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RAPID PROCEDURES FOR THE ROUTINE DETERMINATION OF ORGANOPHOSPHORUS INSECTICIDE RESIDUES IN VEGETABLES

I. DETERMINATION OF HEXANE-SOLUBLE INSECTICIDES BY GAS–LIQUID CHROMATOGRAPHY AND TOTAL-PHOSPHORUS PROCEDURES

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SUMMARY

Two procedures are described for the determination of the less polar organophosphorus insecticides in vegetables, based on acetone–hexane extraction and alumina column clean-up techniques previously reported. Details are given of a screening method based on the colorimetric determination of pesticidal phosphorus in cleaned-up extracts and capable of screening for eleven insecticides at the 0.06–0.1 p.p.m. level, depending on the insecticide. A second procedure using the phosphorus-specific thermionic detector to identify and quantitatively measure residues of these same eleven compounds is also described. When the latter technique is combined with the previously reported method for organochlorine insecticide residues[1], the combined method has been shown to be suitable for the determination of thirty-one insecticide residues. Thirty of these insecticides can be readily determined at the 0.01 p.p.m. level and most insecticides in most vegetables at the 0.002 p.p.m. level. Azinphos methyl can be determined at the 0.02 p.p.m. level.

INTRODUCTION

In recent years there has been widespread introduction of legislation to control residues of insecticides in vegetables and vegetable products, accompanied by an increasing use of such chemicals, particularly organophosphorus insecticides. This has placed a burden on food manufacturers to ensure that products offered for sale to the public comply with statutory limits and has created an urgent need for methods to survey a wide range of food products and raw materials. Such methods must be rapid and sensitive, capable of detecting residues of a wide range of compounds in a wide range of vegetables/products and capable of operation by assistant staff with the minimum of supervision.

The analysis of insecticide residues is complicated by the wide range of properties exhibited by this class of pesticides and particularly by the variety of toxic

metabolites produced in plants from organophosphorus insecticides, which are considerably more polar than the parent insecticides from which they derive. Thus whereas all organochlorine and an appreciable number of organophosphorus insecticides are preferentially soluble in hexane and only sparingly soluble in water, nearly all the toxic metabolites and many parent species of organophosphorus insecticides are preferentially soluble in water and only sparingly soluble in hexane. We have preferred to determine these two groups of compounds, *i.e.* 'hexane-soluble' and 'water-soluble' insecticides, in separate extracts as in our experience this is more rapid and considerably simplifies the problem of clean-up compared to the single-extraction procedures of LAWS AND WEBLEY[2], and HAMENCE[3]. The procedure developed for 'hexane-soluble' insecticides is based on that previously reported for organochlorine residues[1]. To make the procedure of use to the widest range of laboratories two alternative methods for detection of insecticides are given. The insecticides studied have been chosen to include those most widely used and likely to constitute a hazard on home-grown and imported crops.

The procedure developed for determination of "water-soluble" organophosphorus insecticides and toxic metabolites will be published shortly as Part II of this paper.

EXPERIMENTAL

Methods for the extraction and clean-up of hexane-soluble organophosphorus insecticides closely parallel those for the organochlorine insecticides which have previously been discussed[1]. Determinative procedures include colorimetric analysis[2,4], measurement of anticholinesterase activity[5,6], paper and thin-layer chromatography[7–9], and more recently GLC using a variety of detectors[10–13]. To meet the requirements of different laboratories we have used two alternative determinative procedures: a total-phosphorus colorimetric procedure using standard laboratory equipment and a more expensive and sophisticated GLC system incorporating a phosphorus specific "thermionic" detector.

Total-phosphorus procedure

All total-phosphorus procedures used for residue analysis consist of a preliminary oxidation stage followed by colorimetric determination of the orthophosphate formed. We decided that the procedure proposed by SALIMAN[14] would be most suitable for our purpose. Using both his oxidation and molybdenum blue colorimetric methods the data shown in Table I were obtained. To obtain acceptable reagent blanks we found it essential to distil the hydriodic acid solution used in the oxidising reagent. A serious disadvantage of the colorimetric procedure used by SALIMAN is that maximum colour development is only obtained after heating the final 5-ml solution on a steam-bath for 10 min, which resulted in variable evaporation losses. Although not as sensitive, better replication and lower blank values were obtained when the modified molybdenum blue procedure of MURPHY AND RILEY[15] was adapted for use. The basic advantages of this modification are that all the reactants are combined in a single solution, thus reducing the number of transfers required and by incorporating potassium antimonyl tartrate in the reagent, a stable colour develops within 10 min at ambient temperature.

TABLE I

COMPARISON OF REAGENTS USED BY SALIMAN AND BY MURPHY AND RILEY

	Saliman	*Murphy and Riley*
Absorption maxima (nm)	820	880–885
Optical density for 1 μg phosphorus (nett)	0.178	0.150
Recovery of 1 μg phosphorus on 23 occasions (%)	95 ± 10	97 ± 4
Optical density of reagent blank	0.016 ± 0.005	0.011 ± 0.002

TABLE II

RECOVERY OF INSECTICIDES THROUGH DETERMINATIVE PROCEDURE

Insecticide	*Quoted purity of standard* (%)	*Number of occasions*	*Recoveries* (%)	
			Range	*Mean*
Azinphos methyl	94	4	87–94	91
Bromophos	none quoted	15	89–99	95
Chlorfenvinphos	none quoted	6	89–95	91
Diazinon	"pure"	7	86–94	90
Dioxathion	none quoted	5	96–104	99
Disulfoton	97.3	11	84–100	96
Fenitrothion	99.8	6	95–99	98
Malathion	96.3	11	90–103	100
Parathion	99.9	15	90–99	97
Phorate	92.5	11	88–105	100
Thionazin	99	7	88–98	94
Potassium hydrogen phosphate	99.5	23	93–101	97

Using this procedure replicate determinations of organophosphorus insecticides equivalent to 1 μg phosphorus were obtained over a three-week period. Acceptable recoveries of at least 90 % of the quoted purity of the standards used were obtained as shown in Table II with a maximum spread of ± 8 %. Reagent blank values obtained over a four-month period averaged 0.013 optical density units with a spread of 0.005 units.

GLC with the phosphorus thermionic detector

An Aerograph Model 1520 gas chromatograph fitted with the Varian-Aerograph phosphorus detector was used. The position of the polarising electrode relative to the flame, both vertically and horizontally, was found to have a very marked effect on signal-to-noise ratios and optimum operating conditions depended primarily on the relative positions of the flame and electrode. In general the optimum air flow rate was higher and the hydrogen flow rate lower than those recommended by HARTMANN[11]. The background noise level decreased rapidly with reducing hydrogen flow rates. Working at low flow rates of hydrogen, therefore, we have been able to use the high inherent sensitivity in the amplifier to its maximum. The low hydrogen flow rates used required correspondingly low carrier gas flow rates for optimum performance. Surprisingly, under these conditions markedly less base-line drift occurred during temperature programming of the column and it has been possible to extend the scope of the GLC method by programming.

With the low carrier gas flow rates employed, it was necessary during isothermal operation to use higher column temperatures to reduce retention times. The new OV series of stationary phases has been found ideal for operation at these higher temperatures. In addition, certain insecticides that were strongly adsorbed on SE-30 and QF-1 column packings, notably dioxathion and chlorfenvinphos, gave much better peak shapes on the OV series column packings. The stationary phase found most useful for primary analysis was the slightly polar OV-17 (methylphenyl silicone) which gave a column of higher efficiency and longer useful life than the more polar silicones, OV-210 (trifluoropropyl methyl silicone fluid), and OV-225 (cyanopropylmethylphenyl methyl silicone fluid), which were used for confirmatory analysis. As shown in Table III complete resolution of all eleven insecticides could be achieved using these stationary phases under the conditions given in ANALYTICAL PROCEDURE.

TABLE III

SENSITIVITY AND RELATIVE RETENTION DATA FOR THREE TYPES OF STATIONARY PHASE

Stationary phases and column lengths: (A) OV-17, 5 ft.; (B) OV-210, 10 ft.; (C) OV-225, 10 ft.

Insecticide	*Relative retention times*			*Detection limit*[a] *(pg)*
	A	*B*	*C*	
Thionazin	0.76	0.93	0.88	3
Phorate	1.00 (2 min)	1.00 (1.4 min)	1.00 (2 min)	13
Diazinon	1.29	0.98	0.94	6
Disulfoton	1.54	1.36	—	6
Dioxathion	1.63	1.41	1.79	18
Malathion	3.0	3.2	3.4	19
Fenitrothion	3.0	4.0	4.3	13
Parathion	3.2	5.1	4.5	10
Bromophos	3.5	2.48	3.2	21
Chlorfenvinphos	4.3	4.7	4.5	42
Azinphos methyl	8.6	>9	>9	6000

[a] Equivalent to a peak height of 2% f.s.d. on attenuation setting 1 × 4 (1.2×10^{-9} A full scale).

The long retention time and poor response obtained for azinphos methyl under isothermal conditions made detection at levels below 0.25 p.p.m. in vegetables impossible. A great improvement in response for azinphos methyl was obtained by running the column for 1 min at 175° and then programming the temperature at 20°/min to 280°. This is shown by comparing the figures in Table IV with those in

TABLE IV

DATA OBTAINED ON TEMPERATURE-PROGRAMMED OV-17 COLUMN

Insecticide	*Relative retention time*	*Detection limit (pg)*
Phorate	1.00 (4.5 min)	22
Malathion	1.47	14
Chlorfenvinphos	1.60	27
Azinphos methyl	3.0	250

TABLE V

ELUTION OF INSECTICIDES FROM ALUMINA COLUMN

Eluting agent	*Fraction (10 ml)*	*Distribution of insecticides (%)*										
		Azinphos methyl	*Bromophos*	*Chlorfenvinphos*	*Diazinon*	*Dioxathion*	*Disulfoton*	*Fenitrothion*	*Malathion*	*Parathion*	*Phorate*	*Thionazin*
Hexane	1	—	—	—	—	—	—	—	—	—	—	—
	2	—	95	—	—	—	—	—	—	—	90	—
	3	—	5	—	—	—	100	—	—	—	10	—
2% acetone in hexane	1	—	—	—	50	—	—	5	20	—	—	—
	2	—	—	—	50	95	—	95	80	95	—	100
	3	—	—	—	—	5	—	—	—	5	—	—
	4	50	—	10	—	—	—	—	—	—	—	—
	5	40	—	35	—	—	—	—	—	—	—	—
	6	10	—	50	—	—	—	—	—	—	—	—
	7	—	—	5	—	—	—	—	—	—	—	—
	8	—	—	—	—	—	—	—	—	—	—	—

Table III above. Under these conditions the base-line drift was negligible and the time taken to cool the column oven between injections (5 min) was sufficient for the attainment of a steady background current.

Extraction and clean-up

The extraction and clean-up procedures previously reported for organochlorine insecticide analysis[1] were found suitable with slight modification for organophosphorus residue analysis.

GLC procedure. Organophosphorus insecticides were retained more strongly on an 8 g activity V alumina column than were organochlorine insecticides. Table V shows the elution volumes required for the various organophosphorus insecticides. Excellent recoveries were obtained through the column for 5 μg insecticide, equivalent to 1 p.p.m. in vegetables. Satisfactory, though lower, recoveries were also obtained when the equivalent of 0.2 p.p.m. insecticide in vegetables was added to 40 ml water and taken through the complete process, as shown in Table VI.

TABLE VI

RECOVERY OF INSECTICIDES

Insecticide	*Recovery through alumina column* (%)	*Recovery through complete process* (%)	
		GLC procedure	*Total-phosphorus procedure*
Azinphos methyl	91	87	83
Bromophos	100	93	92
Chlorfenvinphos	97	92	88
Diazinon	101	88	81
Dioxathion	101	87	78
Disulfoton	95	91	92
Fenitrothion	97	93	93
Malathion	94	88	77
Parathion	97	94	92
Phorate	98	90	80
Thionazin	98	85	76

Total-phosphorus procedure. The very much lower inherent sensitivity of the total-phosphorus procedure meant that the maximum volume of extract had to be cleaned up in order to achieve a satisfactory screening limit. From practical considerations the optimum volume of hexane extract was 150 ml, equivalent to 37.5 g original vegetable, which gave high blank values after elution from an 8 g alumina column. The size of this blank, however, was reduced to an acceptable level when, prior to column clean-up, the hexane extract was washed with sodium sulphate solution three times instead of once. The rate of elution from the column during clean-up was also found to be critical with a maximum acceptable rate of 3 ml/min. At higher flow rates an increasingly large amount of interfering co-extracted material was eluted from the column, giving rise to very high optical densities on final analysis. Both the acetone and hexane (A.R. and G.P.R. grades) used as extractants and eluting agents were found to contain variable amounts of impurity that were not removed at the clean-up stage of the process and produced a blue colour with the MURPHY AND RILEY reagent. These impurities were readily removed by simple distillation.

TABLE VII

BLANK VALUES IN THE TOTAL-PHOSPHORUS PROCEDURE

Blank	*Value expressed as optical density of final extract*		
	Range	*Mean*	*Total blank value*
Reagent	0.009–0.017	0.013	—
Column	0.003–0.010	0.008	—
Process	0.003–0.011	0.006	0.027
Vegetable			
Pea	0.010–0.025	0.022	0.049
Carrot	0.012–0.023	0.021	0.048
Br. sprout	0.010–0.027	0.019	0.046
Gr. bean	0.012–0.017	0.013	0.040
Br. bean	0.013–0.018	0.015	0.042
Broccoli	0.014–0.026	0.019	0.046
Spinach	0.015–0.020	0.017	0.044
Mean vegetable blank	0.010–0.027	0.018	0.045

Using these modifications, various blank values were determined on a minimum of ten occasions over a period of several months. The size and spread of these values, given in Table VII, show that the vegetable blank, 0.018 ± 0.005 optical density units, obtained by taking 50 g of pesticide-free vegetable through the process, was virtually independent of the type of vegetable analysed. The total blank value of 0.045 ± 0.008 O.D. units is equivalent to approximately 0.08 p.p.m. insecticide in vegetables and defines the screening limit and precision of which the method is capable. Recoveries of insecticides taken through the process at the 0.2 p.p.m. level were mostly in agreement with results obtained using the GLC procedure (Table VI) but five insecticides in particular gave significantly lower recoveries. These differences have been shown to be due to the different evaporation procedures used to concentrate column eluates. In the GLC procedure the eluate was collected in fractions which were conveniently concentrated in tubes in a water bath at 70° under a stream of nitrogen. In the total phosphorus procedure the total eluate was concentrated in Kuderna–Danish apparatus in live steam. The method of concentration has been shown to have little effect on insecticides other than diazinon, dioxathion, malathion, phorate and thionazin, which incurred significant losses by volatilisation during Kuderna–Danish evaporation. Attempts to reduce these losses were unsuccessful, as shown in Table VIII. Volatilization increased if the collector tube was allowed to go to dry-

TABLE VIII

EFFECT OF METHOD OF CONCENTRATION ON PHORATE RECOVERY

Method of concentration	*Recovery* (%)
(1) Kuderna–Danish evaporation in live steam	87
(2) As (1) but under a stream of nitrogen	79
(3) Kuderna–Danish evaporation in water bath at 75°	84
(4) Evaporation from a tube in water bath at 75° under N_2	103
(5) Rotary evaporation under vacuum at 35°	96

ness and it was essential to ensure that solvent was always present in the tube during the final stage of evaporation. Although rotary evaporation gave much better recoveries of pesticides it is unsuited to routine application and Kuderna–Danish evaporation was retained in the procedure.

ANALYTICAL PROCEDURE

Total-phosphorus procedure

Reagents

Hexane. The hexane fraction should be laboratory reagent grade. Redistil from potassium hydroxide pellets, 4 g/l.

Acetone. Acetone should be G.P.R. grade. Redistil before use.

Sodium sulphate. Sodium sulphate should be A.R. grade, granular, anhydrous, and in the form of a 2 % w/v aqueous solution

Alumina. Alumina should be activity V grade. To approx. 1 kg of technical alumina trihydrate, Gibbsite (British Aluminium Co.), in a 5-litre beaker add approx. 3 l of distilled water and stir well. Allow the alumina to settle for 2–3 min and pour off the supernatant liquid and suspended fine material. Repeat this washing process a further two times. Transfer the alumina to a No. 1 or 2 sintered filter and filter under vacuum. Wash the alumina well with acetone and allow to air dry. Spread the alumina in an approximately 1/2 in. layer and activate to Brockmann activity I by heating at 525–675° for a minimum of 4 h, conveniently overnight. Cool in a desiccator and transfer a suitable quantity to a stoppered flask. Add 12 ml distilled water per 88 g of adsorbent. Close the flask and shake until no visible lumps remain and the exothermic reaction has ceased. Equilibrate for 24 h in the closed flask before use.

Digestion reagent. Prepare phosphorus-free hydriodic acid by adding 10–20 g iodine to 125 ml 55 % w/w hydriodic acid, A.R. grade. Distil and collect the first 110–120 ml distillate.

Add 50 ml distilled hydriodic acid, 0.6 g calcium hydroxide, 50 ml distilled water and 500 g phenol, A.R. grade, to a 1-litre volumetric flask and make to the mark with glacial acetic acid, A.R. grade.

Colorimetric reagent. Dissolve 30 g ammonium molybdate, A.R. grade and 0.686 g potassium antimonyl tartrate, A.R. grade, in approximately 2 l of distilled water. Add 2500 ml of 5 *N* sulphuric acid (prepared by diluting 350 ml concentrated sulphuric acid, A.R. grade, to 2.5 l with distilled water) and mix. Make up to 5 l with distilled water and remix.

Immediately before use, dilute 20 ml of the above stock solution to 125 ml with distilled water and add 0.1 g ascorbic acid. This final solution is unstable and must be prepared daily.

Standard phosphorus solution. Dissolve 0.351 g dry potassium dihydrogen phosphate, A.R. grade, in distilled water and make to 1 l. Pipette 5 ml of this solution into a second 1-litre flask and make to volume with distilled water. This solution contains 0.4 μg P/ml.

Extraction

Mince and well mix a bulk sample of vegetable. Macerate a representative 50-g sub-sample in a 500-ml beaker with 50 ml acetone and 200 ml hexane using a top-drive

macerator for 2–3 min. If necessary transfer the bulk of the macerate to a 250-ml centrifuge cup and centrifuge briefly at 2400 r.p.m. to pack down vegetable tissue and assist separation of the two phases. Transfer the upper hexane–acetone layer to a 1-litre separating funnel and wash successively with 1 × 500 ml and 2 × 200 ml of sodium sulphate solution. Remove 150 ml of the hexane extract, ensuring no aqueous phase is present in the aliquot, and transfer to a Kuderna–Danish evaporator fitted with a 10-ml graduated collector tube. Add a few crystals of sodium sulphate and evaporate the contents on a steam-bath to approximately 5 ml. Disconnect the collector tube and continue the evaporation to between 1 and 2 ml in a water bath at 70° under a stream of nitrogen, ensuring that the extract does not evaporate to dryness.

Clean-up

Prepare a slurry of 8 g grade V alumina in a little hexane and transfer with a rapid swirling motion to a clean chromatography column (30 × 1 cm). Add approximately 1 g anhydrous sodium sulphate as a layer on top of the column. Wash the column with 10–20 ml hexane and adjust the hexane level to that of the of the sodium sulphate layer. Transfer the concentrated hexane extract to the top of the column with the minimum of hexane rinsing, allowing the hexane level to fall to that of the sodium sulphate layer between additions. Discard the eluate. Change the receiver and elute twice with 2 ml hexane, followed by a further 25 ml hexane and 80 ml 2% acetone in hexane at a rate of less than 3 ml/min. Concentrate the eluate to 5 ml in a clean Kuderna–Danish evaporator. To avoid contamination at this stage, it is essential that different Kuderna–Danish apparatus are used to concentrate the hexane extract and the column eluate.

Digestion

Transfer the concentrated eluate to a 25-ml pyrex beaker with the minimum of rinsing and add 2 ml digestion reagent. Place the beaker on a steam bath and after 30 min transfer it to a hot-plate situated in a fume-chamber to complete the volatilisation of solvents and reagents. Transfer the beaker to a muffle furnace at 700° for 10 min to oxidise the remaining carbonaceous matter. Finally remove the beaker from the muffle and allow it to cool.

Phosphorus determination

Add 5.0 ml of the colorimetric reagent, agitate the beaker to dissolve residue and set aside for 10 min to develop maximum colour. Determine the optical density of the solution in a 1-cm cell at 882 nm against a water blank.

Determine the reagent blank associated with the procedure by adding 2 ml digestion reagent to each of three 25 ml beakers and taking them through the procedure detailed above. Prepare a standard phosphorus graph by evaporating volumes of the standard potassium dihydrogen phosphate solution, equivalent to 0.2–2 μg phosphorus, to dryness in a series of 25 ml beakers and adding 5.0 ml colorimetric reagent etc.

Calculation of results

For screening purposes a gross optical density of 0.080 units or above in the

final extract indicates that, depending on the insecticide, at least 0.06–0.1 p.p.m. residues is present in the original vegetable.

Phosphorus contents of extracts can be readily determined from the standard graph after subtraction of the total blank value. 0.027 × the phosphorus content gives the p.p.m. of pesticidal phosphorus in the original vegetable. Provided the history of the crop is known, p.p.m. insecticide in the original vegetable can be calculated using the conversion factors listed in Table X.

GLC procedure

Reagents, extraction and clean-up on 8 g activity V alumina are as previously reported[1], except that 80 ml rather than 30 ml of 2 % acetone in hexane is used for elution.

Operating conditions found most satisfactory for analysis with the Varian-Aerograph phosphorus thermionic detector were as follows

Columns: 1/8 in. × 5 ft. or 10 ft. pyrex glass with pyrex glass injection inserts.

Column packings: (A) 3 % OV-17; (B) 3 % OV-210; (C) 3 % OV-225. All on AW-DMCS 80–100 mesh Chromosorb G.

Column temperature: 225°, 230° and 240° for columns A–C respectively. Detector and injector were maintained 10° above the column temperature.

Gas flow rates: Air, 200 ml/min; hydrogen, approximately 11 ml/min; nitrogen, 10–15 ml/min.

Sensitivity: Attenuation settings, 1 × 8 or 1 × 16.

Stability of base-line and replication of response are good provided the flame is left burning and gas flow rates are not reset, and for these reasons the instrument is best run continuously. It is essential to inject a standard mixture of insecticides at intervals during a series of analyses to assess column and detector performance. A 5-μl injection of a mixture containing 0.01–0.1 p.p.m. insecticides is suitable for this purpose.

RESULTS AND DISCUSSION

Total-phosphorus procedure

Aliquots of standard insecticide solutions were added to samples of minced vegetables to establish recoveries through the procedure. Levels of addition were chosen to cover the range of concentration over which a screening method would have to operate and produce significant results. As shown in Table IX recoveries tended to decrease as the level of insecticide added was reduced but at least two thirds were recovered at the 0.1 p.p.m. level or below. The number of positive results, *i.e.* optical densities of $\geqslant$ 0.080 in the final extracts, obtained in the recovery tests confirms the adequacy of the procedure for screening crops at the 0.1 p.p.m. level. Because of the different phosphorus contents of the insecticides studied their response in the screening test varied. The final optical density was also affected by the varying recoveries of different insecticides through the process. Allowing for these variations screening limits ranged from 0.06–0.10 p.p.m. depending on the insecticide as shown in Table X. It must be stressed that meaningful results at these low levels can only be obtained if strict precautions are observed and the care and hygiene associated with micro-methods of analysis adhered to.

TABLE IX

RECOVERIES OF INSECTICIDES FROM VEGETABLES

Insecticide	*Vegetable*	*Level of addition (p.p.m.)*	*Recovery by total-phosphorus*		*Recovery by GLC (%)*
			%	*O.D. of extract*	
Azinphos methyl	Br. sprout	0.15	74	0.100	
	Pea	0.10	65	0.084	72
	Pea	0.05	—	—	70
Bromophos	Pea	0.22	96	0.144	
	Br. bean	0.11	94	0.098	87
	Gr. bean	0.02	—	—	84
Chlorfenvinphos	Br. sprout	0.24	102	0.157	
	Br. sprout	0.10	89	0.089	91
	Br. sprout	0.05	—	—	86
Diazinon	Broccoli	0.28	93	0.186	
	Pea	0.10	—	—	85
Dioxathion	Pea	0.23	86	0.206	
	Carrot	0.11	77	0.114	92
	Pea	0.03	—	—	81
Disulfoton	Br. sprout	0.17	92	0.127	
	Carrot	0.17	103	0.150	
	Carrot	0.085	80	0.088	90
	Pea	0.03	—	—	79
Fenitrothion	Pea	0.24	96	0.177	
	Br. bean	0.16	87	0.133	85
	Carrot	0.09	88	0.090	88
Malathion	Gr. bean	0.2	82	0.122	
	Spinach	0.7	82	0.350	
	Spinach	0.3	85	0.186	
	Spinach	0.1	81	0.089	92
	Spinach	0.05	93	0.067	86
Parathion	Pea	0.18	99	0.151	
	Br. sprout	0.18	82	0.130	
	Br. sprout	0.09	80	0.091	87
	Br. bean	0.09	67	0.083	85
	Br. bean	0.045	—	—	81
Phorate	Br. sprout	0.17	74	0.133	
	Pea	0.10	71	0.092	96
	Br. sprout	0.01	—	—	79
Thionazin	Pea	0.22	84	0.158	
	Br. bean	0.14	81	0.122	
	Carrot	0.14	71	0.114	
	Carrot	0.09	72	0.095	83
	Carrot	0.01	—	—	74

GLC procedure

Recoveries of insecticides added to minced vegetables and taken through the GLC procedure are shown in Table IX. The results show the satisfactory recovery of insecticides obtained down to the 0.01 p.p.m. level. The higher results obtained for thionazin, malathion, phorate and dioxathion using the GLC procedure reflect the different concentration techniques used in the two procedures. This was confirmed in replicate recovery experiments using the total-phosphorus procedure with malathion added to green beans at the 0.2 p.p.m. level. 94% recovery was obtained using

TABLE X

INSECTICIDE DATA

Pesticide	*Molecular weight*	*Conversion factor*	*Data relating to 0.1 p.p.m. insecticide in vegetable*			*Concentration equivalent to an O.D. of 0.08 in extracts (p.p.m.)*
			Phosphorus content of 150 ml extract (μg)	*Theoretical nett O.D.*	*Actual nett O.D. corrected for process loss*	
Bromophos	366	11.8	0.31	0.048	0.046	0.08
Chlorfenvinphos	360	11.62	0.32	0.049	0.045	0.08
Malathion	330	10.65	0.36	0.054	0.045	0.08
Azinphos methyl	317	10.25	0.37	0.056	0.037	0.10
Diazinon	304	9.81	0.39	0.058	0.053	0.065
Parathion	291	9.4	0.40	0.061	0.042	0.085
Fenitrothion	277	8.95	0.42	0.064	0.056	0.06
Disulfoton	274	8.84	0.42	0.065	0.058	0.06
Phorate	259	8.36	0.45	0.069	0.052	0.065
Thionazin	258	8.0	0.47	0.072	0.054	0.065
Dioxathion	456	7.36	0.51	0.077	0.057	0.06

rotary evaporation compared to 81 % recovery using Kuderna–Danish evaporation. The low recovery of 0.1 p.p.m. azinphos methyl obtained with the total-phosphorus procedure is confirmed by GLC analysis.

The much greater sensitivity and the specificity of the GLC procedure makes this the method of choice provided the expensive captital outlay can be justified. The method is rapid and allows for positive identification of individual insecticides at levels ranging from 0.001 p.p.m. thionazin to 0.01 p.p.m. chlorfenvinphos under normal operating conditions. Azinphos methyl can only be detected down to the 0.25 p.p.m. level under these conditions but can readily be determined at the 0.02 p.p.m. level using temperature programming. The major advantage of the GLC procedure is that it can be easily linked with the procedure for organochlorine insecticides[1] to provide a comprehensive method for hexane-soluble insecticides in vegetables. The scope of this procedure has further been extended by taking an additional ten organochlorine insecticides through the method. The elution of these insecticides into different fractions from the column is given in the procedure shown schematically in Fig. 1.

General

The combined procedure for organochlorine and hexane-soluble organophosphorus insecticides has been successfully used to determine residues in crops of known history and is currently being used to survey a range of commercial vegetables and vegetable products. The total-phosphorus screening procedure is capable of detecting all but phorate at or below the stringent limits imposed in certain West European countries. Phorate, which is not mentioned in the West German regulations and has a "zero" tolerance in the Dutch regulations, can, however, be readily detected at the 0.002 p.p.m. level using the GLC procedure.

Attempts were made to replace the British Aluminium Company's technical alumina trihydrate used in the process with alternative ready activated material. Activity I neutral alumina from M. Woelm, G.F.R., was found to give identical results after deactivation with 15 % water but was expensive for routine use. Peter Spence,

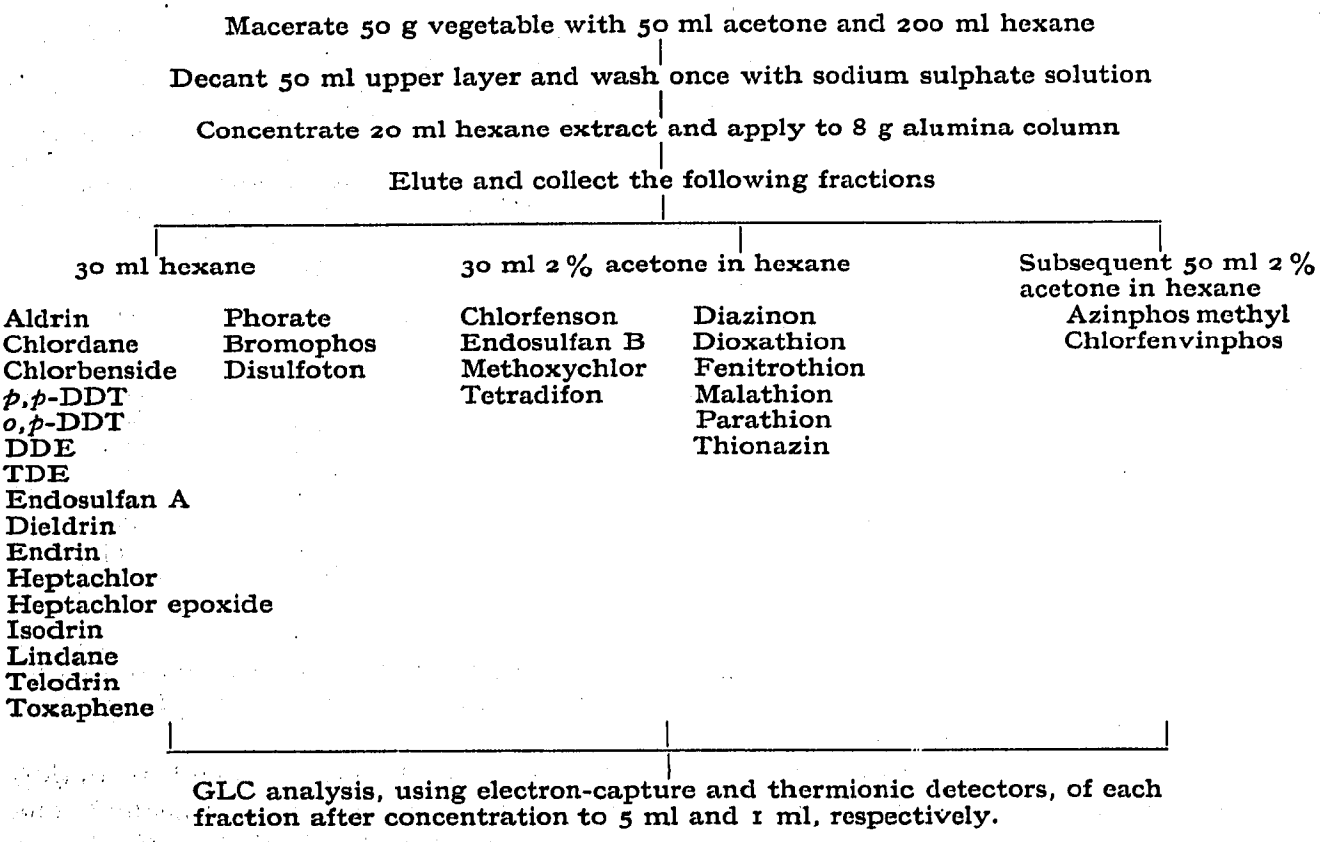

Fig. 1. Procedure for multiple analysis of hexane-soluble insecticides.

Type O and Type H, aluminas, both much cheaper sources of ready activated material, were investigated and found to be unsuitable for use with the total-phosphorus procedure. Optical densities up to 0.700 were obtained from pea extracts after clean-up on activity V materials with even higher optical densities resulting from clean-up on more active grades of these aluminas. This phenomenon of decreasing retention of vegetable co-extractives with increasing activity of alumina was also demonstrated for the B.A.C. alumina. The reason for this and the different characteristics of the various aluminas investigated are not fully understood but have to do with different methods of production and activation procedures.

REFERENCES

1 D. J. Sissons, G. M. Telling and C. D. Usher, *J. Chromatog.*, 33 (1968) 435.
2 E. Q. Laws and D. J. Webley, *Analyst*, 86 (1961) 249.
3 J. H. Hamence, *Chem. Ind. (London)*, (1966) 1255.
4 E. D. Chilwell and P. T. Beecham, *J. Sci. Food Agr.*, 11 (1960) 400.
5 W. P. McKinley and S. I. Read, *J. Assoc. Offic. Agr. Chemists*, 45 (1962) 467.
6 H. Michel, *J. Lab. Clin. Med.*, 34 (1949) 1564.
7 J. A. R. Bates, *Analyst*, 90 (1965) 453.
8 Ali el Refai and T. L. Hopkins, *J. Agr. Food. Chem.*, 13 (1965) 477.
9 N. A. Smart and A. R. C. Hill, *J. Chromatog.*, 30 (1967) 626.
10 L. Guifrida, *J. Assoc. Offic. Agr. Chemists*, 47 (1964) 293.
11 C. H. Hartmann, *Bull. Environ. Contam. Toxicol.*, 1 (1966) 159.
12 M. E. Getz, *J. Gas Chromatog.*, 5 (1967) 377.
13 C. A. Bache and D. J. Lisk, *J. Assoc. Offic. Anal. Chemists*, 50 (1967) 1246.
14 P. M. Saliman, *Anal. Chem.*, 36 (1964) 112.
15 J. Murphy and J. P. Riley, *Anal. Chim. Acta*, 27 (1962) 31.