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ASSAY OF METHYL PARATHION AND FENITROTHION IN CROP EXTRACTS USING VAPOUR-PHASE SEPARATION IN CONJUNCTION WITH GAS CHROMATOGRAPHY

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Methyl parathion and Fenitrothion were separated and assayed by electron capture gas chromatography using stationary phase of 5% DC–200 on Celite at 180°C. Crop extracts contained substances which interfered with the gas chromatographic assay of these insecticides. Vapour-phase separation removed these interfering materials so that 0.1 p.p.m. of Methyl Parathion and Fenitrothion could be assayed in spinach, cabbage, apple, and potato and 1.0 p.p.m. in sugar-cane and cotton.

The organophosphorus compounds Fenitrothion [*O*, *O*-dimethyl-*O*-(3-methyl-4-nitrophenyl phosphorothioate)] and methyl parathion [*O*,*O*-dimethyl-*O*-(4-nitrophenyl phosphorothioate)] are widely used insecticides.

Although closely related, they differ much in their toxicity, Methyl Parathion being more toxic than Fenitrothion. It is, therefore, important to be able to distinguish between the two substances and to have a reliable and sensitive method for analysis of their mixtures.

The methods described todate do not distinguish between the two compounds. The colorimetric method of Averell and Norris and its subsequent modifications[1–6] is based on the reduction of the nitro group to amino group by zinc powder with the formation of the diazo derivatives and coupling with *N*-(1-naphthyl)ethylenediamine measuring the colour formed by absorbance at 555 mμ. The method can be used for either of these insecticides separately but because both of them give a similar colour the method cannot be used for mixture of the two. The alternative polarographic method[7,10–13] based on the reduction of nitro-group to amino-group at the cathode is also unsuitable for the analysis of the mixture because the half-wave potential of Fenitrothion is $E_{\frac{1}{2}}$=0.58V, almost the same as the half-wave potential of Methyl Parathion ($E_{\frac{1}{2}}$=—0.55V) when a Kolthoff buffer (pH=5.0) is used.[18]

The two compounds may be distinguished by IR spectrophotometry but the method lacks sufficient sensitivity to measure residual quantities.

Gas–liquid chromatography is powerful method for separating substances[14–17] but no suitable system has been described which may be used for distinguishing and determining Fenitrothion and Methyl Parathion together. But extracts of various plants often contain materials which interfere with gas–liquid chromatographic assay. Thus, an effective method, for separating the compounds from coextracts, is also needed for general use. Many methods have been described for clean-up based on selective adsorption, solvent partitioning and other physical and chemical methods.[19–21] Each is efficient and useful for a particular problem but they lack general application. Of all the methods adsorption column chromatography is probably the most widely used but the disadvantage of this technique is that it provides variable recoveries. Farrow[22] reported a vacuum-sublimation clean-up method but the method also gives variable recoveries. Gunther *et al.*[23] described a vapour-phase clean-up adopted and modified in turn by Storherr and Watts and also Kim *et al.*[9] to obtain improved recovery and wider application.

This paper describes a gas chromatographic method for separating and assaying Methyl Parathion and Fenitrothion, and its use in conjunction with a vapour-phase clean-up apparatus (Fig. 1) built in this laboratory to separate Methyl Parathion and Fenitrothion from substances extracted from plants which interfere with the gas chromatography of the two insecticides.

Experimental

Material and Methods

Separating funnel 250 ml capacity; rotatory film evaporator; nitrogen high purity, oxygen free; sodium sulphate anhydrous, Analar grade; acetone redistilled fractionally; n-hexane redistilled fractionally; chloroform Analar grade.

All the solvents were fractionally redistilled in all-glass apparatus at least once to remove traces of impurities which effect electron capture detectors and interfere with the assay of pesticides. Each redistilled solvent was checked for impurities by gas–liquid chromatography of a 5 μl sample from 30–50 ml of the solvent concentrated to 0.1–0.5 ml.

Standard stock solutions of 1 mg/ml of both Methyl Parathion and Fenitrothion in n-hexane

*From Rothamstead Experimental Station, Harpenden, U.K. under U.K. Colombo Plan.

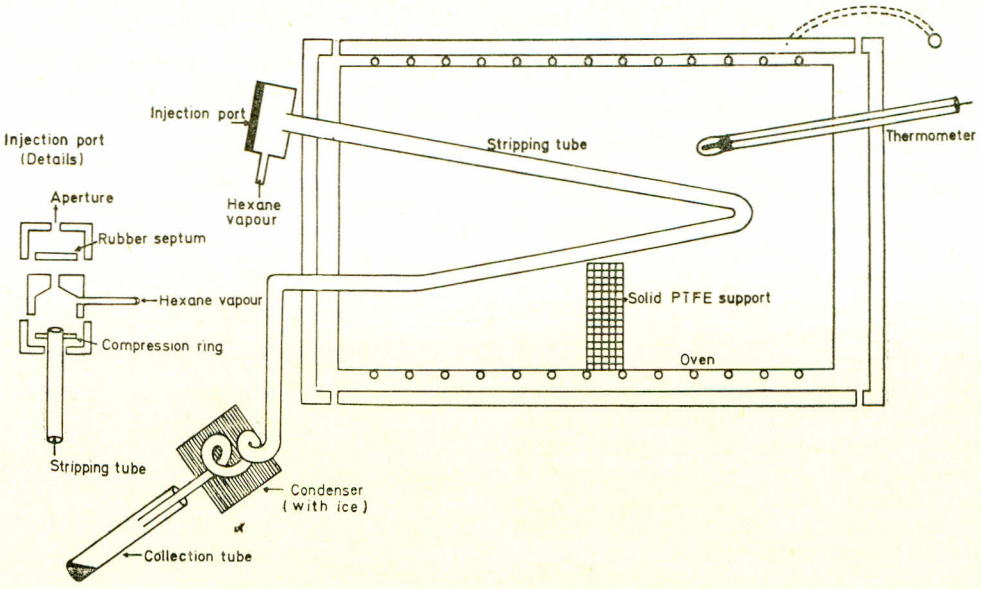

Fig. 1.—Vapour-phase separation apparatus.

were prepared from analytical grade samples supplied by Sumitomo Chemical Co. Ltd., Japan.

The gas chromatograph used was a Philips Model PV 4000 Series fitted with a coiled stainless steel preconditioned column, 2 mm internal dia 1.2 m long packed with 5% DC-200 on Celite and an electron capture detector operating at 180°C. Pure nitrogen was used as carrier gas flowing at the rate of 40 ml/min, using a pressure of 25 lb/in^2.

Extracts were prepared by cutting 50 g of the crops into small slices and macerating with 100 ml of acetone in a Waring Blender for about 2 min. The extract was filtered through a Buchner funnel using a 541 Whatman filter paper and the acetone evaporated under reduced pressure using a rotary film evaporator at 60°C. The residual aqueous extract was filtered through a Buchner funnel and transferred to a separating funnel. 50 ml of chloroform was added and the liquids were swirled for 2 min and the chloroform layer separated. The aqueous layer was then extracted with a further quantity of 50 ml chloroform. Each chloroform layer was washed in turn with about 10 ml of distilled water in a second separating funnel. The chloroform extracts were combined and dried by filtering through anhydrous Na_2SO_4 into a round-bottomed flask and concentrated to 10 ml.

Addition of Insecticides to Extracts.—To check the distillation in the apparatus, known amounts, 0.5, 5.0 and 10 μg, of each insecticide were added to 1 ml extract to give concentrations equivalent to 0.1, 1.0 and 2.0 p.p.m. respectively in the starting material. The insecticide was added at this stage to avoid losses in extraction and processes other than the distillation procedures.

Vapour-phase Separation.—The apparatus is essentially a heated glass tube (Fig. 1) through which a stream of hexane vapour is passed and into which the sample is injected, through a metal injection port fixed to the tube with a silicone-rubber compression seal. Volatile materials in the extracts condense with the solvent vapour and in this way insecticides are separated from the less volatile materials in the extracts. The insecticides distilled over were assayed by gas chromatography.

The heated distillation or stripping tube is housed and supported in a flat metal box which slides in a slightly larger box or oven (28 × 16 × 5.5 cm) wrapped in heating tapes (Fig. 1). The oven is held centrally in a larger aluminium box (35 × 18 × 10 cm) by aluminium spacers in order to provide insulation and to diminish heat losses. The temperature of the oven is regulated by a rheostat. The heated part of the stripping tube is about 60 cm long, bent in a V-shape with an angle of 20–25°C between the two approximately equal arms. The tube is supported in a vertical plane with each arm approximately equally inclined to the horizontal. The lower arm is bent so that approximately the last 7 cm in the oven is horizontal. At right angle to this a further 15 cm of tube projects down from the oven and connected to a 15-cm coil of PTFE tubing immersed into ice to condense vapour. Condensate in this tubing is led into a receiver.

Operations.—Recovery of insecticides under different conditions of operations were determined experimentally and eventually standardized with an oven temperature of 180°C and a flow of hexane vapour of approximately 2 ml liquid/min (Table 1). The vaporization of hexane was controlled by regulating the heat supplied to the vapour generator.

The effect of different hexane flow rates on recovery were examined using replicate injections of a standard amount of Fenitrothion and Methyl

Parathion. With increasing rates of flow the time to recover the insecticide completely from the stripping tube decreased, but it was difficult to condense the vapours when the rate of flow was above 2 ml liquid hexane/min. Hence, 2 ml liquid/min was selected as a convenient and satisfactory rate for the experimental conditions used, because it was convenient to distill the insecticide in the shortest possible time and because prolonged exposure to high temperatures is liable to cause decomposition and consequent low recovery. Before use, the apparatus was cleaned by passing solvent vapour through the tube. The condensed liquid was tested by gas chromatography to check that materials which interfered with residue analysis were not being washed from the stripping tube. To purify, an extract up to 250 µl of a concentrated extract was injected with a Hamilton microsyringe into the stripping tube at a point beyond the entrance of the hexane vapour.

As the injected material flowed down the slope of the tube, new surface was continuously developed from which the pesticide was vaporised and swept into the condenser by the hexane vapour used as carrier. The nonvolatile substances remained behind.

The volatile materials, including the pesticide residues and the hexane vapour were condensed and collected in a graduated test tube. With a flow of hexane of 2 ml liquid/min at an oven temperature of 180°C it took about 20 ml of hexane to wash out all insecticides.

Several replicates of samples could be distilled through the apparatus before the stripping tube filled with nonvolatile residues, and needed to be cleaned. A visible colour in the distillate collected in the test tube indicated that the stripping tube was becoming too contaminated to adequately separate insecticides from interfering materials.

The condensate was concentrated by evaporation of the hexane with gentle heating and the pesticide assayed by gas chromatography. To check the recovery of insecticide from the distillation apparatus, known amounts of insecticides were added to extracts of untreated crops before distillation (Table 2). Insecticides in experimental solutions were assayed by comparison of peak heights with those from standard solutions containing known amounts of insecticides. Insecticides were identified by their retention times.

Results and Discussion

The operating conditions of the gas chromatograph were varied until a satisfactory separation of Methyl Parathion and Fenitrothion was obtained. Using a column packed with 5% DC-200 on Celite at 180°C with a nitrogen flow of 40 ml/min the retention time of Methyl Parathion was 5.5 and of Fenitrothion it was 6.6 min which were sufficiently different to clearly distinguish these compounds. Subnanogram quantities of both insecticides could be detected using an electron capture detector.

Table 1.—Assay of Residues of Methyl Parathion and Fenitrothion in Crop Extracts (Residues equivalent p.p.m.).

Crop	Methyl Parathion		Fenitrothion	
	Added	Found	Added	Found
Cotton	0.1	—	0.1	—
	1.0	0.99	1.0	0.99
	2.0	1.98	2.0	1.99
Potato	0.1	0.099	0.1	0.102
	1.0	0.99	1.0	1.02
	2.0	2.00	2.0	2.03
Cabbage	0.1	0.099	0.1	0.1
	1.0	1.01	1.0	1.00
	2.0	2.01	2.0	2.06
Apple	0.1	0.099	0.1	0.099
	1.0	1.00	1.0	0.99
	2.0	1.96	2.0	2.00
Sugar-cane	0.1	—	0.1	—
	1.0	0.99	1.0	1.00
	2.0	1.98	2.0	2.01
Spinach	0.1	0.98	0.1	0.10
	1.0	1.00	1.0	0.99
	2.0	2.00	2.0	2.00

Table 2.—Effcet of Temperature of the Stripping Tube on Percentage Recovery of Insecticides in 20 ml of Distillate.

Column temp.	160°	170°	180°	190°C
Methyl Parathion	57–60	80–83	98.1–101	*Decomposition
Fenitrothion	50–60	75–80	99.5–102	Decomposition

* Low recovery and multiple peaks.

Crude plant extracts containing materials which interfere with the chromatograms by emerging as long retained broad peaks. These substances also contaminate the column and change its properties. In addition they are liable to contaminate electron capture detector and diminish sensitivity. Vapour-phase separation removed these substances from the 6 kinds of crop extracts (Table 1 and 2) so that they did not adversely effect performances of the gas chromatograph even after repeated injections. At the same time, Methyl Parathion and Fenitrothion added to the extracts were recovered almost quantitatively by the distillation (Table 1 and 2). The separation of insecticides from interfering substances extracted from all 6 crops was sufficiently good to permit the assay of 1 p.p.m. insecticide in the crops and 0.1 p.p.m in four crops. However, some interfering substances distilled from the two waxy crops—sugar-cane and cotton—which prevented assay of these smaller amounts of insecticides.

Conclusions

Gas chromatography may be used to separate, identify and assay Methyl Parathion and Fenitrothion. However, some materials are extracted from crops which interfere with gas chromatographic assay but they were removed by vapour-phase separation.

Vapour-phase separation may be used routinely because it requires only small quantities of readily available solvents in addition to laboratory-made apparatus.

A combination of vapour-phase separation and gas chromatography was successfully used for the assay of residual quantities of Methyl Parathion and Fenitrothion in extracts of six crops.

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