Table III. Recovery of Karathane from 50 Grams of Fruit

	Cleanup Procedure			
	Florisil Column Chromatography		Sulfuric Acid Treatment	
Karathane Added, μg.	Karathane found, μg.	Recovery, %	Karathane found, μg.	Recovery, %
		APPLE		
50	41.8	83.6	44.8	89.6
30	26.8	89.3	28.5	95.0
10	9.1	91.0	8.6	86.0
		GRAPE		
50	42.8	85.6	44.6	89.2
30	27.2	90.7	26.3	87.7
10	9.1	91.0	9.6	96.0
		STRAWBERRY		
50	41.4	82.8	44.7	89.4
30	27.1	90.3	26.9	89.7
10	9.3	93.0	8.8	88.0
		Av. 88.6		90.0

were obtained when the solvent was removed under reduced pressure at 50° to 60° C. with the flash evaporator. The flasks can remain on the evaporator for at least 10 minutes after the last visible traces of solvent have been removed with no significant loss of the residue (Table I). Increased water bath temperatures have little effect on recoveries (Table II).

Color Development and Interferences. Following the addition of *N,N*-dimethylformamide to Karathane residues, a yellow color develops which has a peak absorption at 444 mμ. Maximum color development is obtained in 20 minutes and the color which is formed remains stable for at least 1 hour (Figure 1). Standard curves prepared by the procedure obey Beer's law up to at least 50 μg. of Karathane in 4 ml. of *N,N*-dimethylformamide. Under these conditions, standard solutions containing 5, 10, 20, and 50 μg. of Karathane have absorbances of 0.045, 0.090, 0.180, and 0.440, respectively, when measured in cuvettes (1-cm. light path) with a Beckman DU spectrophotometer.

Compounds such as parathion, 2,6-dichloro-4-nitroaniline, 1,3,5-trichloro-2,4-dinitrobenzene, and 1,3-difluoro-4,6-dinitrobenzene do not form measurable colors under conditions of maximum color development for Karathane. In addition, very little interference is obtained from unfortified check samples. Blank samples prepared from 100 grams of unfortified fruit have very low absorbances, ranging from 0.035 to 0.050.

Recovery and Sensitivity. With the florisil column, the average recovery of Karathane from treated fruits is 88.6%, and the recovery is 90% when the sulfuric acid procedure is used (Table III). Moreover, as low as 0.05 p.p.m. of Karathane on strawberries can be detected with an average recovery of 86% (std. dev. ±5.4). Thus, it is apparent that either cleanup procedure can be used with very satisfactory results.

Field-Treated Samples. In order to test the reliability of the procedure, field experiments were conducted. A test plot of strawberries was sprayed 21 days before harvest (the recommended time of application) with a commercial preparation of Karathane (6 ounces of Karathane per acre), and representative samples of the treated fruit were analyzed for residues. In Figure 2, the degradation curve shows a logarithmic decline, and only 0.05 p.p.m. of Karathane remains after 16 days following application.

Acknowledgment

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NEMATOCIDE RESIDUES

Determination of 1,2-Dibromo-3-chloropropane and Tetrachlorothiophene in Crops

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ANALYSIS of nematocides is often complicated by their low boiling points or high vapor pressures. Recovery of these compounds from crop material is often accompanied by high losses which occur during concentration of the extracts prior to final analysis. The need for specific analytical methods to detect the presence of traces of the original compound is apparent. Previous attempts to develop such methods have often lacked sensitivity.

Specific residue methods for the determination of 1,2-dibromo-3-chloropropane (various trade names) have not been reported. Analytical data based on total bromine content of agricultural crops have been used for an estimation of residues of this nematocide (*3*). Archer *et al.* (*1*) showed that several nematocides could be readily detected using gas chromatography. They showed that dibromochloropropane could be detected in 6 minutes using an 8-foot, 1/4-inch o.d., 20% silicone grease column at 132° C. with a thermal conductivity detector. The study was a comparison of two types of columns with two types of detectors. A similar technique has been found applicable to

Procedures for the analysis of 1,2-dibromo-3-chloropropane and tetrachlorothiophene in agricultural crops are described. The analysis is made by gas-liquid chromatography, and the nematocide is detected by an electron capture unit. Direct analysis of the crop extract has eliminated the need for any concentration steps and has also allowed a sensitivity of 0.01 p.p.m. to be obtained. The procedures for extraction and cleanup are described. Good recoveries are obtained in the picogram concentration range for both nematocides on all crops tested. Applications of this procedure are suggested for other types of studies.

the analysis of tetrachlorothiophene (*2*).

The availability of a gas chromatographic system with an especially sensitive detector has made possible the analysis of several materials. Reliable data on residue levels may now be obtained at a low sensitivity value.

The need for accurate and sensitive analytical data on the residue content of crops treated with dibromochloropropane or tetrachlorothiophene for nematode control was imperative in order for the material to obtain registration and official recommendation for use in California.

Experimental

Dibromochloropropane. Since no methods were available for the direct detection of the presence of dibromochloropropane in agricultural crops, an investigation to develop a method was initiated. Ordinary methods of sample processing and analysis were not adequate to handle a compound of such high volatility. Although the boiling point is 196° C., the vapor pressure is so high that ordinary concentration of a crop extract caused great losses of the compound.

The recent introduction of the electron capture detector for use with gas chromatography prompted the authors to investigate the potential of this detector for the analysis of dibromochloropropane.

The response of the detector was excellent, and under the conditions of instrument operation, the material chromatographed in 5 minutes at 75° C. This is more than 100° C. less than the boiling point of the compound, which emphasizes its high vapor pressure and volatility. The sensitivity, or minimum detectable quantity, was in the picogram range.

Method development to produce a lower detectable limit of 0.05 to 0.01 p.p.m. was sought. With an adequate cleanup procedure, a sensitivity of 0.01 p.p.m. could be achieved without concentration of the extract.

Two types of samples were studied—a vegetable, Brussels sprouts, and a high oil product, walnut meats. These were chosen to demonstrate the broadness of the applicability of the method and to test the crop difference in residue storage.

Tetrachlorothiophene. The report from the Pennsalt Chemicals Corp. describes an analytical method for tetrachlorothiophene using a gas chromatograph with a thermal conductivity detector. An involved sample preparation procedure is necessary to achieve a sensitivity of 0.1 p.p.m. The method uses a 6-foot, $^1/_4$-inch stainless steel column packed with 30% Dow Corning 550 silicone fluid on C-22 firebrick at 190° C. and 30 pounds of helium pressure. A retention time of approximately 9 minutes was noted.

Work in this laboratory has shown that the optimum conditions for proggrammed temperature gas chromatography analysis show a response at 140° to 145° C. with a 2-foot stainless steel $^1/_4$-inch column packed with 20% SE-30 silicone gum rubber on 40- to 60-mesh Chromosorb P. The program rate was 15° C. per minute with an initial temperature of 75° C.

By using a gas chromatographic system equipped with a microcoulometer detector, as little as 0.1 μg. of tetrachlorothiophene may be detected. This will show a 10% scale response on the detector. The instrument was operated with a 140° C. column, a 200° C. injection block, a carrier gas flow rate of 100 cc. per minute. Response was recorded in 6 minutes.

Greater sensitivity was achieved with a gas chromatograph equipped with an electron capture detector. The instrument was operated at 10 pounds of nitrogen pressure and at 100° C., which gave a flow rate of 35 cc. per minute. A molecular sieve (13X) was included in the gas line. A 10% scale deflection on the recorder was observed with 15 picograms of tetrachlorothiophene (1 picogram is 1×10^{-6} μg.).

Method

Equipment. An Aerograph Hy-Fi with an electron capture detector head was the basic piece of equipment. The instrument was equipped with a 5-foot, $^1/_8$-inch diameter stainless steel column packed with 5% SE-30 silicone gum rubber on 60- to 80-mesh Chromosorb W

These operating conditions gave the greatest detector response consistent with a reasonable retention time. The instrument was operated at its highest sensitivity settings and the detector response was fed into a 1 mv. per second recorder.

Plant Materials. BRUSSELS SPROUTS. Plants were treated with 100 p.p.m. of dibromochloropropane or tetrachlorothiophene in the transplant water at the rate of one pint per plant. Samples were taken for analysis 170 days later together with untreated control samples of the same growth history, and stored in the freezer.

PERSIAN WALNUTS. The orchard soil was treated with a 44.9% concentrate of dibromochloropropane at the rate of 5 gallons per acre. Similar applications had been made annually since 1956. Appropriate control samples were also collected. The nut meats were held for analysis in a freezer.

Extraction. Crop samples of whole frozen Brussels sprouts or frozen nut meats were transferred to a homog-

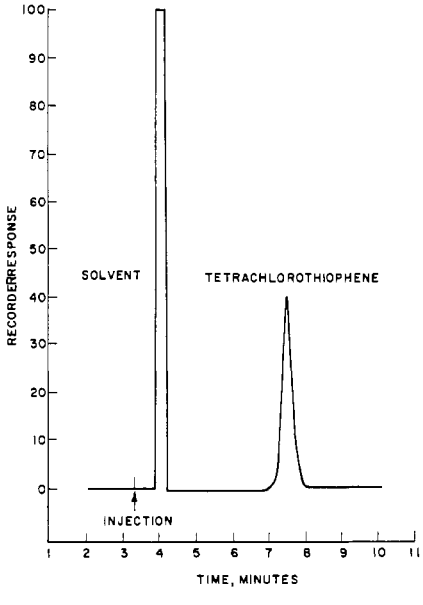

Figure 1. Gas chromatogram of 40 picograms of tetrachlorothiophene showing a 40% scale deflection on a 165-mm. wide chart driven at 1 cm. per minute

Table I. Residue and Recovery Data for Tetrachlorothiophene in Brussels Sprouts

Sample	Tetrachlorothiophene Residue, P.P.M.[a]	Recovery of Fortified Check, % Florisil Cleanup 0.05 P.P.M.	Florisil Cleanup 1.0 P.P.M.	Charcoal cleanup, 1.0 P.P.M.
Check 1	0.0	90	95	89
Treatment 1	<0.01	..	..	..
Check 2	0.0	94	94	93
Treatment 2	<0.01	..	..	..
Check 3	0.0	92	96	91
Treatment 3	<0.01	..	..	..

[a] Sensitivity set at 0.01 p.p.m. of tetrachlorothiophene.

Table II. Residue and Recovery Data for 1,2-Dibromo-3-chloropropane in Brussels Sprouts and Walnut Meats

Sample	Dibromochloropropane Residue, P.P.M.[a] Brussels sprouts	Walnuts	Recovery of Fortified Check, % Brussels Sprouts 0.05 P.P.M.	Brussels Sprouts 1.0 P.P.M.	Walnuts 0.05 P.P.M.	Walnuts 1.0 P.P.M.
Check 1	0.0	0.0	95	94	97	97
Treatment 1	<0.01	<0.01	..	..	..	..
Check 2	0.0	0.0	92	96	97	95
Treatment 2	<0.01	<0.01	..	..	..	..
Check 3	0.0	0.0	94	96	95	100
Treatment 3	<0.01	<0.01	..	..	..	..

[a] Sensitivity set at 0.01 p.p.m. of 1,2-dibromo-3-chloropropane.

enizer, and a volume of redistilled petroleum ether equal to 10 times the amount of sample was added. After blending for 2 minutes at high speed, the mixture was filtered through fluted paper.

Cleanup. DIBROMOCHLOROPROPANE. Walnuts and Brussels Sprouts. The crop extract was cleaned up using activated Florisil (heated 1 hour at 270° C.). A column 6 inches × $^1/_2$ inch with a reservoir top was packed to a depth of $5^1/_2$ inches and wetted with petroleum ether. A 50-ml. aliquot of the extract was passed through the column and discarded. A second 50-ml. aliquot was put onto the column and the eluate saved for analysis.

TETRACHLOROTHIOPHENE. Fifty milliliters of the crop extract were passed through a column of activated Florisil previously wetted with petroleum ether. The first 25 ml. of extract that passed through the column was discarded and the balance collected for analysis.

A second effective cleanup procedure was the use of charcoal. Nuchar C-190N removed all the pigment and most of the background recorder response due to the crop extract. The tetrachlorothiophene was not removed from the solution. Two grams of charcoal were mixed with 100 ml. of extract and shaken for 30 minutes before filtration.

The Florisil cleanup was considered superior on the basis of removal of material that contributed to background response of the detector.

Analysis. Extracts of treated samples, check samples, and check samples fortified at levels of 1.0 and 0.05 p.p.m. were prepared and cleaned up for analysis. The unconcentrated extracts were used for analysis. The fortified check samples contained a concentration equivalent to 100 picograms or 5 picograms in each microliter. The lower limit of detectability of 1,2-bromo-3-chloropropane or tetrachlorothiophene (Figures 2 and 3) is about 10 picograms. On this basis, an aliquot of extract equivalent to a milligram of sample that contained 10 picograms of residue would give a sensitivity of 0.01 p.p.m. The 10 μl. required to represent 1 mg. of sample gave no background response, indicating that this amount could easily serve as the sample size and the basis for calculations.

Results and Discussion

Since only selected compounds will be detected by the electron capture detector, the specificity of a method may be improved dramatically by the proper cleanup and sample handling. Proper sample handling extends to field operations prior to laboratory processing. In the case of tetrachlorothiophene, a lower limit of detection is about 5 picograms (5×10^{-6} μg. is obtainable). A more practical limit in the presence of crop extracts is twice this amount or 1×10^{-5} μg. This, as well as a representation of the linearity of the instrument at its highest sensitivity settings, is shown in Figure 2.

The recovery of tetrachlorothiophene from crop material taken through the

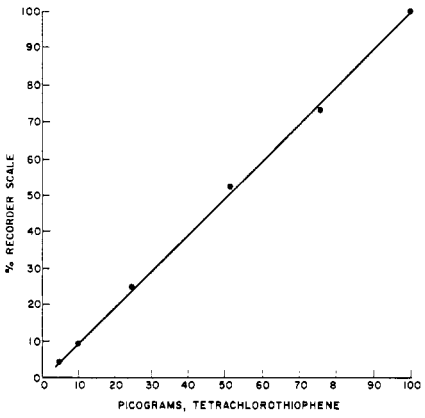

Figure 2. Linearity of detector response to picogram amounts of tetrachlorothiophene

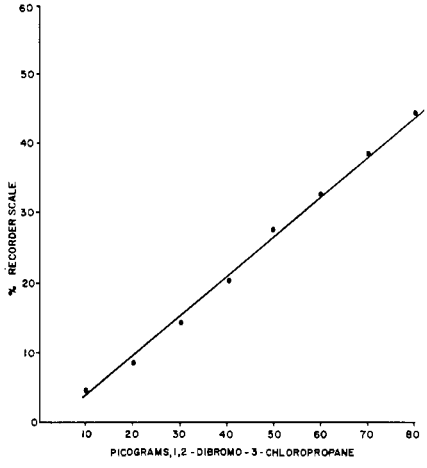

Figure 3. Detector response and linearity for picogram amounts of 1,2-dibromo-3-chloropropane

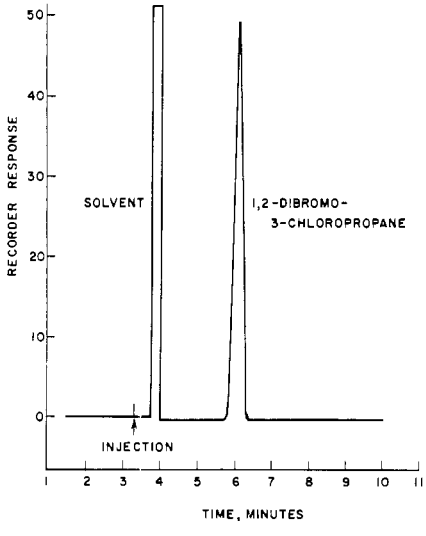

Figure 4. Gas chromatogram of 1,2-dibromo-3-chloropropane, 100 picograms showing approximately 50% scale deflection on a 165-mm. wide chart driven at 1 cm. per minute

two cleanup procedures is compared in Table I along with the residue data for the Brussels sprouts.

The ability to detect the presence of such extremely small amounts of material provides the advantage of reducing the sample size while still allowing a sensitivity of 0.01 p.p.m. Lower sensitivity could be obtained by using larger samples, but at the expense of the life of the column and detector.

Representative gas chromatograms for picogram amounts of both nematocides are shown in Figures 1 and 4.

The cleanup and analysis procedures are adaptable to other studies involving these nematocides. Studies such as soil penetration, movement in irrigation water, effective dosages, and plant uptake should be possible.

The method sensitivity referred to under sample analysis is depicted for dibromochloropropane in Figure 3. The illustration shows that a linear relationship between detector response and sample size exists at least over the range shown.

Recovery and residue analysis for dibromochloropropane data are shown in Table II for both Brussels sprouts and walnut meats. In no case was a residue above the sensitivity of the method detected. Recovery data were considered to be excellent for the walnuts and Brussels sprouts. The background recorder response for check or treated samples was such that as little as 10 picograms of dibromochloropropane could have been detected.

The extraction, cleanup, and analysis procedures of these diverse crops gave indications of its broader applicability. The speed of analyses would make the analysis of these nematocides in soil, irrigation water, and uptake by plants possible areas for study.

Acknowledgment

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INSECTICIDE RESIDUES

Insecticide Residues in Peppermint and Their Distillation with Peppermint Oil

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Analyses of peppermint hay and peppermint oil after treatment of the crop with DDT, aldrin, dieldrin, or Dibrom indicate that each of these pesticides will persist through the processing of the hay. Both field and laboratory distillation experience indicate that the amount of residue found in peppermint oil depends in part on the severity of the distillation,—i.e., the amount of steam used. Up to 60 p.p.m. of dieldrin was found in peppermint oil recovered in a small still when excessive amounts of steam were used. Oil recovered with conventional distillation procedures contained less than 1 p.p.m. dieldrin. Peppermint grown in aldrin-treated soil contains more dieldrin than aldrin. Maximum oil residues using commercial stills were: DDT, 10.6 p.p.m.; dieldrin, 1.9 p.p.m.; and Dibrom, 36.4 p.p.m. Microcoulometric gas chromatography has been successfully applied to all samples with sensitivities as low as 0.01 p.p.m. attainable with fresh and spent hay. Special sample preparation methods are described.

During the period of active growth, oil accumulates in the peppermint plant, until at harvest time it constitutes about 0.5% of the fresh weight. At harvest the crop is cut, field cured for 2 to 4 days, chopped, and subjected to steam distillation to remove the oil. The hay residue remaining after this distillation process may be returned to the field and used as green manure or it may be fed to livestock.

The oily nature of this crop and the method of recovering the oil provide special circumstances under which the behavior of pesticide residues may differ from that found in other crops. It might be expected, for example, that residues of oil-soluble pesticides applied to this crop would persist and that the concentrating effect of steam distillation would lead to excessive residues in the final product. The purpose of the study reported here was to investigate this and other questions in the case of aldrin applied to the soil before the peppermint growth begins, and dieldrin, DDT, and *O,O*-dimethyl 1,2-dibromo-2,2 dichloroethyl phosphate (Dibrom, registered trademark of the California Chemical Corp.) applied to peppermint foliage during the growing season.

Residue investigations on peppermint oil have been conducted by Gould (*3*), who reported dieldrin residues up to 4.3 p.p.m., DDT to 9.8 p.p.m., heptachlor to 3 p.p.m., and aldrin to 2.4 p.p.m. No foliage or spent hay analyses were included.

Experimental

Aldrin Soil Treatment. Single 5-acre plots, located in western Oregon, were treated with aldrin emulsible at 2.5, 5, and 10 pounds active per acre in April 1960. At this time, peppermint is in a near dormant stage with growth of not more than 1 or 2 inches. The aldrin was applied with a boom-type weed sprayer and the ground disked in two directions immediately after the application. The plots were harvested 142 days later at which time fresh hay samples were collected for aldrin and