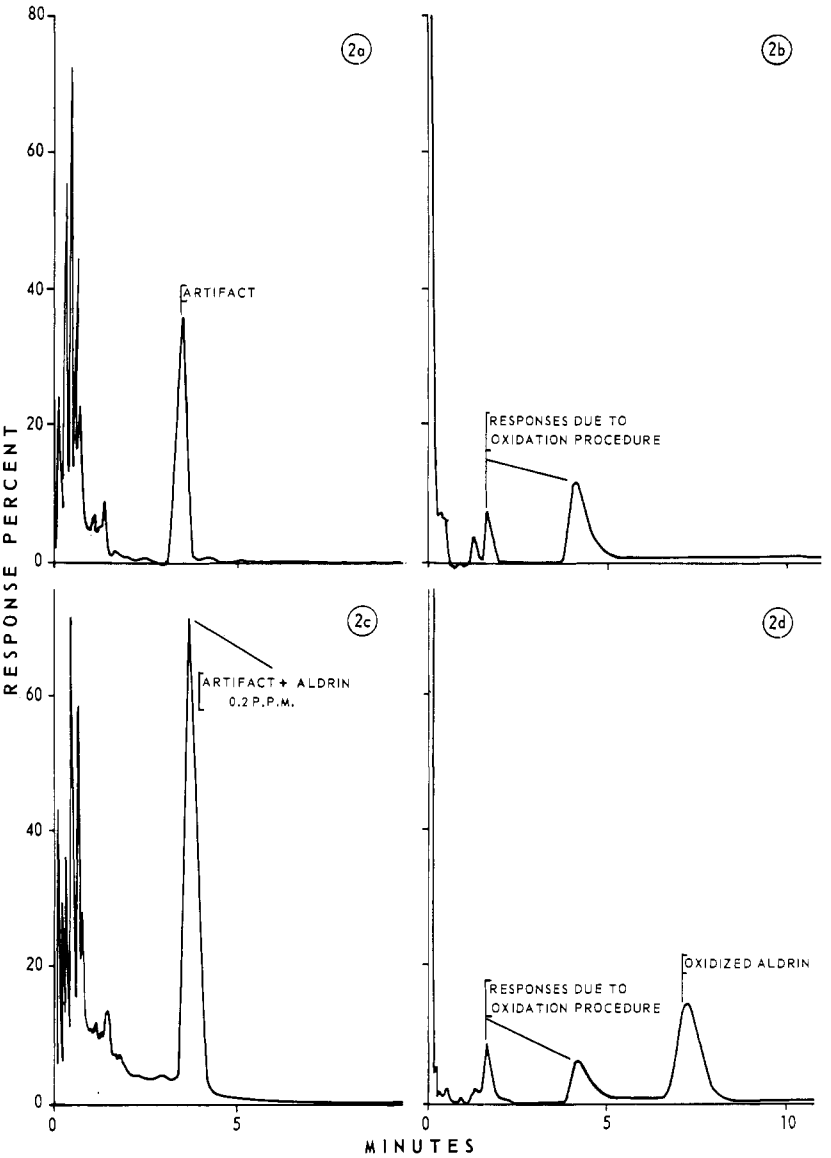

Figure 2. Chromatograms of aldrin in presence of turnip artifact

a. Artifact in turnip extract representing 1 gram per ml. of turnip, 2 μl. injected
b. Oxidized turnip extract, (*a*), 2 μl. injected
c. Turnip artifact + 0.2 p.p.m. of aldrin (*a* + aldrin), 2 μl. injected
d. Oxidized fortified turnip extract (*c*), 2 μl. injected

prior to packing into the column. Condition further 48 hours at 240° C. before use at 155° C., 25 cc. per minute of nitrogen flow, attenuation 8, and detector at 30 volts (chromatogram, Figure 1*e*).

Column Chromatography

Prepare the Florisil column as follows: Add 2 grams of anhydrous sodium sulfate, followed by 30 grams of Florisil. Tap the column gently with a wooden dowel or spatula handle until the Florisil level is constant, then add 5 grams more of sodium sulfate. Prewash the column with 50 ml. of benzene, followed by two successive additions of 25 ml. of petroleum ether. Allow the column to drain until dripping ceases. Using a 50-ml. graduate as a receiver, apply the petroleum ether extract containing pesticides (approximately 5 to 10 ml.) to the column. Rinse the flask with petroleum ether several times and add the rinsings to the column. Discard the first 40 ml. of eluate. Using a 250-ml. Erlenmeyer flask as a receiver, elute the column with solvents in the order given, collecting volumes as follows: 200 ml. of petroleum ether (first fraction), 200 ml. of 5 to 1 benzene–petroleum ether (second fraction), 200 ml. of chloroform (third fraction), and 150 ml. of acetone (fourth fraction). A constant flow rate of 6 ml. per minute based on petroleum ether is optimum. Six columns may be run simultaneously using the constant liquid level adapters.

Begin and terminate fraction collections when the liquid level in the column reaches the upper sodium sulfate layer. Reduce the volumes from the first and second fractions to about 3 ml., transfer to 10-ml. volumetric flasks with rinsings, and make up to volume

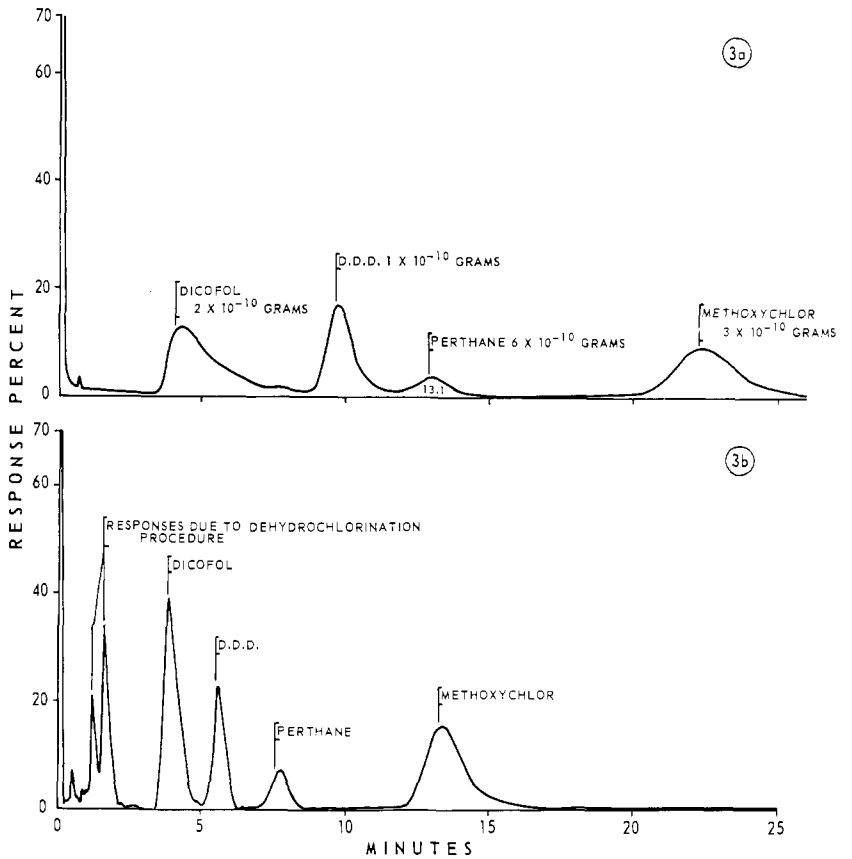

Figure 3. Chromatograms of insecticides and their dehydrochlorinated products

a. Insecticide standards
b. Dehydrochlorinated insecticide standards

with petroleum ether. Evaporate the third and fourth fractions just to dryness using a water bath and manifold with positive air pressure (*11*). Take up the residue in petroleum ether and transfer to a 10-ml. volumetric flask as described above.

Standardize the Florisil column by applying two composite insecticide solutions containing different components and fractionate according to the procedure outlined above. The first fraction should contain the components of solution I: heptachlor, aldrin, isobenzan, DDE, and technical DDT; the second fraction, solution II components: lindane, heptachlor epoxide, γ-chlordan, dieldrin, endrin, DDD, and methoxychlor.

Oxidation Procedure

Evaporate the solvent from an appropriate sample just to dryness in a large test tube. Add 4 ml. of oxidation solution (reagent B) and place in a 77° C. water bath for 12 minutes. Remove and cool. Add 10 ml. of petroleum ether and swirl. Add approximately 70 ml. of distilled water, stopper, and shake for 15 seconds. Allow the phases to separate and remove the aqueous layer by aspiration. Repeat the washing process twice. After the last aspiration add 3 grams of anhydrous sodium sulfate and swirl. Decant the petroleum ether portion to a convenient container and inject into a gas chromatograph.

Dehydrochlorination Procedure

Evaporate the solvent from an appropriate sample just to dryness in a large test tube. Add 5 ml. of 2% alcoholic potassium hydroxide (reagent A). Stopper and place in a 77° C. water bath for 15 minutes. Cool, add 10 ml. of petroleum ether, and continue as in the oxidation procedure.

Results and Discussion

Identification or quantitation would not be possible using the crowded chromatogram obtained when 19 pesticides were injected without prior fractionation (Figure 1*a*). Florisil column fractionation separates the insecticides so that the charts (Figure 1, *b*, *c*, and *d*) display peaks of 6, 10, and 3 insecticides, respectively. It is thus possible to obtain clear separation between peaks and leave room for other responses. Malathion and parathion have identical retention times on the Dow 11 column at the temperature and nitrogen flow used. The insert (Figure 1*e*) illustrates their resolution when the QF-1 column is used. This author used the Florisil column to separate insecticides at the 10-μg. level. Larger quantities have been applied, but tailing into other fractions has been noted in some instances. Table I, which lists the fractions and gas chromatographic retention times of 32 pesticides, illustrates the ease with which mixtures with similar retention times

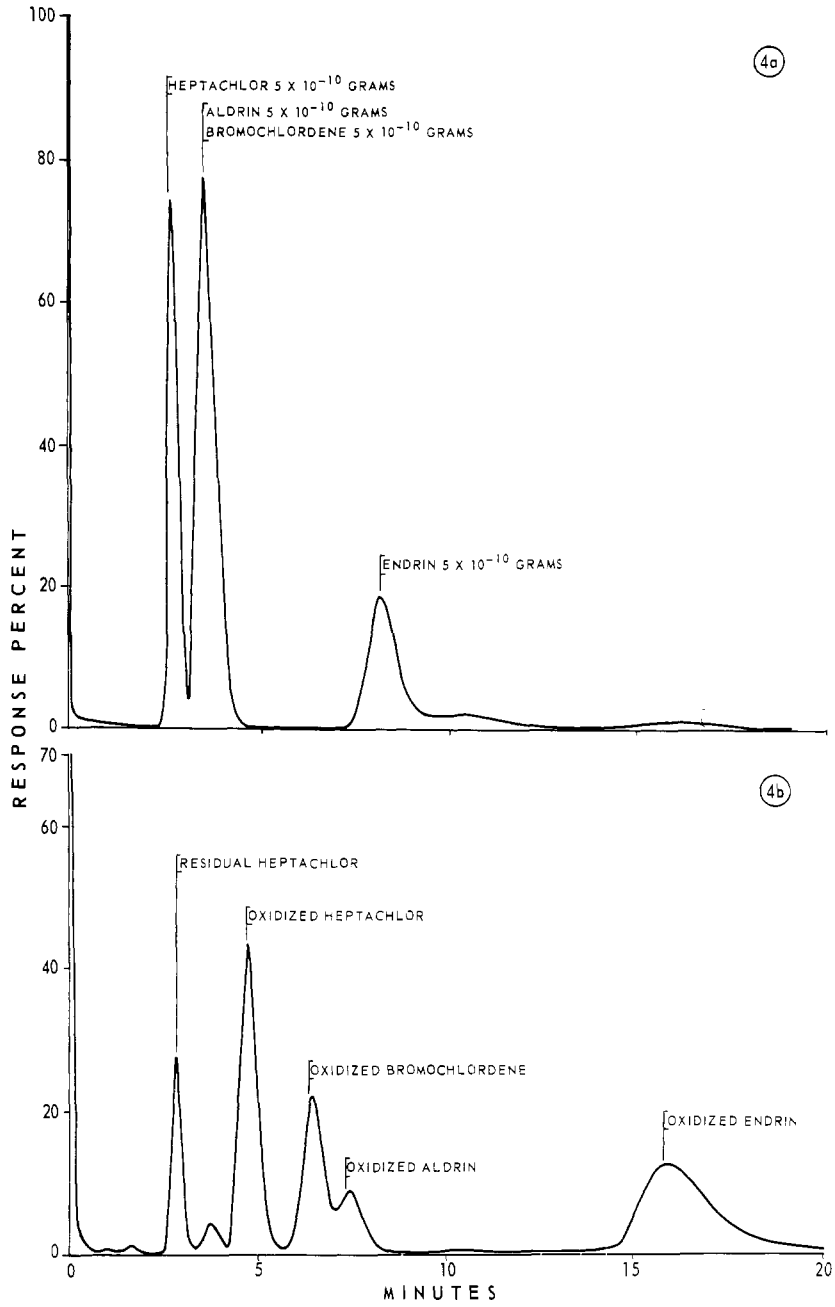

Figure 4. Chromatograms of insecticides and their oxidized products

a. Insecticide standards
b. Oxidized insecticide standards

can be separated—e.g., diazinon-disulfoton, dicofol-parathion, dieldrin-DDE, and *o,p'*-DDT–DDD. Not listed is the fourth fraction which contains the metabolites of azinphosmethyl (*12*) and diazinon. It is possible that metabolites of similar organophosphorus insecticides would also elute in this fraction.

Since individual lots of Florisil may have different activities, the weight of adsorbent and volumes of solvents can be used only as a guide. The analyst will have to adjust these so that the insecticides of composite I do not elute in the second fraction and those of composite II do not elute in either the first or third fraction. When extreme activity was encountered, this author found the addition of 0.5 ml. of absolute alcohol to the column prewash procedure an excellent way to obtain the desired deactivation. Increasing the volume of alcohol makes the Florisil less adsorbent, a factor that can be precisely controlled to suit individual requirements.

The Dow 11 silicone column is used for primary detection and quantitative evaluation. Dieldrin and endrin, for accurate quantitative work, can be separated by reducing the nitrogen flow rate when the Dow 11 column is used or by injection onto the QF-1 column.

Table I. Florisil Fractionation of Some Insecticides and Their Gas Chromatographic Retention Times in Minutes

Dow 11				QF-1			
	Fraction				Fraction		
Insecticide	1	2	3	Insecticide	1	2	3
Azinphosmethyl			0.5	Azinphosmethyl			0.8
Phorate		1.0		Phorate		0.8	
Lindane		1.5		Heptachlor	0.9		
Diazinon			1.7	Lindane		0.9	
Disulfoton		1.7		Aldrin	1.1		
Nemacide		2.2		Bromochlordene	1.2		
Heptachlor	2.6			Disulfoton		1.3	
Aldrin	3.5			Isobenzan	1.5		
Bromochlordene	3.8			Diazinon			1.6
Dicofol		3.6		Nemacide		1.8	
Parathion			3.7	γ-Chlordan		2.6	
Morestan			3.8	BAY 37289		2.7	
BAY 37289		3.9		Heptachlor epoxide		3.0	
Isobenzan	4.0			DDE	3.2		
Malathion			4.3	Bromochlordene epoxide		3.4	
Heptachlor epoxide		4.6		Perthane	4.0		
Captan			4.7	*o,p'*-DDT	4.4		
γ-Chlordan		5.4		Endosulfan I		4.4	
Endosulfan I		6.1		Morestan			4.5
Bromochlordene epoxide		6.4		Dieldrin		4.9	
Dieldrin		7.4		Dicofol		5.0	
DDE	7.6			Malathion			5.5
Endrin		8.1		Endrin		5.6	
Endosulfan II			9.3	DDD		6.6	
DDD		9.9		*p,p'*-DDT	7.4		
o,p'-DDT	10.4			Parathion			11.1
Ethion		11.2		Carbophenothion		9.5	
Carbophenothion		13.0		Ethion		10.9	
Perthane	13.5			Methoxychlor		15.6	
p,p'-DDT	13.6			Endosulfan II			17.2
Methoxychlor		22.6		Tetradifon			48.0
Tetradifon			23.2				

Because of the different retention times and order of elution (Table I), this latter column is also used either to augment identification or to resolve spurious responses. For example, on the Dow column the order of elution for some of the more common insecticides is diazinon, Nemacide, heptachlor, heptachlor epoxide, γ-chlordan, dieldrin, DDE, ethion, and carbophenothion. By contrast, the order of elution on the QF-1 column is heptachlor, diazinon, Nemacide, γ-chlordan, heptachlor epoxide, DDE, dieldrin, carbophenothion, and ethion. The QF-1 column is not used for primary detection because of poor resolution of a number of insecticides.

Identification and evaluation of questionable sample responses are further substantiated by subjecting them to the oxidation or dehyrochlorination procedures, thus converting the parent materials to their metabolites to distinguish them from artifacts—for example, an artifact present in turnips has an identical retention time to aldrin. The oxidation procedure destroys the artifact while producing a response due to the aldrin oxidation product, which presumably is dieldrin (Figure 2), because of its identical column fractionation and gas chromatographic retention characteristics. Gannon and Bigger (*4*) have shown aldrin to be susceptible to conversion to dieldrin. Heptachlor, bromochlordene, and endrin can also be converted by the oxidation procedure to produce alternate column and gas chromatographic elutions (Figure 4, *a* and *b*). The water bath temperature and time element are critical, particularly when mixtures of insecticides are present in one sample. A higher temperature yields more complete conversion of heptachlor to its epoxide but reduces the quantity of converted aldrin product. The converse is true when a lower temperature is used.

The conversion of dicofol to *p,p'*-dichlorobenzophenone (*6*) and DDD, *o,p'*- and *p,p'*-DDT, Perthane, and methoxychlor to their olefins (*10*) is attained by dehydrochlorination (Figure 3, *a* and *b*). The gas chromatographic responses obtained serve as a more specific identification when compared with those of the parent compound. The oxidation and dehydrochlorination procedures produce some early gas chromatographic responses which can be eliminated by Florisil column fractionation prior to injection. This also serves as an additional verification of the identity of the

insecticide. The conversion procedures can be applied to materials other than those mentioned above.

Other spurious responses, with retention times identical to some insecticides, have also been detected—e.g., undistilled petroleum ether, benzene, and alcohol. When chromatographically pure solvent was passed through filter paper, an interfering response was also obtained. Benzene exhibits two responses; one is removed by reflux and distillation over 9% active sodium-lead alloy, the other by Florisil column chromatography prior to use. The removal of these solvent contaminants is essential in situations which require concentration of large volumes of solvents.

The combined procedures outlined above tentatively identify pesticides by column fractionation, chemical conversion, and gas chromatographic retention time; resolve pesticides which otherwise would have identical retention times; and afford cleanup of extracts to reduce contamination of gas chromatographic columns and detectors. During the past 2 years these methods have worked satisfactorily with extracts of various soil types (*9*), alfalfa, carrots, corn, onions, flue-cured tobacco, radishes, and turnips.

Chemical Designations of Proprietary Materials

BAY 37289. *O*-Ethyl *O*-2,4,5-trichlorophenyl ethylphosphonothioate.

1-Bromochlordene. 1-Bromo-4,5,6,7,8,8-hexachloro-3*a*,4,7,7*a*-tetrahydro-4,7-methanoindene.

Morestan. 6-Methyl-2,3-quinoxalinedithiol cyclic *S*,*S*-dithiocarbonate.

Nemacide. *O* - (2,4 - Dichlorophenyl) *O*,*O* - diethyl phosphorothioate.

Perthane. 1,1 - Dichloro - 2,2 - bis(*p* - ethylphenyl) ethane.

Acknowledgment

The author thanks J. R. W. Miles and other members of the laboratory staff for their advice in preparing this paper.

References

(1) Bonelli, E. J., Hartmann, H., Dimick, K. P., J. AGR. FOOD CHEM. **12,** 333 (1964).
(2) Bowman, M. C., Schechter, M. S., Carter, R. C., *Ibid.*, **13,** 360 (1965).
(3) Burke, J., Giuffrida, L., *J. Assoc. Offic. Agr. Chemists* **47,** 326 (1964).
(4) Gannon, N., Bigger, J. A., *J. Econ. Entomol.* **51,** 1 (1958).
(5) Goulden, R., Goodwin, E. S., Davies, L., *Analyst* **88,** 941 (1963).
(6) Gunther, F. A., Blinn, R. C., J. AGR. FOOD CHEM. **5,** 517 (1957).
(7) Gutenmann, W. H., Lisk, D. J., *Ibid.*, **11,** 301 (1963).
(8) Gutenmann, W. H., Lisk, D. J., *J. Assoc. Offic. Agr. Chemists* **46,** 859 (1963).
(9) Harris, C. R., Sans, W. W., Miles, J. R. W., J. AGR. FOOD CHEM. **14,** 398 (1966).
(10) Klein, A. K., Watts, J. O., *J. Assoc. Offic. Agr. Chemists* **47,** 311 (1964).
(11) Miles, J. R. W., *Chemist-Analyst* **48,** 100 (1959).
(12) Miles, J. R. W., *J. Assoc. Offic. Agr. Chemists* **47,** 882 (1964).
(13) Moats, W. A., *Ibid.*, **46,** 172 (1963).
(14) Murphy, R. T., Barthel, W. F., J. AGR. FOOD CHEM. **8,** 442 (1960).
(15) Schechter, M. S., Haller, H. L., *J. Am. Chem. Soc.* **66,** 2129 (1944).
(16) Skerrett, E. J., Baker, E. A., *Analyst* **85,** 606 (1960).
(17) Watts, J. O., Klein, A. K., *J. Assoc. Offic. Agr. Chemists* **45,** 102 (1962).

Received for review June 21, 1966. Accepted September 26, 1966. Contribution 73, Entomology Laboratory, Research Branch, Canada Department of Agriculture, Chatham, Ontario.