Vapor Phase Cleanup Method for Gas Chromatographic Determination of Pesticide Residues in Plant Extractives

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A vapor phase method for cleanup of plant extractives intended for pesticide residue determination by gas chromatography features use of the superheated vapor of a normally liquid solvent to separate the pesticide vapor from the non- or less-volatile extractives of plant materials. Quantitative condensation of the vapor mixtures resulted in quantitative recovery of the volatilized pesticides. For 11 chlorinated hydrocarbon insecticides and a chlorinated phenoxyalkyl acid herbicide in citrus fruit extracts and oils, the average recoveries ranged from 91.7 to 107.2% of the amounts introduced into the cleanup system. Data show effective cleanup with high recovery and replicative results.

In the gas chromatographic analysis of pesticides, various difficulties have been experienced with the separation of pesticide residues from naturally occurring plant substances which cause instrumentation interference.

More specifically, extractives derived from plants contain, besides the organic pesticide residues, volatile substances and non- or less-volatile substances. The nonvolatile substances such as waxes and fixed oils do not pass through the column and, thus, do not contaminate the stationary phase in the column to make the analysis difficult.

Citrus fruit and products therefrom are no exception, and most of the following work was done in the presence of citrus fruit extractives. The removal of interfering plant waxes and other non- or less-volatile substances from pesticide residue extracts not only extends column life by reducing the residue left in the injection block and column, but also provides improved chromatographic results.

Numerous cleanup procedures have been suggested, based on selective adsorption, solvent partitioning, and other physical and chemical methods (*1*, *3–4*, *5*, *7*, *13*, *15*). Each has demonstrated its efficiency and usefulness for particular problems.

Adsorption column chromatography is probably the most widely accepted. Its disadvantages are variable recoveries of pesticides due to substantial retention of some pesticides on the column and, in some cases, problems relating to the use of mixed adsorbents or elution solvent systems (*14*). No single chromatographic adsorbent or elution solvent system has been found suitable for cleanup of all samples with multiple pesticide contamination. It would be more difficult for a residue analyst to apply a column chromatographic cleanup method for a sample with little or no record of pesticide treatment.

Mills (*11*) reported elution difficulty with the aldrin group, kelthane, and several other compounds from a column of activated Florisil. McKinley *et al.* (*9*, *10*), Eidelman (*3*), Moats (*12*), and Baetz (*1*) separately reported elution and recovery difficulties with various adsorbents.

Farrow (*5*) has reported on a vacuum sublimation cleanup method. The results indicate considerable variation in the recoveries of the pesticides tested.

Gunther, Blinn, and Ott (*7*, *15*) reported on the use of a cleanup device employing vaporization of pesticide residues using nitrogen as the carrier gas. Their nonselective recoveries of pesticides ranged from 80 to 93%.

Recently Storherr and Watt (*16*) reported a method similar to that of Gunther, using apparatus similar to a prototype constructed in this laboratory. Though this type of cleanup device presented good recovery with extracts of samples of low wax or fixed oil content, our experience has been that extracts containing large amounts of plant fats and/or waxes gave erratic results.

Because low and variable recoveries of pesticides often result from various types of cleanup procedure, replicative high recovery of pesticide was the objective. This paper describes the use of readily condensable and nonreactive vapor of *n*-hexane as a carrier gas in a simple operation.

Materials and Methods

Reagents. Standard 1 μg. per μl. pesticide solutions employed for fortification were prepared in xylene from analytical grade γ-benzene hexachloride, heptachlor, kelthane, aldrin, ovex, dieldrin, Chlorobenzilate, Thiodan, DDT, methoxychlor, Tedion, and the isopropyl ester of 2,4,5-trichlorophenoxyacetic acid. These pesticides were furnished by various pesticide manufacturers and used without further purification. Xylene, *n*-hexane, and Socal No. 2 solvent were redistilled in the laboratory and chromatographed to confirm freedom from interfering organic chlorides prior to use.

Apparatus. Cleanup apparatus (Figure 1), a microfractional concentrator (Figure 2), and a Dohrmann gas chromatograph instrument equipped

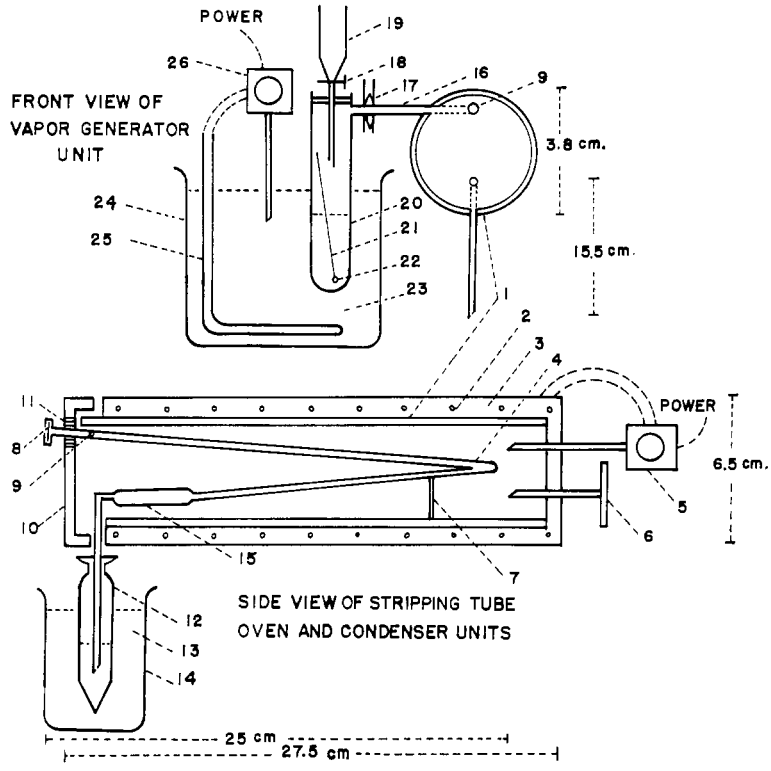

Figure 1. Schematic diagram of vapor phase cleanup apparatus

1. Aluminum tube
2. Nichrome heating wire
3. Insulation
4. Stripping tube
5, 26. Thermostat controls
6. Thermometer
7. Tube support
8. Injection port
9. Hexane vapor inlet
10. Removable cap of housing
11. Glass wool plug
12. Vapor trapper
13. Ice bath
14, 24. 250-ml. beakers
15. Enlargement
16. Side neck
17. Ball joint with metal clamp
18. Stopcock
19. Hexane reservoir
20. Hexane vapor generator
21. Wire
22. Boiling chip
23. Low viscosity silicone oil
25. Heater

with T-100 silver microcoulometric detector were used. The gas chromatographic column consisted of a 200-cm. × 6.4-mm. o.d. (6-feet × 1/4-inch) aluminum column packed with 20% (w./w.) Dow-Corning high vacuum silicone grease on 60- to 80-mesh acid-treated Chromosorb P.

Cleanup Apparatus. The experimental cleanup apparatus consists of a thermostatically controlled oven, a vapor generator, a stripping tube, and a vapor condenser. The arrangement of these components is shown in Figure 1.

The oven was constructed from a 25-cm. length of aluminum tubing (3.8-cm. o.d.). The tube was covered with thin sheet asbestos as electrical insulation and wound with a commercially manufactured 300-watt (at 115 volts) Nichrome wire covered with another layer of asbestos. This assembly was housed in a 6.5-cm. o.d. sheet metal tube and the space between the heater and the tube was filled with glass wool. Temperature regulation of the oven was provided by a thermostat.

The vapor generator, 20, was made from a 20-mm. borosilicate glass test tube with side neck to which a standard 12/3 size ball joint, 17, had been sealed. The vapor generator was heated in a silicone oil bath equipped with a 250-watt thermostatically controlled immersion heater. A tiny fragment of wood, held with a wire to the bottom of the vapor generator tube, acts as a boiling chip to ensure smooth boiling of the hexane. The rate of vapor evolution and flow can be regulated by adjusting the temperature of the bath. The quantity of vapor used to elute the pesticide residue in vapor form is controlled by putting into the vapor generator tube only the desired amount of hexane.

The stripping tube, 4, was made from a piece of Pyrex tubing (4-mm. i.d., Corning No. 7740) bent into a V shape in such a way that each arm of the V is approximately 25 cm. in length. The upper end of the V is provided with a silicone rubber septum for sample injection, 8, and 2.5 cm. down the tube (at a point inside the oven) as noted, 9, a side arm for introduction of hexane vapor comes in from the top. At the other end of the V, an enlargement, 15, of the tube provides a small reservoir for accumulation of the nonvolatile residues. When installed as shown in Figure 1, the V shape provides a continuous gradual downward slope from the point of sample injection to the enlargement near the end.

The condenser is simply a 15-ml. graduated centrifuge tube to which a socket portion of ball joint had been sealed to permit direct connection to the microfractional concentrator (Figure 2). It is cooled by being floated in ice water. The ball joint, which does not function as a part of the condenser, is used subsequently to connect the centrifuge tube with its load of hexane and the volatiles from a sample to the microfractional concentrator (Figure 2). The hexane can then be distilled off under vacuum without the necessity of transfer to another container.

Operation. An operating oven temperature of 245° C. generally has been used. Experience with the Dohrmann gas chromatograph at this temperature indicates that most chlorinated hydrocarbon- and sulfur-containing pesticides are stable and have a satisfactory vapor pressure. The flow of hexane vapor was adjusted to a rate of approximately 2 ml. (as liquid) per minute by regulation of the vapor generator thermostat, 26.

Prior to the use of the vapor phase cleanup process, extraction solvent was distilled off using the Kuderna-Danish evaporator (*2*). In the case of citrus oils, most of the terpenes were removed by the microfractional concentrator (Figure 2) in place of the evaporator. With negligible loss of pesticide residue, this concentration process greatly reduced the volume of sample that must be injected into the vaporizer and prevented the flashing that would result if a large volume of highly volatile material were to come in contact with the hot tube.

A volume of up to 0.5 ml. of concentrated sample, equivalent to 5 ml. of the original citrus oil, was found to be a suitable quantity to inject into the stripping tube. A hypodermic needle long enough to reach beyond the point of entrance of the hexane vapor, 9, was used to accomplish this.

As the injected sample flows downward along the slope of the tube, new surface is continuously developed from which the pesticide vapor is stripped. The pesticide residue vapor is swept into the condenser by the hexane used as the carrier vapor. The nonvolatile substances remain behind and are collected in the enlargement, 15, of the tube, located just before the tube exit from the oven.

The volatile materials, including the pesticide residues and the hexane vapor, are condensed in the graduated centrifuge tube. The necessary elution time was established at approximately 8 minutes after injection on the basis of the maximum recovery of a known quantity of aldrin from lemon oil. At the end of the elution period, about 200 μl. of Socal No. 2 solvent was slowly injected. This solvent has a high boiling point, and its vapor condensed on the inside of the stripping tube just after it left the oven. The resulting film of liquid efficiently flushed out traces of pesticide residue that had condensed inside the cool end of the tube.

Several samples, or replicates, may be run through the apparatus before the

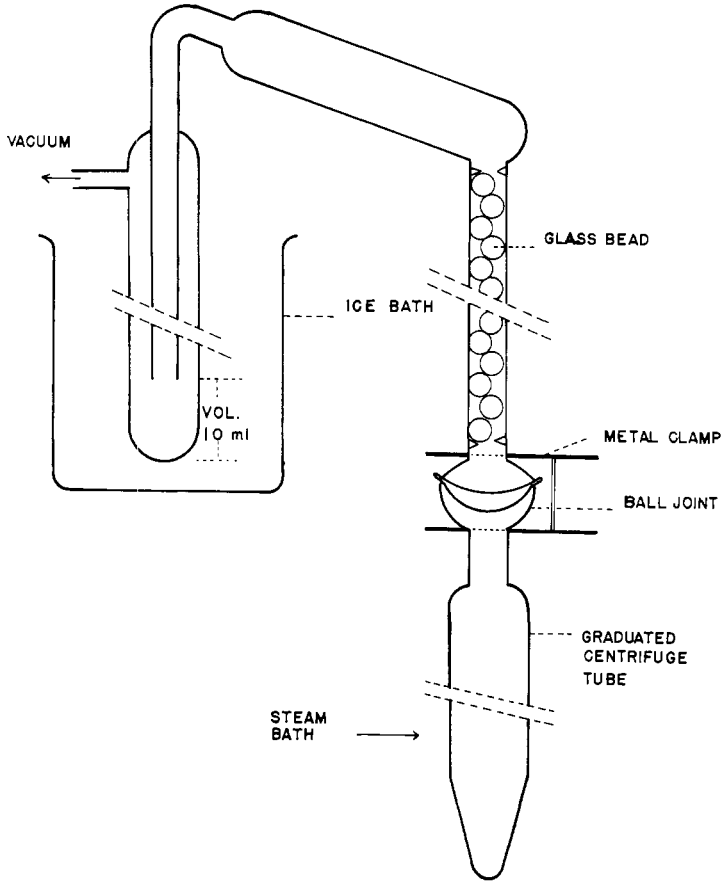

Figure 2. Microfractional concentrator

enlargement at the end of the stripping tube becomes filled with nonvolatile residue, thus requiring insertion of a clean stripping tube. Presence of visible distillate color in the centrifuge tube is an indication that the stripper is filled with residue and that the last sample must be repeated after insertion and thermal equilibration of a clean tube.

The desired small volume of condensate required for the gas chromatographic analysis was obtained by vacuum evaporation of the hexane at ambient temperature.

Standard recoveries were calculated from the peak area of a known quantity of pesticide in xylene added to the appropriate citrus oil which had been precleaned by the procedure described here, prior to the addition of pesticide. The recovery data for cleaned samples obtained by comparison of the peak areas of the standard and cleaned samples, and the final results, are expressed in terms of per cent recoveries of the pesticide.

Results and Discussion

In early work with a device similar to that of Gunther (*7*) using nitrogen gas as carrier, the authors were unable to obtain acceptable or consistent recovery of several pesticides.

On the theory that incomplete nucleation allowed traces of pesticide to escape collection along with the carrier gas in the form of fog or vapor, the nitrogen gas was replaced by totally condensable hexane vapor. Recovery was sharply increased and the results were much more consistent, as shown in Table I. The average recovery of aldrin from nitrogen gas and hexane vapor was found to be 43.0 and 90.3%, respectively. The much higher recovery in hexane vapor would appear, at least in part, to be related to the condensability of hexane.

During the experimental testing of this apparatus, small but significantly consistent losses of pesticides were noted. It was reasoned that a substantial proportion of the unrecovered pesticide might have been deposited on the inside surface of the cool end of the stripping tube.

If this were the case, an injection of a high boiling solvent at the end of each run should wash out any material so deposited by condensation in the outlet end of the stripping tube. Oil-soluble pesticides, in general, are more readily soluble in aromatic than in aliphatic solvents (*6*), and since only a small quantity of solvent could be used, a high boiling aromatic seemed indicated. A highly aromatic petroleum derivative, Socal solvent No. 2 with a boiling range between 140° and 170° C., was selected. Actually, xylene, boiling at 144° C., might have been equally satisfactory. This injection of a high boiling solvent after passage of the hexane vapor increased the average recovery of aldrin from 90.3% to 95.0 and 99.7% when the load in the vaporizer tube was only 2 and 3 μg., respectively. This indicates the necessity for elution of pesticide condensate from that part of the stripping tube exposed to cooling where the temperature is high enough to maintain hexane in vapor form but low enough to allow deposition of some of the pesticide residue.

To observe the reproducibility of results with the condensing system, samples containing 2 and 3 μg. of aldrin in xylene were successively processed as previously described. Table II presents data on reproducibility of aldrin determinations after cleanup. Consistent recoveries of aldrin averaging 99.7% were obtained at the 3-μg. level, and slightly irregular recoveries (average of 95.0%) at the 2-μg. level. Of course, recoveries will be increasingly irregular as the quantity of material to be detected approaches the limit of detection.

Application of Method

To Citrus Oil. An experimental recovery for a mixture of four pesticides in lemon oil sample was conducted (Table III).

The average recoveries of γ-BHC, kelthane, ovex, and Chlorobenzilate were 96.4, 100.0, 101.4, and 101.4%, respectively. Although the recoveries of ovex and Chlorobenzilate are slightly high, the over-all recoveries are very satisfactory, and a high degree of reproducibility is demonstrated.

To Citrus Peel Extractive. Recovery experiments of eight additional pesticides—2,4,5-T, heptachlor, kelthane, dieldrin, Thiodan, DDT, methoxychlor, and Tedion—in orange peel extractive were conducted. The pesticides were processed and determined in the presence of one or more additional

Table I. Comparison of Aldrin Recovery from Cleanup Using Nitrogen Gas and Hexane Vapor as Carriers

(3 μg. aldrin)

Nitrogen Gas		n-Hexane Vapor	
Found, μg.	Recovery, %	Found, μg.	Recovery, %
1.62	54.0	2.97	99.0
1.24	41.3	2.40	80.0
1.24	41.3	2.26	95.3
1.06	35.5	2.60	86.7
Av. 1.29	43.0	2.56	90.3

Table II. Recovery of Aldrin from Cleanup Using Hexane Vapor Plus Socal No. 2 Solvent Injection

Taken, μg.	Found, μg.	Recovery, %
2.0	1.92	96.0
	1.70	84.9
	2.12	106.1
	1.81	90.9
	1.70	84.9
	1.86	92.9
	1.92	96.0
	1.96	98.0
	1.98	99.0
	1.92	96.0
	2.00	100.0
Av. 2.0	1.90	95.0
3.0	2.99	99.7
	2.99	99.7
	2.99	99.7
	2.99	99.7
	2.99	99.7
	2.99	99.7
Av. 3.0	2.99	99.7

compounds, except methoxychlor which was processed alone.

The recovery results and the per cent mean values with standard deviation are presented in Table IV. Over-all average recoveries are quantitative and the per cent recoveries of pesticides range from a minimum of 92.2 % to a maximum of 107.2%. In most cases, standard deviation of individual pesticide recoveries is well below ±5%, a value that is commonly found in a similar experiment (7) and considered acceptable.

The efficiency of the cleanup method was approximated by determining the evaporation residues of known amounts of the lemon and orange oils in the stripping tube after cleanup. Table V indicates that an average of 1.50% of the weight of the lemon oil injected remained in the stripping tube; in the case of the orange oil, 2.78%.

Although the separated pesticide eluate contains traces of the non- or less-volatile substances that were fed into the column during gas chromatographic determination, the column efficiency is well maintained with no adverse effect.

The major portion of non- or less-volatile substances, such as stearoptenes, sterols, paraffin hydrocarbons, and high molecular weight waxy esters, remain in the stripping tube of the cleanup apparatus, and the more highly volatile substances are eluted with the pesticides.

The high recoveries of pesticides shown are mainly due to the total condensability of the hexane vapor, but the favorable effect of continuous development of new surfaces of the extractives during the stripping process undoubtedly is also a factor.

If a liquid is being stripped by a stream of gas, the interface will be poorer in strippable materials than the interior of the liquid phase. Means to ensure continuous presentation of a new surface for stripping will increase the efficiency of the operation but, of course, complete stripping can be approached though never actually achieved.

Though a counterflow method would theoretically be better, it is practically very difficult to achieve for batch operation with very small volumes of liquid phase. Parallel flow actually gives very good efficiency if it can be continued long enough without adversely affecting recovery of the pesticides from the dilute stripping gas.

The authors feel that, without the employment of continuous new surface development, it would be difficult to obtain near-complete recovery of finite amounts of pesticides of less-volatile nature in the presence of large amounts of plant waxes and oils from citrus peel, avocado, etc.

Absorption (the mechanics of which is the exact opposite of stripping) must become progressively less efficient as the stripping gas becomes leaner as a result of depletion of the source material. Complete absorption is as unobtainable as complete stripping. Since the system being considered involves only trace amounts of pesticides and these have very low vapor pressures, the stripping gas must perforce carry extremely minute proportions of these substances and efficient absorption will consequently be very difficult to achieve.

In the present work, losses during concentration of this condensate are very low because of the great difference in vapor pressures of the low boiling liquid and the pesticide-containing residue.

The vapor phase method described was developed for a general cleanup of pesticide residue in citrus extractives and has been applied in this laboratory for several years to the routine cleanup of samples with multiple pesticide contamination.

When the vapor phase cleanup apparatus is employed as described, the procedure is relatively simple and less time-consuming than most column chroma-

Table III. Recoveries of Mixtures of Pesticides from Lemon Oil after Cleanup

Sample	Pesticide	Added, μg.	Found, μg.	Recovery, %
1	γ-BHC	0.60	0.55	91.7
	Kelthane	1.80	1.80	100.0
	Ovex	3.00	3.00	100.0
	Chlorobenzilate	3.00	3.00	100.0
2	γ-BHC	0.80	0.78	97.5
	Kelthane	2.40	2.49	103.8
	Ovex	4.00	4.05	101.3
	Chlorobenzilate	4.00	4.17	104.3
3	γ-BHC	0.80	0.80	100.0
	Kelthane	2.40	2.31	96.3
	Ovex	4.00	4.11	102.8
	Chlorobenzilate	4.00	4.00	100.0

Table IV. Recoveries of Mixtures of Pesticides from Hexane Extract of Orange Peel after Cleanup

Sample	Pesticide	No. of Samples	Added, P.P.M.	Found (Av.), P.P.M.[a]	Recovery, % ±[b]
1	Isopropyl ester	4	2.23	2.23	100.0 ± 2.4
	of 2,4,5-T	4	2.94	2.94	100.0 ± 3.5
	Dieldrin	4	3.38	3.38	100.0 ± 2.0
		4	4.01	3.70	92.3 ± 1.3
	Tedion	4	1.52	1.44	94.7 ± 2.5
		4	1.31	1.38	105.3 ± 0.0
2	Heptachlor	6	2.44	2.42	99.2 ± 4.4
	Thiodan	6	2.40	2.44	101.7 ± 2.4
3	Kelthane	4	1.81	1.70	93.9 ± 3.4
	DDT	4	7.40	7.20	97.3 ± 3.0
4	Methoxychlor	4	5.03	4.88	97.0 ± 3.3

[a] Based on fresh peel weight.

[b] ± indicates standard deviation $\sigma = \sqrt{\frac{\Sigma(X - \bar{X})^2}{n}}$.

Table V. Per Cent Weight of Non- or Less-Volatile Matter Removed from Orange and Lemon Oil Samples

Oil Sample	Taken, Mg.	Non- or Less-Volatile Residual Matter, Mg.	Non- or Less-Volatile Residual Matter, %
Orange	2725	72.7	2.67
	2725	68.8	2.53
	1821	57.4	3.15
	1821	51.4	2.82
Av.	...	...	2.78
Lemon	2731	38.8	1.42
	1835	29.5	1.61
	9105	140.3	1.51
	9105	131.0	1.44
Av.	...	...	1.50

tographic cleanup methods. The authors' years of experience with the apparatus (*8*) indicate that up to six to eight samples of 0.5 ml. each of concentrated citrus extractives can be cleaned up before the stripping tube is overloaded. From the injection of the sample to the final recovery of the solvent-free concentrated extractive, which is directly usable for gas chromatographic analysis, not more than 30 minutes of elapsed time are required. Since it is hoped that this procedure will also be found advantageous for the cleanup of volatile pesticide-containing extracts prior to paper or thin-layer and gas chromatographic analysis, construction details of the apparatus are given.

Acknowledgment

The authors gratefully acknowledge the opportunity and encouragement given in this experimental study by W. E. Baier, Director of Research and Development Division, Sunkist Growers, Inc.

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Received for review April 25, 1966. Accepted August 28, 1966.

RESIDUE DETERMINATION

Determination of Fenthion Residues in Plant and Animal Tissues by Electron-Capture Gas Chromatography

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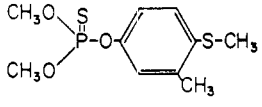
A specific residue method is described for the determination of fenthion and its five metabolites in plant and animal tissues. The method is based on oxidation of the various compounds to the oxygen analog sulfone which in turn is hydrolyzed to the corresponding phenol. The phenol is brominated and acetylated prior to detection measurement by electron-capture gas chromatography. The method is sensitive to 0.1 p.p.m. of the compound.

Fenthion—(*O,O*-dimethyl *O*-[4-(methylthio)-*m*-tolyl] phosphorothioate—is the common name for the organic phosphorus insecticide and parasiticide sold under the registered trademarks Baytex, Entex, and Tiguvon. The structural formula is as follows:



The metabolism of fenthion in plants has been studied by Niessen, Tietz, and Frehse (*5*) who found that the compound was converted to its sulfoxide and sulfone as well as the oxygen analog sulfoxide and sulfone. The oxygen analog was not observed. However, Rawson and Arthur (*6*) reported detection of small quantities of the oxygen analog in cotton plants.

Brady and Arthur (*2*), in their study of the metabolism of fenthion in rats, found that the compound was rapidly oxidized to the sulfoxide and sulfone, and to the oxygen analog and its sulfoxide and sulfone.

Knowles and Arthur (*4*) found that dermal application of P^{32}-labeled fenthion to dairy cows resulted in small amounts of radioactivity in the milk. Fractionation of the acetonitrile-soluble portion of this activity on Celite partition columns gave several distinct peaks, one of which corresponded to fenthion, another to the sulfoxide and oxygen analog, and a third to the oxygen analog sulfoxide and oxygen analog sulfone. Acetonitrile-soluble activity in tissues was negligible. Intramuscular injection resulted in the same number of acetonitrile-soluble activity peaks as obtained for the dermal application. The method developed, therefore, was designed to measure not only fenthion but also its five metabolites.

The primary concern in the development of the method was adequate sensitivity and specificity for all of the compounds. Preliminary experiments involved the use of a Dohrmann microcoulometric gas chromatograph equipped with a sulfur-sensitive titration cell. Although acceptable results were obtained for fenthion, attempts to chromatograph some of the metabolites were unsuccessful.

A suitable gas chromatographic procedure based on the phenol of the oxygen analog sulfone was finally developed. Acetylation of the phenol gave a moderately sensitive response in a gas chromatograph equipped with an electron-capture detector. As expected, sensitivity was increased further by brominating the phenol prior to acetylation. This acetylated phenol sulfone as well as the halogenated derivative undoubtedly could have been determined using a Dohrmann chromatograph, but this was not attempted because electron capture offered greater sensitivity. Using electron capture, the desired sensitivity of 0.1 p.p.m. and specificity were easily achieved.

Analytical Method

Apparatus and Reagents. An F & M Model 700 gas chromatograph equipped with a pulsed-type electron capture detector set at a pulse interval of 15 μseconds was used. The electrometer range and attenuation set-