A RAPID AND SENSITIVE PROCEDURE FOR THE ROUTINE DETERMINATION OF ORGANO-CHLORINE PESTICIDE RESIDUES IN VEGETABLES

D. J. SISSONS, G. M. TELLING AND C. D. USHER
Unilever Research Laboratory, Colworth House, Sharnbrook, Beds. (Great Britain)
(Received October 24th, 1967)

SUMMARY

A rapid and sensitive procedure is described for the quantitative determination of residues of organo-chlorine pesticides in a wide range of vegetables, based on an acetone–hexane extraction, clean-up with Nuchar Attaclay or alumina and identification by GLC using an electron-capture detector. These pesticides include the most toxic and/or persistent organo-chlorine compounds and constitute the most likely hazard on commercial crops grown in this country or imported.

The sensitivity of the procedure varies with different pesticides and vegetables but all pesticides can be determined at the 0.01 p.p.m. level and most pesticides in most crops at the 0.002 p.p.m. level. Recoveries of pesticides added to vegetables at the 0.1–0.01 p.p.m. level averaged 90% with a spread of ±10 %. The procedure has been successfully used to identify and determine residues on crops of known history and is being at present used to routinely survey a range of crops of unknown history.

INTRODUCTION

In recent years with the advent of the GLC electron-capture detector and of TLC it has been possible to develop rapid and sensitive procedures for the routine determination of a range of pesticides in a variety of foodstuffs. Very few such methods have, however, been published in the literature. The most rapid of these was developed by GOODWIN *et al.*[1] In its basic form it required 50 min per analysis but due to interference from coextracted material the limit of sensitivity was 0.1 p.p.m. Increased sensitivity, in some instances to 0.01 p.p.m., was claimed after clean-up of extracts by passage through a column of alumina. Other methods, notably those of HAMENCE[2] and MILLS *et al.*[3] for multiple pesticide residue analysis in vegetables, although more sensitive than GOODWIN's, take considerably longer. The MILLS' method was adopted by the Association of Official Analytical Chemists[4] and is at present under investigation by the Federal Drug Administration[5].

We wished to develop a rapid and sensitive procedure for the determination of certain organo-chlorine pesticide residues in a wide range of vegetables. The procedure should be robust and capable of operation by assistant staff with the minimum of training and supervision. The investigation was confined to lindane, dieldrin, DDT, DDE, heptachlor, heptachlor epoxide, endosulfon A and B isomers, aldrin

and endrin. Residues of these insecticides constitute the most likely hazard in home-grown and imported crops.

EXPERIMENTAL

Extraction

The extraction of organo-chlorine pesticide residues has been widely studied and many different solvent systems advocated. Acetonitrile has been widely used as an extracting agent in the U.S.A.[6,7]. Less toxic and cheaper solvents are more frequently used in this country. GOODWIN and HAMENCE both used acetone as extractant but systems composed of a mixture of a water-immiscible and a water-miscible solvent would appear to be most suitable for complete extraction[8,9]. However, such systems are not widely used, possibly because of emulsification problems, and difficulties associated with the removal of a quantitative aliquot for analysis. CASSIL[10] obtained good recoveries of several organo-chlorine pesticides from vegetables extracting with a benzene–isopropanol mixture: using the same extractant, KLEIN[11] demonstrated that macerating was more efficient than either tumbling or Soxhlet extraction. SERGEANT[12] used acetone–light petroleum to extract several organo-chlorine pesticides from certain vegetables, and MATTICK *et al.*[13] used ethanol–hexane to extract endrin from cabbage. These mixed solvents gave recoveries of at least 70 % for the particular pesticides studied.

SERGEANT obtained low recoveries of organo-chlorine pesticides from plant material using light petroleum as extractant. We have found similar low recoveries of DDT (45–55 %) from peas at the 0.05 p.p.m. level using GOODWIN's method. This loss of DDT is due to absorption on to the vegetable tissue: additional extraction of the pea residues recovered a further 20–30 % of pesticide. Recoveries of 90–95 % of DDT from peas were obtained by CASSIL's procedure. Because of the toxicity of benzene we investigated mixtures of other water-miscible and -immiscible solvents and the acetone–hexane system was chosen for further study.

We found it possible to macerate a range of vegetables with acetone–hexane mixtures with no emulsification problems and always obtained a clean separation into two layers. Complete removal of acetone from the resulting upper hexane layer without loss of pesticide was obtained by shaking with 2 % aqueous sodium sulphate solution. It was also demonstrated that after maceration pesticides were evenly distributed throughout the hexane, whether the hexane was present in the upper layer or dissolved in the lower aqueous acetone layer. DDT (0.2 p.p.m.) was added to replicate 50 g samples of pesticide-free peas. Each sample was extracted with 50 ml acetone and a volume of hexane varying from 50 ml to 300 ml. Fig. 1 shows that the concentration of pesticide extracted into the hexane in the upper layer is constant and independent of the volume of hexane recovered after maceration. This fact is important in considerably reducing the time of the extraction stage; an aliquot of hexane removed at this stage for subsequent clean-up is representative of the whole hexane extract and complete extraction of pesticide into the upper layer by time-consuming re-extraction of the vegetable residues is unnecessary. In order to facilitate the extraction of pesticides into the upper layer the ratio of hexane: acetone was fixed at 4:1 at which level over 90 % of both hexane and pesticides were recovered.

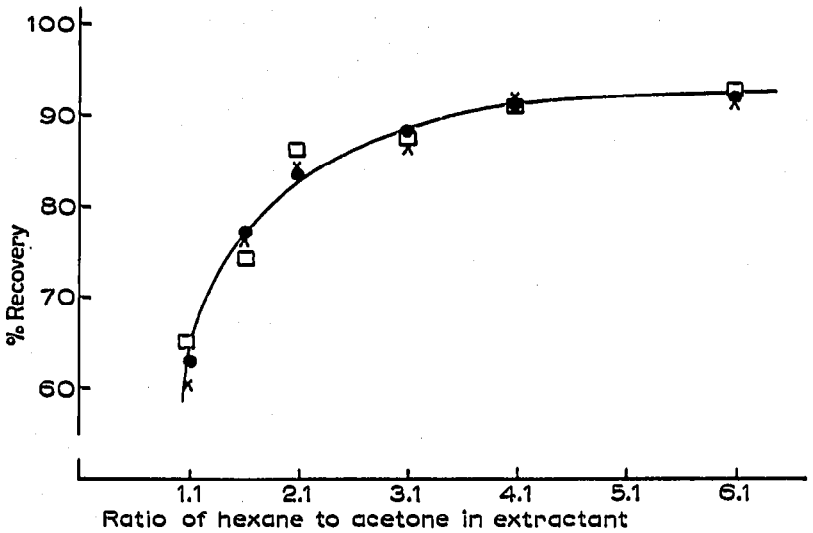

Fig. 1. Recovery of hexane (—●—●—), *o,p'*-DDT (—□—□—) and *p,p'*-DDT (—×—×—) in the upper layer after maceration with increasing ratios of hexane: acetone as extractant.

Clean-up

The degree of clean-up necessary depends on the vegetable under examination, the solvent used and the final method of determination. A simple partitioning as used by GOODWIN is sufficient for relatively high residue levels in some crops but for sub-p.p.m. levels a more rigorous clean-up is required. The extract is usually chromatographed on a column of adsorbent material such as florisil[6,7], magnesium oxide[5], aluminium oxide[1,2] etc., either singly or mixed. This treatment results in dilution of the extract, which must then be re-concentrated by evaporation. When large volumes are required for elution the process is time-consuming and has the added hazard that solvent impurities which are also concentrated may seriously interfere with subsequent analysis. GOODWIN and HAMENCE considerably reduced the time required for adsorbent clean-up by using small columns of alumina (2–5 g) eluted with small volumes of solvent. CASSIL used an alternative but not widely used adsorbent, Nuchar Attaclay. This mixed activated carbon and attapulgus clay was shaken for 30 sec with a benzene extract of the vegetable and filtered off. The process was not claimed to be as efficient as, for example, a florisil clean-up but its speed and simplicity would make it ideally suited to a routine procedure.

Nuchar Attaclay. We have used CASSIL's method and obtained 90–95 % recoveries of pesticide from peas. As less toxic alternatives to benzene in this method we investigated toluene, chloroform, hexane, diethyl ether and ethanol but only from toluene were acceptable recoveries obtained. It was also shown that the presence of up to 50 % of hexane in the toluene did not adversely affect recovery. Therefore evaporation of hexane extracts to dryness before addition of toluene was unnecessary, and both the time taken and the risk of loss of volatile pesticides were reduced. With the exception of endosulfan B, which gave an average recovery of 75 %, recoveries of 92–107 % were obtained for pesticides added to green beans at the 1.0 to 0.01 p.p.m. levels. However, for other vegetables this clean-up procedure showed considerable variation in efficiency. On peas, broad beans, turnips and green beans accurate determination of residues at the 0.01 p.p.m. level and lower was possible. In most of the remaining vegetable extracts however there was some residual inter-

J. Chromatog., 33 (1968) 435–449

TABLE I

ELUTION OF PESTICIDES FROM AN ACTIVITY V ALUMINA COLUMN

Eluting agent	*Fraction (5 ml)*	*Distribution of pesticides (%)*									
		Lindane	*Dieldrin*	*DDT*		*Heptachlor*	*Heptachlor epoxide*	*Endosulfan*		*Aldrin*	*Endrin*
				o,p'	*p,p'*			*A*	*B*		
Hexane	1	—	—	68	52	47	—	—	—	—	72
	2	77	1	28	43	53	—	15	—	17	28
	3	22	22	4	5	—	77	82	—	69	—
	4	1	67	—	—	—	23	3	—	14	—
	5	—	10	—	—	—	—	—	—	—	—
	6	—	—	—	—	—	—	—	—	—	—
2% Acetone in hexane	1–3	—	—	—	—	—	—	—	—	—	—
	4	—	—	—	—	—	—	—	29	—	—
	5	—	—	—	—	—	—	—	69	—	—
	6	—	—	—	—	—	—	—	2	—	—

ference but accurate determination at the 0.05 p.p.m. level was still possible. The level of this unremovable co-extracted material increased with increasing vegetable maturity and in carrots, parsnips, leeks and onions accurate residue analysis at the 0.5 p.p.m. level ultimately became difficult.

Alumina. GOODWIN used a column of 5 g alumina, Brockmann grade I–II, to clean-up extracts and eluted pesticides with 1 % acetone in hexane. We have investigated aluminas of various activity and found that many pesticides, notably *p,p'*-DDT, decomposed on the more active grades (I–III). We found a much better combination was activity V alumina with pure hexane, which gave cleaner extracts and enabled us to work with smaller quantities of pesticides. The optimum conditions determined for clean-up on activity V alumina were 8 g adsorbent eluted with 30 ml hexane: endosulfan B required a further 30 ml 2 % acetone in hexane for elution. Although numerous naturally-occurring compounds were eluted by the polar solvent from some vegetable extracts, no interference in the analyses of endosulfan B resulted. By applying pressure to the column complete elution of all pesticides was achieved within 7 min (14 min for endosulfan B).

The order of elution of pesticides from the alumina column is given in Table I and the recoveries obtained, in Table II. At least two replicates of each pesticide were taken through the process at each level quoted in the table. These levels were chosen to correspond to likely concentrations in vegetables; for example, 0.2–0.0016 p.p.m. lindane and 1.0–0.02 p.p.m. DDT. Using the same procedure clean extracts were obtained from a wide range of vegetables permitting the determination of residues at the 0.01 p.p.m. level and below.

Alumina + fuller's earth. The difficulties experienced previously with some mature crops, *viz.*, carrots, parsnips, leeks and onions, were reduced but not eliminated by alumina treatment. However a column of activity V alumina incorporating a little fuller's earth mixed in the ratio of approx. 15:1 removed almost all of the interferences found in these vegetables: accurate determinations to the 0.01 p.p.m. level and lower were then possible. The mixed adsorbent was effective in retaining β-carotene, and colourless extracts were obtained from all vegetables studied including mature carrots—the presence of carotenes in some extracts eluted from alumina alone did not appear to interfere with GLC analysis. Endrin was only partially eluted from the mixed column at low concentration; about 0.4 μg being irreversibly adsorbed. This caused significant errors in crops containing 0.2 p.p.m. or less of endrin. Endrin however could be quantitatively determined in eluates from the alumina column. The relative efficiencies of the three different clean-up procedures examined above are illustrated in Fig. 2 which shows gas–liquid chromatograms obtained from extracts of mature carrots after clean-up.

Determination of pesticides

Because of its extreme sensitivity, GLC analysis with an electron-capture detector is the preferred method of determination of organo-chlorine pesticides. Retention data obtained from a single GLC column are however inconclusive evidence of identification of pesticides and in this study we have used retention data derived from more than one stationary phase to confirm identification. To facilitate this identification a dual column Aerograph Model 1520 instrument fitted with dual concentric-tube detectors operated in the DC mode was used.

TABLE II

RECOVERY OF PESTICIDES AFTER TREATMENT WITH ALUMINA

Pesticide	*Lindane*	*Dieldrin*	*DDT*		*Heptachlor*	*Heptachlor epoxide*	*Endosulfan*		*Aldrin*	*Endrin*
			o,p'	*p,p'*			*A*	*B*		
Level of application (μg)	0.2	1.0	5.0	5.0	0.4	0.8	1.0	1.2	0.4	5.0
Mean recovery (%)	97	106	100	96	99	97	99	100	106	97
Level of application (μg)	0.008	0.02	0.1	0.1	0.04	0.08	0.1	0.1	0.04	0.1
Mean recovery (%)	105	98	97	100	96	101	97	99	98	103

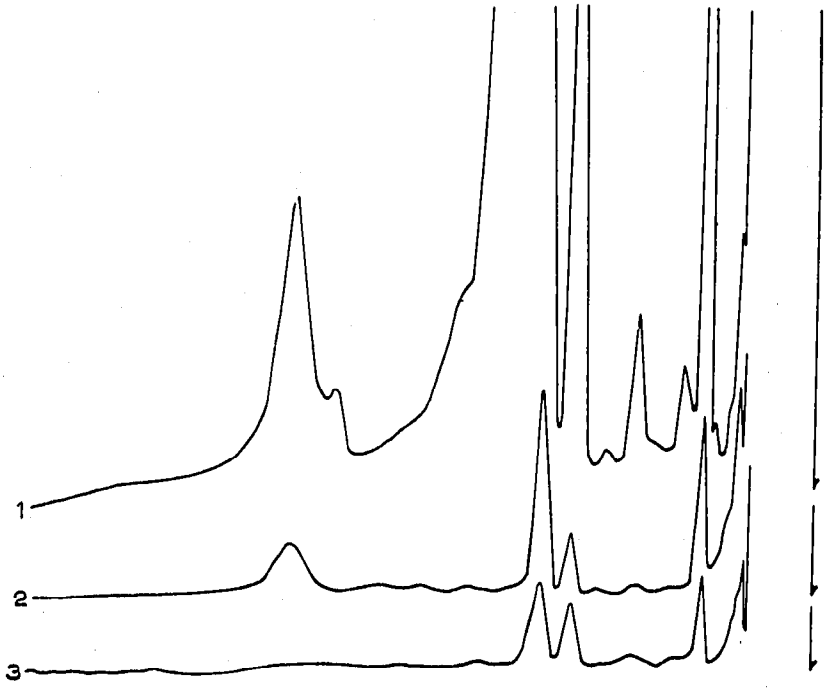

Fig. 2. Chromatograms of carrot extracts after clean-up on (1) Nuchar Attaclay; (2) alumina column; (3) alumina + fuller's earth column.

(*a*) *Column packings.* Numerous supports and stationary phases ranging in polarity from silicone grease to polyethylene glycols have been investigated. Silanization of supports was found to be essential and acid-washed DMCS treated Chromosorb G has been used throughout. The three stationary phases found most useful for routine application were, those most widely used by other workers: SE 30 methylsilicone gum rubber and QF1 fluorosilicone gum, and the more recently introduced XE 60 cyanosilicone gum. Incorporation of a small amount of Epikote resin with each stationary phase helped to reduce the activity of the support. The SE 30 column gave the best resolution of pesticides (see Table III) and as it also had by far the longest useful life, being relatively unaffected by extraneous material present in extracts, it was used for primary analysis. The more polar QF1 and XE 60 columns lasted for shorter periods and gave poorer resolution of pesticides. However both were useful as confirmatory columns and in the quantitative determination of aldrin and, in some less clean extracts, lindane, heptachlor and heptachlor epoxide. Aldrin could not be determined in the SE 30 column because varying amounts of dibutyl phthalate, which has a similar retention time, are present in extracts. This wide-spread contaminant derives from polyvinyl acetate based materials and is virtually impossible to remove[14]. Other compounds derived from certain vegetables and the solvents used can also interfere with the low-level determination of the more-volatile pesticides on the SE column. Elution of these compounds is more rapid from polar columns, resulting in less interference. The peak for dibutyl phthalate on the QF1 column coincides with that for DDE, but on the XE 60 column this compound does not interfere with any of the pesticides under consideration.

(*b*) *Retention data.* Retention times relative to that of aldrin of the various

pesticides on the three stationary phases are given in Table III. The data were obtained using the conditions given in the analytical procedure. These conditions were not the optimum for column efficiencies which were between 150 and 200 plates/ft. Considerable improvement in column efficiencies, up to 400 plates/ft. could be obtained at higher temperatures but breakdown of some pesticides occurred under these conditions. The long retention time and low sensitivity of endrin on QF1 made this column unsuitable for confirmation of low levels of this pesticide.

TABLE III

RETENTION DATA FOR THREE TYPES OF STATIONARY PHASE

Pesticide	*Stationary phase*		
	SE 30	*QF 1*	*XE 60*
Lindane	0.44	0.85	1.32
Heptachlor	0.78	0.9	0.95
Aldrin	1.00 (5′)	1.00 (4′)	1.00 ($2^1/_2$′)
Heptachlor epoxide	1.30	1.90	2.33
Endosulfan A	1.67	2.59	2.60
Dieldrin	2.01	3.10	3.60
p,p′-DDE	2.15	2.06	2.97
Endrin (major peak)	2.27	>6	4.05
Endosulfan B	2.33	4.77	7.65
o,p′-DDT	2.89	2.72	4.03
p,p′-DDT	3.74	4.33	7.13

Sensitivity and linear dynamic range

Because of the characteristics of the particular electron-capture detector used it was essential to determine the linear response range for each individual pesticide. As shown in Table IV the response was only proportional to concentration for very small amounts of pesticide. Deviation from linearity began above concentrations equivalent to 0.1 p.p.m. lindane and 1.0 p.p.m. DDT in vegetables and it was important to dilute extracts to below these concentrations for analysis.

TABLE IV

LINEAR DYNAMIC RANGE OF PESTICIDES

Pesticide	*Detection limit* (ng)*	*Linear response range (ng)*	
		min. studied	*max.*
Lindane	0.0007	0.003	0.5
Aldrin	0.0013	0.005	0.75
Heptachlor	0.0012	0.005	0.6
Heptachlor epoxide	0.0015	0.006	0.7
Dieldrin	0.002	0.008	2.5
Endosulfan A	0.002	0.008	0.7
Endosulfan B	0.0025	0.01	1.0
p,p′-DDE	0.003	0.01	1.5
Endrin	0.015	0.05	3.5
o,p′-DDT	0.015	0.05	5.0
p,p′-DDT	0.015	0.05	5.0

* Equivalent to a peak of height 2 % fsd on attenuation setting 1 × 4 (1.2×10^{-9} A full scale).

Analytical procedure

(*a*) *Reagents*

Hexane. Hexane fraction, laboratory reagent grade. Redistil from potassium hydroxide pellets (4 g/l) within a day or so of use and store in well stoppered vessels. This step has been found necessary due to the variable level of impurities present in different batches of this reagent.

Acetone. AR grade.

Toluene. Laboratory reagent grade, sulphur free. Shake with tap water to saturate before use.

Sodium sulphate. AR grade granular, anhydrous; and 2% w/v aqueous solution.

Fuller's earth. For adsorption purposes BDH.

Nuchar Attaclay. Varian Aerograph.

Alumina. Activity V grade. Activate alumina trihydrate (British Aluminium Company) by heating at 525–675° for a minimum of 4 h, cool and add 12 ml of water per 100 g of adsorbent in a stoppered flask. Shake until no visible lumps remain and the exothermic reaction has ceased. Equilibrate for a minimum of 24 h in the closed flask at ambient temperature. A convenient but more expensive alternative is neutral aluminium oxide (M. Woelm, Eschwegen, Germany) supplied ready activated to grade I. Deactivate by the addition of 15 % water and equilibrate as above.

All apparatus must be scrupulously clean and rinsed with acetone and hexane before use. Working surfaces must be wax and polish free and the use of PVC or polythene avoided. Time of extraction and clean-up should be kept to a minimum.

(*b*) *Extraction*

Macerate a representative 50 g sample of vegetable with 50 ml acetone and 200 ml hexane in a top-drive macerator for 2–3 min. 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 a 50 ml aliquot of the upper hexane–acetone layer to a 1 l separating funnel and wash once with 500 ml sodium sulphate solution. Run the lower aqueous acetone phase to waste with a little of the hexane layer. Transfer a 20 ml aliquot of the hexane layer into a Kuderna–Danish evaporator fitted with a 10 ml graduated tube, ensuring no water is present in the aliquot. Add a few crystals of anhydrous sodium sulphate and evaporate the contents to a few ml taking care that the solution is not evaporated to dryness. Disconnect the graduated tube and continue the evaporation to between 1 and 2 ml on a steam bath under a stream of nitrogen. (Alternatively carry out the complete evaporation in a 25 ml tube on a steam bath under a stream of nitrogen.)

(*c*) *Clean-up procedures*

(i) Add at least 2.5 ml of water-saturated toluene to the concentrated hexane extract prepared above to bring the final volume to 5.0 ml. Add 0.5 g of Nuchar Attaclay, stopper the tube and shake the contents for 30 sec. Filter the extract through a No. 42 paper into a tube containing a little anhydrous sodium sulphate. Stopper the tube immediately and reserve for GLC analysis.

(ii) Prepare a slurry of 8 g Brockmann grade V alumina in a little hexane and transfer to a clean chromatographic column (30 × 1 cm) with a rapid swirling motion. Wash the column with 3 × 5 ml aliquots of hexane and adjust the hexane level to just above the surface of the adsorbent. Transfer the 1–2 ml concentrated hexane extract to the column with the minimum of hexane rinsing. Elute under slight pres-

sure with 6 × 5 ml aliquots of hexane at the rate of 4–5 ml/min and collect the total eluate. Change the receiver and elute the column with 6 × 5 ml aliquots of 2 % v/v acetone in hexane, again collecting the total eluate. Evaporate each eluate in a Kuderna–Danish evaporator, and adjust the final volume to 5.0 ml. Stopper the tube immediately and reserve for GLC analysis.

(iii) Prepare the mixed fuller's earth–alumina column as in section (ii) above with the addition of 0.5 g fuller's earth to the 8 g of alumina slurried in hexane. The order of elution of pesticides from this column is the same as from the alumina column.

(d) GLC analysis

The operating conditions found most satisfactory for pesticide analysis on the Aerograph 1520 instrument were as follows:

Columns: 1/8 in. × 5 ft. stainless steel with Pyrex glass insert.

Column packings: (A) 2.5 % SE 30; (B) 1.0 % QF 1; (C) 1.5 % XE 60. All + 0.01 % Epikote resin on AW-DMCS treated, 80–100 mesh Chromosorb G.

Column temperature: 185°.

Detector temperature: 200°.

Injector temperature: 200°.

Nitrogen flow: 40–60 ml/min.

Sensitivity: Attenuation settings 1 × 4 or 1 × 8.

It is essential to inject a standard mixture of pesticides at intervals during a series of analyses to assess column and detector performance. Suitable standard mixtures and concentrations are conveniently prepared in hexane as follows:

Mixture I: 0.02 p.p.m. of lindane, aldrin, endosulfan A, HEOD (dieldrin); 0.1 p.p.m. of endrin and *p,p'*-DDT.

Mixture II: 0.02 p.p.m. of heptachlor, heptachlor epoxide, endosulfan B, *p,p'*-DDE; 0.1 p.p.m. *o,p'*-DDT.

To identify a particular pesticide, chromatograms of an extract must be obtained on at least two columns and compared with the chromatogram of the standard pesticide mixture run closest in time. Positive identification can only be made if peaks have the correct retention times on each column. The concentration of residues is conveniently and accurately determined by peak height measurement. As the concentration of pesticide in the final aliquot of extract injected is approximately the same as that in the original vegetable, provided the same volumes of extract and standard are injected (5 μl) residue levels can be simply and readily determined by direct comparison of peak height measurements. If the amounts determined vary significantly between columns the lowest concentration must be taken as the more accurate result.

RESULTS AND DISCUSSION

Analysis of pesticide-free crops

It was necessary to determine the background levels of naturally-occurring co-extracted material that could be confused with pesticides on chromatographic analysis. For this purpose selected crops were grown in the greenhouse in the complete absence of pesticide and analysed by the above procedure. The results showed that no compounds were present which would interfere with the determination of low levels of most of the pesticides, but that varying interference could occur with heptachlor,

heptachlor epoxide and particularly lindane, which have low retention times on the SE 30 column. The most interfering compound was present in extracts of carrot and parsnips in the position of lindane and gave a peak of maximum height equivalent to 0.008 p.p.m. On the more polar stationary phases however the two peaks were resolved and no significant interference was encountered in determining 0.002 p.p.m. lindane. Varying levels of interference from dibutyl phthalate occurred in every extract. These results stress the importance of confirmatory analysis on other stationary phases for residues at low levels. Chromatograms of extracts from pesticide-free peas and carrots are shown in Figs. 3 and 4 (chromatograms 1).

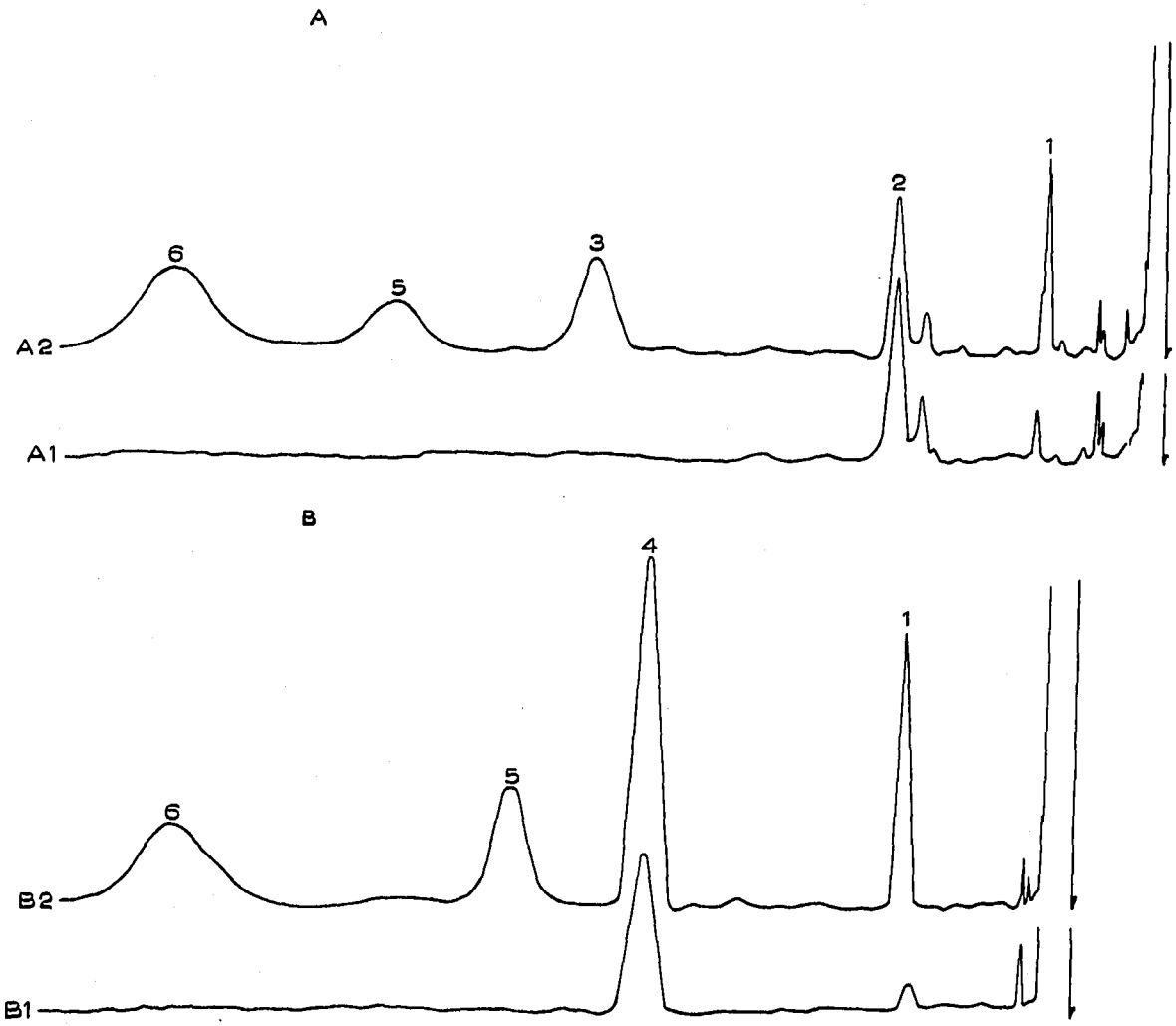

Fig. 3. Extracts of pesticide-free (1) and sprayed peas (2) analysed on an SE 30 (A) and a QF 1 (B) column. Peak 1 = lindane (0.003 p.p.m.); peak 2 = major impurity peak (dibutyl phthalate); peak 3 = *p,p'*-DDE (0.005 p.p.m.); peak 4 = combined *p,p'*-DDE + major impurity peak; peak 5 = *o,p'*-DDT (0.01 p.p.m.); peak 6 = *p,p'*-DDT (0.02 p.p.m.).

Recovery experiments

Known quantities of pesticides were added to minced vegetables which were then macerated and taken through the analytical procedure. The typical recoveries given in Table V demonstrate the satisfactory and reproducible recovery of low levels of pesticides from vegetables after clean-up by the recommended procedures. With

TABLE V

RECOVERIES OF PESTICIDES ADDED TO VEGETABLES

Vegetable	*Type of clean-up*	*Recovery of pesticide* (%)										
		Lindane		*Dieldrin*	*DDT*		*Heptachlor*	*Heptachlor epoxide*	*Endosulfan*		*Aldrin*	*Endrin*
		(*i*)	(*ii*)		*o,p'*	*p,p'*			*A*	*B*		
Brussels sprout	Alumina	93	96	102	103	97	96	92	99	102	95	85
Carrot (mature)	Alumina	122	300	98	95	92	96	93	92	105	86	85
Carrot (mature)	Alumina + fuller's E	100	93	95	97	89	96	97	98	107	75	12
Cauliflower	Alumina	97	110	97	106	99	92	98	96	108	92	88
Leek (mature)	Alumina	275	—	95	96	92	92	103	94	95	80	95
Leek (mature)	Alumina + fuller's E	95	91	97	95	89	85	91	92	100	73	18
Celery	Alumina	95	104	100	104	100		not determined				
Parsnip (mature)	Alumina	160	350	107	103	96		not determined				
Parsnip (mature)	Alumina + fuller's E	97	117	97	95	90		not determined				
Green bean	Alumina	97	95	101	98	104	94	102	103	101	98	98
	Nuchar-Attaclay	92	93	98	99	96	106	100	101	73	104	104
Level of addition (p.p.m.)		0.01	0.002	0.01	0.02	0.02	0.01	0.02	0.03	0.03	0.005	0.05

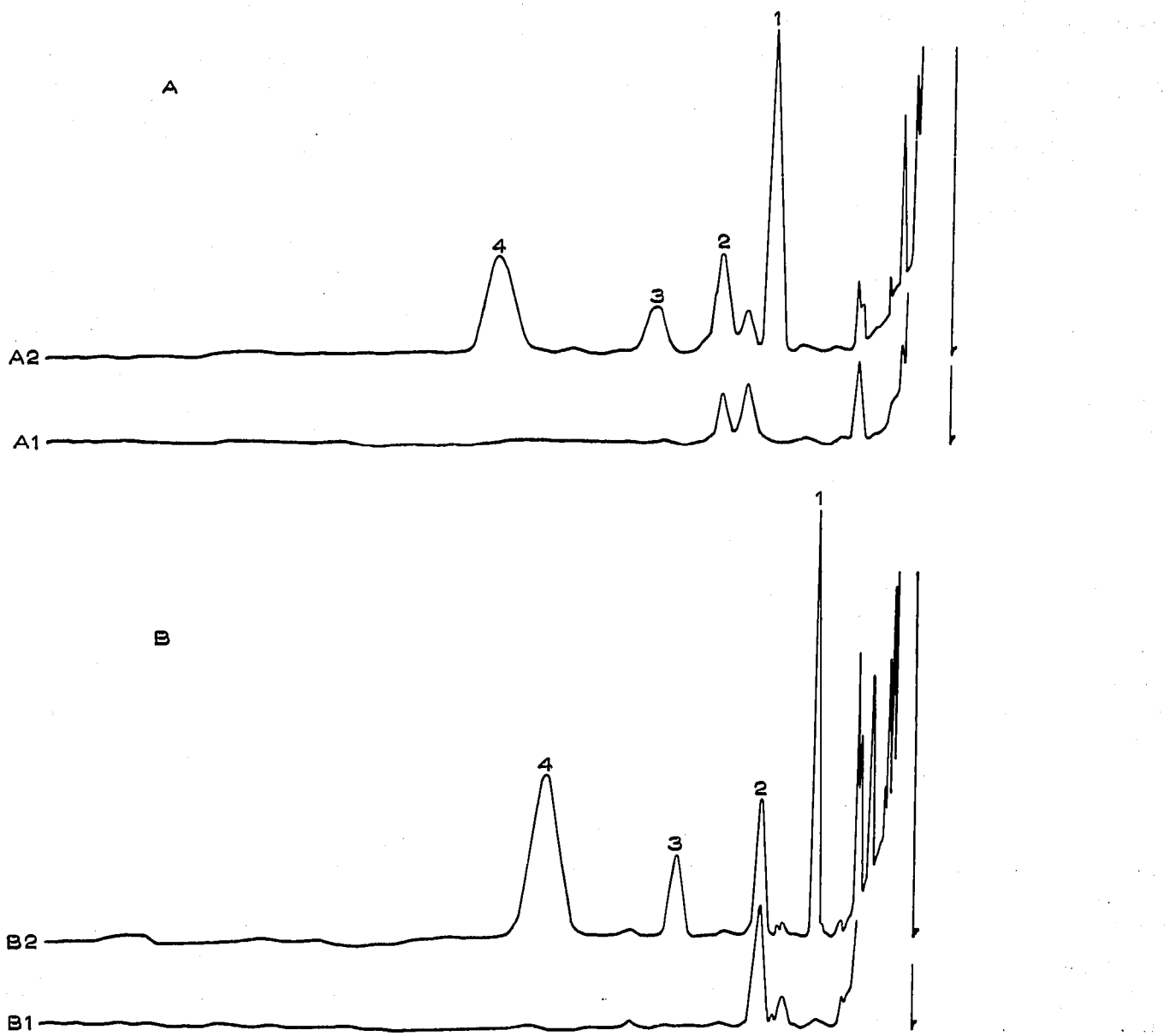

Fig. 4. Extracts of pesticide-free (1) and sprayed carrots (2) analysed on an SE 30 (A) and a XE 60 (B) column. Peak 1 = heptachlor (0.01 p.p.m.); peak 2 = major impurity peak; peak 3 = heptachlor epoxide (0.002 p.p.m.); peak 4 = dieldrin (0.01 p.p.m.).

two exceptions the average recoveries of pesticides were 90 % or better with a maximum spread of ±10 %. The exceptions were aldrin through the alumina column, when recoveries ranged from 70–110 % with a mean recovery of 88 %, and endosulfan B after treatment with Nuchar Attaclay when recoveries ranged from 68–85 % with a mean recovery of 75 %. The ability of fuller's earth to remove significant levels of interference from certain vegetable extracts permitting the low-level determination of lindane, and the consequent losses of endrin are clearly shown. Even after this treatment interference in the determination of lindane at the 0.002 p.p.m. level was sometimes encountered in parsnips, but was always insignificant at the 0.01 p.p.m. level.

Analysis of crops of known and unknown history

A wide range of crops including peas, green beans, broad beans; cabbage, Brussels sprouts, cauliflower; potatoes, carrots, turnips, parsnips; leeks, onions, spring onions; spinach, lettuce; celery, asparagus, sweetcorn, tomatoes, rice and also several different fruits has been analysed using the proposed procedure. No difficulty was experienced in analysing frozen and dehydrated products, and in many instances the processed products gave cleaner extracts than the parent fresh vegetables. Spanish onions have been the only vegetable that was not adequately cleaned up, interference occurring on all three stationary phases. A limited study of the extraction of residues from vegetables has shown that the acetone–hexane system is as efficient an extractant as the acetonitrile system, used by MILLS *et al.*[3], and shown by BURKE AND PORTER[15] to be superior to many other procedures and comparable to exhaustive extraction. Typical chromatograms of extracts of sprayed peas and carrots are shown in Figs. 3 and 4 (chromatograms 2).

General

While using the procedure for routine pesticide surveys of different crops we have been able to determine the rate of analysis. The time required to analyse a single sample is about 50 min using a Nuchar Attaclay clean-up and 75 min using a column clean-up procedure. Of this time the GLC stage takes 15 to 20 min and is the rate determining factor. Using the dual column instrument a team of three assistants can serially extract and analyse between 75 and 100 samples a week depending on the clean-up required. Storage of extracts overnight for analysis the following morning when the instrument is normally idle for the first 1–1$^1/_2$ h can still further increase the overall rate of analysis. No significant loss of pesticide results if extracts are stored overnight in a refrigerator.

Recently we have investigated Aeropak 30, a support material specially prepared from Chromosorb W by Varian Aerograph, as an alternative to Chromosorb G. With a loading of 5 % QF 1 the column efficiency increased to over 450 plates/ft. and pesticide peaks were six times higher than were obtained on the Chromosorb G column under identical conditions. If similar increases in sensitivity can be obtained with other stationary phases on this support then the final concentration stage in the method could be avoided with a saving of time and no loss of sensitivity.

ACKNOWLEDGEMENT

The authors wish to thank the Laboratory of the Government Chemist and Shellstar Ltd. for providing certain of the pesticide standards used in this investigation.

REFERENCES

1 E. S. GOODWIN, R. GOULDEN AND J. G. REYNOLDS, *Analyst*, 86 (1961) 697.
2 J. H. HAMENCE, *Chem. Ind. (London)*, (1966) 1255.
3 P. MILLS, J. H. ONLEY AND R. A. GAITHER, *J. Assoc. Offic. Agr. Chemists*, 46 (1963) 186.
4 Changes in Official Methods of Analyses, *J. Assoc. Offic. Anal. Chemists*, 49 (1966) 222.
5 J. GAUL, *J. Assoc. Offic. Anal. Chemists*, 49 (1966) 463.
6 U.S. FOOD DRUG ADMIN., *Pesticide Analytical Manual*, Vol. 1, 1965.
7 U.S. PUBLIC HEALTH SERVICE, *Guide to the Analysis of Pesticide Residues*, Vol. 1, 1965.

8 W. W. THORNBURG, *Analytical Methods for Pesticides, Plant Growth Regulators and Food Additives*, Vol. 1, Academic Press, New York, London, 1963, p. 90.
9 K. I. BEYNON AND K. E. ELGAR, *Analyst*, 91 (1966) 143.
10 C. C. CASSIL, *Residue Reviews*, Vol. 1, Springer-Verlag, Berlin, 1962.
11 A. K. KLEIN, *J. Assoc. Offic. Agr. Chemists*, 43 (1960) 703.
12 C. A. SERGEANT, *Analyst*, 83 (1958) 335.
13 L. R. MATTICK, D. L. BARRY, F. M. ANTENUCCI AND A. W. AVENS, *J. Agr. Food Chem.*, 11 (1963) 54.
14 D. F. LEE, J. BRITTON, B. JEFFCOAT AND R. F. MITCHELL, *Nature*, 211 (1966) 521.
15 J. A. BURKE AND M. L. PORTER, *J. Assoc. Offic. Anal. Chemists*, 49 (1966) 1157.