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

II. A SCREENING PROCEDURE FOR WATER-SOLUBLE INSECTICIDES

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SUMMARY

A screening procedure is described for the determination of residues of the more polar organophosphorus insecticides in vegetables. The method is based on aqueous extraction, partition into chloroform, alumina column clean-up and colorimetric determination of total pesticide phosphorus. Screening limits for sixteen organophosphorus insecticides and metabolites were in the range 0.05–0.1 p.p.m., depending on the particular insecticide.

INTRODUCTION

Organophosphorus insecticides are being increasingly used in agriculture, and although legislation to control residues of these insecticides exists in many European countries, there is a lack of methods suitable for routine application to a wide range of residues in vegetables and vegetable products. A large number of the organophosphorus insecticides currently in use and the toxic metabolites derived from them in plants are preferentially soluble in water and are not detected in the procedures for hexane-soluble insecticides previously reported[1]. These polar insecticides and metabolites usually remain in the aqueous phase after maceration but even if some degree of partition into hexane occurs, they are effectively removed again during the washing stage of the process.

All organophosphorus insecticides are to a greater or lesser extent systemic and are readily metabolised in plants to more polar toxic compounds — this usually involves oxidation of sulphur atoms in the molecule and can result in a multiplicity of products. In addition these metabolites are hydrolysed to produce non-toxic ionic phosphate esters. Any method for the determination of organophosphorus insecticide residues must therefore be capable of determining both the parent insecticide and any toxic metabolites formed but not the products of hydrolysis.

This paper describes a rapid and sensitive screening procedure for determining toxic water-soluble organophosphorus residues in vegetables, based on the determination of total pesticide phosphorus in suitably cleaned-up extracts. The insecticides and

metabolites studied cover a relatively wide range of polarities and include those most widely used in this country. A comprehensive screen for organophosphorus insecticide residues in vegetables is obtained when this procedure is used in conjunction with that previously reported for hexane-soluble insecticides. The use of GLC with a thermionic detector for confirmatory analysis is also briefly discussed.

EXPERIMENTAL

Extraction

Many methods which make use of polar solvents such as acetone[2] and acetonitrile[3] for the extraction of organochlorine residues have been shown to be also suitable for the extraction of a range of organophosphorus insecticides and metabolites. Solvent systems capable of extracting compounds exhibiting such a wide range of polarities, however, give rise to extracts that require elaborate clean-up to remove the high level of co-extracted material. For this reason we have preferred to extract water-soluble organophosphorus residues into aqueous solution using a modified form of the method of CHILWELL AND BEECHAM[4]. Using their procedure to determine 0.5 p.p.m. dimethoate in cabbage, spinach and lettuce, we found that the recoveries tended to be inversely proportional to the time taken to extract the insecticide from the vegetable into chloroform. To minimise this loss from aqueous solution and to facilitate removal of tissue we have preferred to macerate the vegetable directly with dilute acetic acid solution and increase the ratio of extractant to vegetable. These modifications resulted in 85 % recovery of dimethoate at the 0.2 p.p.m. level compared to original recoveries of approximately 70 %. We did not neutralise extracts before partition with chloroform as this step could result in loss of insecticide by hydrolysis, is time consuming and did not increase the recovery.

Clean-up

Considerable clean-up of aqueous extracts is readily achieved by extracting insecticides and toxic metabolites into chloroform. This step also serves to remove the non-toxic phosphate esters which are retained in the aqueous phase. For parent insecticides the partition ratios are greatly in favour of chloroform. Toxic metabolites, however, can be markedly more hydrophilic and exhaustive extraction may be required for complete transfer of these species into chloroform.

Although certain crops, notably cabbage, give chloroform extracts that are clean enough for dimethoate analysis by GLC, further clean-up of all extracts is required for total pesticide phosphorus analysis. This is usually achieved by adsorption chromatography using such materials as Florisil[5], silica gel[6], alumina[7] and activated charcoal[8]. Using an 8-g column of activity V alumina, we found that complete retention of all polar organophosphorus compounds occurred under the conditions used for elution of hexane-soluble species[1]. Demeton-S-methyl required 50 ml of 40 % acetone in hexane for complete elution. Dimethoate and oxydemeton-methyl required 50 ml, and the more polar dimethoxon 100 ml, of 50 % chloroform in hexane for complete elution. Using chloroform as eluting solvent, however, elution of the latter metabolite was obtained within 25 ml. Under these conditions complete elution of all insecticides, except dichlorvos, naled and trichlorphon, was achieved with 25 ml chloroform. Randomly low and variable recoveries of the above three insecticides were obtained

through the column. Elution with additional chloroform failed to increase these recoveries. It was demonstrated that the losses only occurred during chromatography; acceptable and reproducible recoveries were obtained for replicates taken through the extraction, partition and Kuderna–Danish evaporation stages in the process, as shown in Table I. It seemed likely therefore that the insecticides were being hydrolysed by the alkalinity of the alumina (British Aluminium Company) used, to produce strongly absorbed orthophosphate esters. The significant improvement in both recovery and replication obtained, when neutral/neutralised aluminas were used, is clearly shown in Table II. For reasons not clearly understood neutralised BAC alumina

TABLE I

FATE OF DICHLORVOS, NALED AND TRICHLORPHON ON ALKALINE ALUMINA

Insecticide	*Recovery through stages in process (%)*				*Recovery from peas (%)*	*Level of addition (p.p.m.)*
	Extraction + partition + K.D.	*Partition + K.D.*	*K.D.*	*Column*		
Dichlorvos	86–88	87–89	85–86	55–90	34–87	0.25
Naled	89–93	90–93	93–94	16–93	14–27	0.18
Trichlorphon	86–88	89–91	95–98	7–74	0–17	0.35

TABLE II

FATE OF DICHLORVOS, NALED AND TRICHLORPHON ON NEUTRAL ALUMINAS

Insecticide	*Recovery (%)*		
	Neutralised BAC, pH 6.7	*Neutral M. Woelm, pH 6.8*	*Neutralised P. Spence Type H, pH 6.6*
Dichlorvos	69–82	84–90	90–95
Naled	57–81	73–79	88–93
Trichlorphon	60–81	76–83	91–93

was still less efficient than neutral alumina (M. Woelm, G.F.R.), which was less efficient than neutralised P. Spence Type H alumina. This latter material gave excellent recoveries of all three insecticides with good replication. The neutralised aluminas were prepared by the addition of glacial acetic acid during deactivation to activity V material. The Type H material gave a product of pH within the range 6.5–7.0 compared to an original value of pH 9.4. Using neutralised activity V Type H alumina satisfactory elution of all insecticides under investigation was obtained in 30 ml chloroform, as shown in Table III. In practice it is easier to collect a measured volume of eluate than to elute with known volumes of solvent. Therefore it was decided to collect 35 ml column eluate to ensure complete elution of trichlorphon from the column. Using this procedure the recoveries given in Table III, for 10 μg insecticide, equivalent to approximately 0.3 p.p.m. in vegetables, were obtained.

TABLE III

DISTRIBUTION AND ELUTION OF INSECTICIDES ON NEUTRALISED ACTIVITY V ALUMINA

Fractions: (A) 10 ml; (B) 5 ml; (C) 10 ml; (D) 5 ml.

Insecticide	*Distribution in chloroform eluate (%)*				*Recovery*
	A	*B*	*C*	*D*	
Demeton	90	10	—	—	97
Demeton-S-methyl	90	10	—	—	89
Thiometon	90	10	—	—	92
Methyl-demeton-methyl	85	15	—	—	91
Oxydemeton-methyl	50	50	—	—	92
Dimethoate	60	35	5	—	93
Formothion	60	40	—	—	86
Mevinphos	90	10	—	—	93
Phosphamidon	95	5	—	—	94
Dimethoxon	55	30	15	—	90
Malaoxon	90	10	—	—	98
Phorate-O-analogue	85	15	—	—	95
Phorate-O-sulphoxide	80	20	—	—	94
Dichlorvos	5	65	25	5	94
Naled	35	50	15	—	91
Trichlorphon	—	30	50	20	93

Determination

The oxidation and colorimetric procedures previously reported[1] were found suitable without modification for the determination of polar insecticides and metabolites. Various blank values were determined on a minimum of ten occasions over a period of three months. The values in Table IV show clearly that the total blank is almost independent of the particular vegetable under analysis and demonstrates the efficiency of the clean-up procedure used. The total blank value of 0.025 $\pm$ 0.006 optical density units is equivalent to approximately 0.05 p.p.m. insecticide in vegetables and defines the screening limit of which the method is capable.

TABLE IV

BLANK VALUES IN THE TOTAL-PHOSPHORUS PROCEDURE

Blank	*Value expressed as optical density of final extract*		
	Range	*Mean*	*Total blank value*
Reagent/beaker	0.009–0.017	0.013	—
Process	0.008–0.012	0.010	0.023
Vegetable			
Pea	0.000–0.007	0.002	0.025
Carrot	0.001–0.009	0.005	0.028
Br. sprout	0.000–0.007	0.003	0.026
Gr. bean	0.001–0.003	0.001	0.024
Br. bean	0.000–0.003	0.002	0.025
Spinach	0.000–0.004	0.001	0.024
Mean vegetable blank	0.000–0.009	0.002	0.025

Recoveries of insecticides were determined through the oxidation/determinative stages and through the complete procedure. Levels of addition were chosen to correspond to concentrations equivalent to approximately 0.2 p.p.m. in vegetables. As shown in Table V, recoveries through the oxidation/determinative stages agree well with the purities of the standards where quoted. Recoveries through the complete procedure were also generally satisfactory. The lower recoveries for formothion, dimethoxon, thiometon and naled were shown to be due to significant volatilisation losses (up to 13 % for formothion), which occurred during Kuderna–Danish evaporation of extracts prior to column chromatography. The lower recovery of trichlorphon was due to a loss of approximately 7 % occurring at the partition stage in the process.

TABLE V

RECOVERY OF INSECTICIDES THROUGH OXIDATION/DETERMINATION AND THE COMPLETE PROCEDURE

Insecticide	*Quoted purity of standard* (%)	*Recovery through oxidation/ determination* (%)	*Recovery through complete process* (%)
Demeton	97	98	94
Demeton-S-methyl	96	96	88
Thiometon	92	97	82
Methyl-demeton-methyl	69	91	91
Oxydemeton-methyl	not quoted	89	92
Dimethoate	99	93	90
Formothion	at least 95	98	78
Mevinphos	not quoted	100	93
Phosphamidon	'pure'	98	90
Dimethoxon	not quoted	86	75
Malaoxon	96	98	98
Phorate-O-analogue	not quoted	65	88
Phorate-O-sulphoxide	not quoted	46	90
Dichlorvos	'pure'	95	92
Naled	'pure'	98	86
Trichlorphon	99	100	84

ANALYTICAL PROCEDURE

Reagents

The following reagents were applied:

Chloroform, reagent grade. Redistil before use.

10 % acetic acid. Dilute 100 ml glacial acetic acid, A.R. grade, to 1 l with distilled water.

Sodium sulphate, A.R. grade, granular, anhydrous.

Alumina, neutral, activity V. Deactivate activity II alumina, 'Peter Spence' Type H, 100–200 mesh, by the addition of 10.5 ml water and 1.0 ml glacial acetic acid to each 88.5 g alumina contained in a suitable flask. Stopper the flask tightly and shake until no visible lumps remain and the exothermic reaction has ceased. Equilibrate for 24 h in the closed flask before use.

Extraction

Mince and well mix a bulk sample of vegetables. Macerate a representative 75-g sub-sample for 2–3 min in a 500-ml beaker with 200 ml distilled water containing 7 ml of 10 % acetic acid solution, using a top-drive macerator. Transfer the bulk of the macerate to a 250-ml centrifuge cup and centrifuge briefly at 2400 r.p.m. to pack down suspended vegetable tissue. Immediately filter the supernatant liquor with suction through a Whatman No. 541 paper on a buchner funnel. Transfer 100 ml filtered extract to a 500-ml separating funnel and extract successively with 1 × 200 ml and 2 × 100 ml chloroform. Combine the three chloroform extracts and evaporate to between 5 and 10 ml in a Kuderna–Danish evaporator fitted with a 10-ml graduated collector tube. Remove the collector tube and further reduce the volume 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 neutralised activity V alumina in a little chloroform 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 chloroform and finally adjust the solvent level to the top of the salt layer. Transfer the concentrated extract to the column with the minimum of chloroform rinsing (combined volume < 5 ml), allowing the chloroform level to fall to the top of the salt layer between additions, and discard the eluate. Replace the receiver under the column with a collector tube calibrated to 35 ml. Elute insecticides from the column with chloroform and collect the first 35 ml eluate. Concentrate the eluate to 5 ml in a water bath at 70° under a stream of nitrogen or air.

Determination of phosphorus

Full details of the reagents and a procedure for the oxidation and colorimetric determination of phosphorus in the concentrated eluate have previously been reported[1].

Calculation of results

For screening purposes a gross optical density of 0.050 units in the final extract indicates that, depending on the insecticide, 0.05–0.1 p.p.m. residues is present in the original vegetable. Phosphorus contents of extracts can be determined from the standard graph after subtraction of the total blank value. 0.035 × the phosphorus content gives the p.p.m. pesticide phosphorus in the original vegetable. Approximate concentration of insecticide in vegetables can be calculated using the conversion factors listed in Table VII, provided the history of a crop is known. Such values are however imprecise due to variable production of metabolites.

RESULTS AND DISCUSSION

Aliquots of standard insecticide solutions were added to minced vegetables to establish recoveries through the procedure. Levels of addition were chosen to cover the range of concentrations over which a screening method would have to operate and produce significant results. The results in Table VI show that recoveries tended to

TABLE VI

RECOVERIES FROM VEGETABLES

Insecticide	*Vegetable*	*Level of addition (p.p.m.)*	*Recovery (%)*	*Optical density of extract*
Demeton	Carrots	0.17	78	0.101
	Br. sprouts	0.11	71	0.067
Demeton-S-methyl	Br. sprouts	0.20	83	0.117
	Peas	0.10	77	0.065
Thiometon	Peas	0.35	45	0.108
	Carrots	0.28	38	0.087
	Br. Sprouts	0.21	34	0.060
	Peas	0.14	18	0.039
Methyl-demeton-methyl	Spinach	0.45	70	0.205
	Br. sprouts	0.09	57	0.059
Oxydemeton-methyl	Peas	0.18	90	0.102
	Carrots	0.10	100	0.079
Dimethoate	Carrots	0.16	83	0.112
	Br. sprouts	0.08	80	0.062
Formothion	Gr. beans	0.19	74	0.083
	Spinach	0.10	63	0.055
Mevinphos	Peas	0.22	86	0.131
	Spinach	0.11	70	0.071
Phosphamidon	Peas	0.18	99	0.115
	Carrots	0.09	70	0.060
Dimethoxon	Carrots	0.17	79	0.115
	Spinach	0.08	68	0.059
Malaoxon	Peas	0.16	85	0.093
	Spinach	0.10	71	0.056
Phorate-O-analogue	Peas	0.14	91	0.087
	Br. sprouts	0.07	82	0.056
Phorate-O-sulphoxide	Spinach	0.20	82	0.113
	Peas	0.14	95	0.090
Dichlorvos	Br. sprouts	0.17	71	0.098
	Spinach	0.08	64	0.061
Naled	Carrots	0.20	72	0.086
	Peas	0.15	71	0.060
	Spinach	0.11	64	0.051
Trichlorphon	Peas	0.19	65	0.090
	Spinach	0.12	65	0.065

decrease as the level of insecticide added was reduced; recoveries of at least 60 % were obtained at the 0.1 p.p.m. level for all insecticides except thiometon. Significant losses of this insecticide occurred at all the levels studied but a satisfactory screening limit of 0.2 p.p.m. was obtained.

The number of positive results, *i.e.* optical densities of 0.050 in the final extracts, obtained in the recovery experiments confirms the suitability of the procedure for screening crops at the 0.1 p.p.m. level (0.2 p.p.m. for thiometon). Because of the different phosphorus contents of the insecticides investigated their responses in the

'ABLE VII

NSECTICIDE DATA

nsecticide	*Molecular weight*	*Conversion factor*	*Data relating to 0.1 p.p.m. insecticide in vegetables*			*Concentration equivalent to an O.D. of 0.050 in extracts*
			Phosphorus content of 100-ml extract (μg)	*Theoretical nett O.D.*	*Actual nett O.D. corrected for process loss*	
Jaled	381	12.3	0.23	0.035	0.024	0.1
Jalaoxon	314	10.1	0.29	0.043	0.031	0.08
Phosphamidon	299.5	9.67	0.30	0.045	0.035	0.07
Phorate-O-sulphoxide	260	8.39	0.34	0.051	0.041	0.06
Demeton	258	8.33	0.35	0.052	0.037	0.07
Trichlorphon	257.5	8.31	0.35	0.052	0.035	0.07
Formothion	257	8.3	0.35	0.052	0.031	0.08
Oxydemeton-methyl	246	7.94	0.36	0.054	0.041	0.06
Thiometon	246	7.94	0.36	0.054	(0.008)	0.2
Phorate-O-analogue	244	7.88	0.37	0.056	0.045	0.06
Demeton-S-methyl	230	7.42	0.39	0.058	0.044	0.06
Dimethoate	229	7.4	0.39	0.058	0.046	0.05
Mevinphos	224	7.23	0.40	0.060	0.042	0.06
Dichlorvos	221	7.14	0.40	0.060	0.040	0.06
Methyl-demeton-methyl	214	6.91	0.42	0.063	0.037	0.07
Dimethoxon	213	6.87	0.42	0.063	0.044	0.06

screening test varied. The final optical density was also affected by different recoveries through the process. Allowing for these variations screening limits ranged from 0.05–0.1 p.p.m. depending on the particular insecticide as shown in Table VII. At this sensitivity the screening procedure is capable of detecting residues of fourteen insecticides/metabolites at or below the stringent levels specified in the regulations of certain Western European countries. Phorate analogues, which are not mentioned in the West German regulations and have a 'zero' tolerance in the Dutch regulations, cannot be detected below the 0.06 p.p.m. level.

Low blank values and meaningful results can only be obtained if the care and hygiene associated with micro-analytical techniques are adhered to. Total blank values using insecticide-free vegetables must be determined through the apparatus until a constant and low value is obtained — if none are available process blanks will provide sufficiently accurate information. During the present study the total blank value fell from an initial level of 0.050 to 0.025 O.D. units. This reduction was almost entirely due to a reduction in the reagent/beaker blank value from 0.035 to 0.013 O.D. units. The exact reason for this reduction is not clear but is associated with the type and make of 25-ml beakers used. It was also necessary to distil chloroform before use because variable amounts of impurity in some batches of this reagent resulted in very high process blank values.

The screening procedure has been successfully used to determine residues in crops of both known and unknown history. Some difficulties have been experienced with certain overmature fresh vegetables, notably peas. To facilitate preparation of extracts in these instances it has been found advantageous to work with a smaller sample weight, *e.g.* 50 g, and take a correspondingly larger volume of macerate for

extraction. To prevent emulsions during the partitioning stage it was occasionally necessary to increase the volumes of chloroform to 200 ml in the second and final extractions.

Because of the wide range of residue limits imposed for different insecticides it is important that residues detected in the screen should be confirmed and identified by an alternative technique. GLC analysis with a thermionic detector, although limited by the poor response of certain metabolites and the ease with which breakdown of certain insecticides/metabolites can occur on the column, has been found to be useful in this connection. In a limited study using an OV-17 column packing under the conditions previously reported[1], satisfactory chromatograms were obtained for all the compounds investigated although individual responses varied widely as shown in Table VIII. Replication of response was generally satisfactory but the variable response from some insecticides/metabolites allowed only semi-quantitative interpretation to $\pm 50\%$. It was difficult to transfer insecticides/metabolites quantitatively from chloroform into a suitable solvent for GLC analysis. Large losses could occur if the chloroform eluate was allowed to go to dryness or near dryness. Satisfactory recoveries were obtained when 5 ml chloroform eluate was carefully evaporated to 1 ml on a water-bath at 70° under a stream of nitrogen, 4 ml methanol added and the extract re-evaporated to 1 ml. Using this technique satisfactory confirmation of dichlorvos, naled and trichlorphon has been demonstrated at the 0.2 p.p.m. level.

TABLE VIII

RELATIVE RETENTION AND SENSITIVITY DATA ON OV-17 COLUMN

Insecticide	*Number of peaks produced*	*Relative retention*[a] *of major peak*	*Response*[b] *(cm/0.5 ng injected)*
Column temp. 225°			
Demeton	1	1.21	10
Demeton-S-methyl	1	0.95	9
Thiometon	3	1.21	25
Methyl-demeton-methyl	3	0.74	30
Oxydemeton-methyl		merged with solvent	
Dimethoate	1	1.8	3
Formothion	3	2.55	13
Mevinphos	1	0.4	16
Phosphamidon	2	2.35	3
Dimethoxon	2	1.26	1 cm/3 ng
Malaoxon	1	2.55	1.5
Phorate-O-analogue	1	0.8	18
Phorate-O-sulphoxide	2	2.9	0.5 cm/4 ng
Dichlorvos		merged with solvent	
Naled	2	1.0	22
Trichlorphon		merged with solvent	
Column temp. 175°			
Oxydemeton-methyl	1	0.15	2 cm/2 ng
Mevinphos	1	0.45	1.5
Dichlorvos	1	0.18	25
Naled	2	1.0	1
Trichlorphon	1	0.18	2

[a] Relative to phorate; 2.0 min at 225°, 11.0 min at 175°.
[b] Attenuation setting 1 × 8 (2.4×10^{-9} A full scale).

TABLE IX

COMPARISON OF EXTRACTION TECHNIQUES

Insecticide	*Addition (p.p.m.)*	*Replicates*	*Recovery (%)*	
			Aqueous extract	*Acetone extract*
Dimethoate	0.2	6	range 76–86 mean 81	77–86 85
Dimethoxon	0.2	6	range 73–84 mean 78	67–80 72
Phosphamidon	0.1	4	range 75–87 mean 84	67–86 81

We expect that this procedure will be capable of confirming the presence of insecticides/metabolites at the levels detectable in the screen, other than phorate-O-sulphoxide, dimethoxon and oxydemeton-methyl for which detection limits will be in the order of 0.25, 0.2 and 0.1 p.p.m., respectively.

Recently the Joint Dimethoate Residues Panel have recommended a method of analysis for dimethoate residues in foodstuffs[9] based on acetone extraction, transfer of residues to chloroform, clean-up on neutral activity V alumina and total pesticide phosphorus determination. In collaborative studies in up to eight laboratories recoveries of dimethoate and dimethoxon at the 0.5 p.p.m. level averaged 90 % and 66 % respectively. The Panel method necessitates the use of rotary evaporation to remove acetone from extracts prior to transfer of residues into chloroform. As shown in Table IX the much simpler aqueous extraction procedure gives very similar recoveries of insecticides/metabolites from peas (both sets of extracts were analysed using our proposed procedure). The Panel method also employs wet oxidation and colorimetric stages that require constant attention and manipulation. We think that the proposed procedure is less time consuming and far more suitable for routine application to large numbers of samples than that recommended by the Panel.

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