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DETERMINATION OF ORGANOCHLORINE INSECTICIDE RESIDUES IN FATTY FOODSTUFFS USING A CLEAN-UP TECHNIQUE BASED ON A SINGLE COLUMN OF ACTIVATED ALUMINA

G. M. TELLING and D. J. SISSONS

Unilever Research Colworth/Welwyn Laboratory, Colworth House, Sharnbrook, Bedford (Great Britain)

and

H. W. BRINKMAN

Unilever Research, Duiven Laboratory, Helhoek 30, Groessen (The Netherlands)

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SUMMARY

A method is described for the extraction and clean-up of fatty foods prior to the determination of organochlorine insecticides and related compounds. Clean-up is based on the use of a single 22-g column of activity-4 alumina and the method is capable of routinely determining cyclodienes, BHC isomers and HCB at the 5–10 μg/kg level and DDT-type compounds at 20–30 μg/kg level.

INTRODUCTION

All organochlorine pesticides are highly lipophilic substances and their estimation and determination in foodstuffs of significant fat content have always presented problems compared with the relatively straight-forward process developed for their determination in vegetables. While food extracts that contain traces of vegetable matter are acceptable for gas chromatographic analysis, traces of fat quickly lead to serious losses in efficiency and sensitivity of the gas chromatographic system owing to both deposition in the injector and on the column and loss of detector performance. Hence quantitative removal of fat from final test solutions is vital and various approaches have been tried: (1) Liquid–liquid partition between solvent pairs such as acetonitrile–light petroleum[1] or dimethylformamide–hexane[2] has the advantage that relatively large (1–5 g) samples of fat can be treated. The disadvantages of these techniques, however, are the potential hazards of solvents, the quantities of solvents and glassware involved and the fact that total removal of fat is not achieved and that a secondary clean-up system based on a column of Forisil[1,3] or alumina[2] is required to remove residual traces of fat. (2) Methods based on a wide range of techniques varying from saponification[4], and freezing-out[5] to sweep codistillation[6] and gel permeation[7] have been proposed, but all have presented problems, especially as routine screening methods. (3) Various workers have reported clean-up techniques

based on single or multiple column chromatography. Column packing materials have included Florisil[8], Celite[9], silica gel[10] and microcolumns of alumina[10,11].

There is an increasing need for rapid, simple, inexpensive and non-hazardous methods for monitoring organochlorine insecticide residues in a wide range of foodstuffs at levels as low as 5–10 μg/kg. None of the methods listed above completely meets these criteria.

In one of our laboratories, we have previously developed methods for insecticide residues in fruits and vegetables based on the use of a single column of active alumina to clean-up hexane extracts of samples[12–14]. We found this adsorbent to be a far more reproducible product than Florisil and to require far smaller volumes of solvent to elute a range of organochlorine and organophosphorous compounds from the column.

We have therefore investigated the suitability of columns of activated alumina for the removal of oils and fats from hexane extracts of fatty foodstuffs and have developed a rapid procedure in which fat is removed on a single column of alumina in a process similar to our method for vegetables.

DEVELOPMENT OF METHOD

Extraction of residues from fatty foodstuffs

Complete extraction of all lipophilic insecticide residues from fatty foodstuffs can only be ensured when all of the fat is extracted. This is particularly important when residue levels are subsequently expressed on a fat basis and has resulted in Soxhlet extraction becoming widely accepted as a standard procedure for pesticide extraction in fatty products. In general the sample is first disintegrated by grinding with sand, sodium sulphate added to remove water and the resulting free-flowing powder extracted with hexane or light petroleum. In our laboratories we have experienced considerable problems with contamination from Soxhlet thimbles which necessitated exhaustive extraction prior to use. We therefore have preferred to extract fat by direct maceration with a hexane–acetone mixed solvent. A mixture in the ratio 4 volumes hexane to 1 volume acetone as used for extraction of fruit and vegetables[12] has been found satisfactory for this purpose.

A comparison of fat levels in various meat products determined by Soxhlet extraction, with hexane or with chloroform–methanol, acid hydrolysis by the Weibul method[22] and direct maceration with hexane–acetone (4:1) are given in Table I.

These results show that agreement between levels found by the various techniques is good when allowance is made for sampling variations.

Clean-up of extracts containing fat

Alumina has a considerable capacity for adsorbing fats and oils. This adsorbtive capacity is dependent on the activity of a particular alumina as shown in Fig. 1. This graph was obtained by preparing a series of aluminas of different activities, as measured on the Brockman scale, by the addition of predetermined amounts of water. Ten-gram quantities of each alumina were added in hexane to a series of 1-cm I.D. chromatography columns and 1 g of refined sunflower oil dissolved in 10 ml hexane added to the top of each column, which was then eluted with 100 ml hexane. No further oil was eluted in a second 100-ml volume of hexane. Each column eluate was

TABLE I

FAT CONTENTS OF DIFFERENT PRODUCTS DETERMINED BY ALTERNATIVE METHODS

Product	*Fat content (%)*			
	Soxhlet extraction		*Hexane–acetone maceration*	*Weibul method*
	Chloroform–methanol	*Hexane*		
Steak	2.8	—	2.7	2.95
Lamb	2.55	—	2.65	2.5
Sausage meat (1)	18.7	—	19.5	20.8
(2)	—	33.0	35.4	34.4
Luncheon meat	—	24.0	27.4	25.8
Lean minced beef	—	3.95	3.85	3.9
Animal feedstuff	—	3.3	3.31	—

evaporated to dryness in a tared vessel to determine, by difference, the weight of fat retained on each column of alumina. These results indicate that oil retention is proportional to activity with up to approximately 0.9 g sunflower oil being retained on a 10-g column of alumina activity 1.

A similar pattern was also noted for retention of organochlorine insecticides. All common organochlorine insecticides are eluted from a column of 8 g activity-5 alumina within 30 ml hexane as previously reported[12], whereas dieldrin was only partially eluted in 350 ml hexane from a similar column of activity-3 alumina. However, fat deactivates alumina in a similar manner to its deactivation with water, and a column of 10 g activity-3 alumina, saturated with sunflower oil, gave complete recovery of dieldrin in 100 ml hexane eluate. Therefore, by suitable choice of the

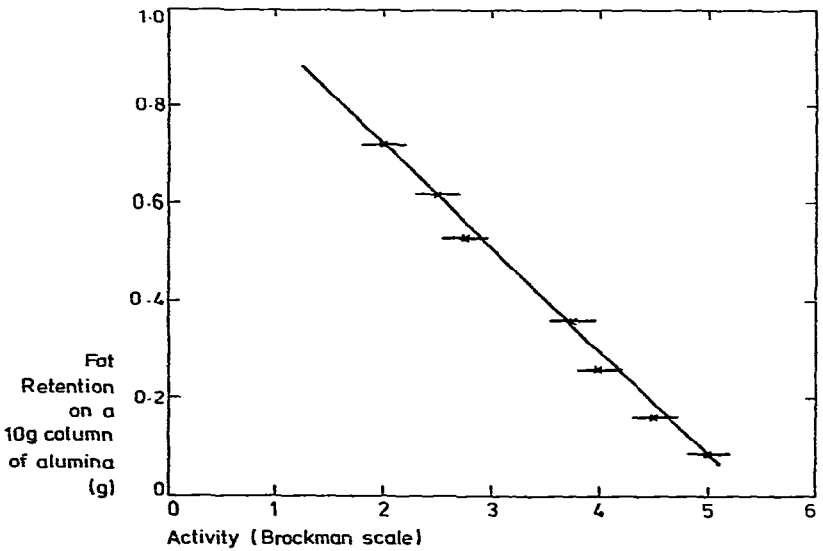

Fig. 1. Effect of activity on fat retention of 10-g alumina columns.

activity and quantity of alumina, it appeared possible to develop a satisfactory single-stage clean-up procedure for residues of organochlorine insecticides in fatty extracts.

It soon became apparent that the distribution of fat between alumina and different eluting solvents varied widely. With solvents more polar than hexane elution of fat from the column occurred to varying extents. Even with hexane a low-level seepage of fat occurred from a column fully saturated with sunflower oil. To ensure complete retention of fat on the column during elution of insecticides it was therefore necessary to provide a small excess of active alumina (unsaturated with fat) at the foot of the column. In our experience this is a critical factor in designing a suitable clean-up system; if the excess of alumina is too large or the alumina too active, elution volumes increase significantly or adsorption may even be irreversible.

Based on the above observations we have evaluated various combinations of activity of alumina and column sizes. Initial experiments indicated that significant degradation of insecticides, particularly DDT, occurred on aluminas of activity greater than 2 on the Brockmann scale. No such breakdown occurred with activity-2½ alumina, and in order to clean-up the maximum quantity of fat our initial investigations were performed with this material. A micro-column of 5 mm diameter containing 1 g of activity-2½ alumina has the capacity to retain 40 mg of extracted fat and completely elute common organochlorine insecticides in 30 ml hexane. This system provides a rapid clean-up from low-fat products such as cod-fillets, but does not have the capacity to give adequate sensitivity in general routine screening where fat contents of extracts are invariably much higher and results are normally expressed on a fat rather than a whole sample basis.

On the other hand, a three-column system capable of cleaning up 1 g of extracted fat and fractionating insecticides into two groups was also developed. This procedure gave excellent sensitivity but was time-consuming and necessitated extraction of large sample weights of many products in order to provide the 1 g fat required for clean-up. Furthermore, small variations in activity of alumina used gave significant variations in the fractionation pattern of insecticides from the columns. Consequently, the following system was developed and has been used successfully for routine screening of a range of products for a number of years.

Column of 4 g activity-2½ alumina

As shown in Fig. 1, a 10-g column of activity-2½ alumina has the capacity to retain approximately 0.6 g sunflower oil. The current West German limits for vegetable oils[15] require a method capable of measuring HCB at the 5 μg/kg level, cyclodienes and γ-BHC at the 10 μg/kg level and other organochlorine compounds at the 20–200 μg/kg levels. In order to detect these levels in electron-capture gas–liquid chromatographic (GLC) systems it is necessary to clean-up 0.15–0.2 g oil. Bearing in mind the need to have a small excess of active alumina at the base of the column after addition of oil, a 4-g column of activity-2½ alumina, with a fat capacity of 0.24 g (0.4 × 0.6) had the required characteristics for a routine screening procedure, to clean-up hexane solutions containing 0.2 g oil (approximately 80% of alumina saturated with fat). Elution of insecticides was achieved in 110 ml hexane with recoveries ranging from 83 to 118% for levels added to sunflower oil at the μg/kg level as shown in Table II. Retention of oil on the column was excellent with less than 0.001 g being co-eluted in the pesticide fraction. This system has been success-

TABLE II

RECOVERY OF ORGANOCHLORINE INSECTICIDES AND THE FUNGICIDE HCB FROM SUNFLOWER OIL USING A 4-g COLUMN OF ACTIVITY-2½ ALUMINA

Pesticide	*Level of addition (μg/kg)*	*Recovery (%)*
γ-BHC	20	92
	8	97
	4	93
HCB	5	83
Heptachlor epoxide	50	92
	20	100
	4	100
Endrin	5	87
Dieldrin	50	98
	10	92
	5	93
p,p'-DDE	50	93
	10	100
p,p'-DDD	50	100
	10	104
	5	118
p,p'-DDT	20	108
	10	85

fully used for routine analysis of a wide range of vegetable oils. Attempts to apply the method routinely to a wider range of fatty substrates and pesticides have, however, not been entirely satisfactory. The weight of different types of fats retained by the activity-2½ alumina column varied widely as shown in Table III. Both crude and refined vegetable and fish oils show similar behaviour, but most other fats of animal origin show large differences in retention. These variations undoubtedly reflect basic differences in the composition of the lipids extracted from different sources, which are not apparent from the crude fat determinations carried out on extracts. Consequently, it is necessary to determine the fat retention characteristics of the alumina column to each new type of fat extract and either to vary the quantity of alumina used for clean-

TABLE III

RETENTION CHARACTERISTICS OF VARIOUS TYPES OF OIL AND FAT ON COLUMNS OF ACTIVATED ALUMINA

Type of oil	*Weight retained on 4-g column activity 2½*	*Weight retained on 22-g column activity 4*
Refined vegetable oils	0.25	0.62
Crude vegetable oils	0.26	0.62
Refined fish oils	0.25	0.61
Crude fish oils	0.22–0.26	0.62
Milk and butter fat	0.20	0.51–0.52
Animal fats		
lard, suet, dripping	0.18–0.20	0.57
crude tallow	0.25–0.36	—
Meat and bone meal	0.24–0.32	—

up, the weight of fat taken for analysis and/or the hexane elution volume. Thus a 4-g column of activity-2½ alumina is suitable for clean-up of both crude and refined vegetable and fish oils with complete elution of organochlorine insecticides in 110 ml hexane. Dairy products, milk and other animal fats are less effectively retained and require a 5.5-g column of activity-2½ alumina to clean-up 0.2 g fat, with elution of insecticide residues in 130 ml hexane. Alternatively, 0.165 g dairy fat can be cleaned-up satisfactorily on a 4-g activity-2½ alumina column, but this reduces the sensitivity of the procedure. These two groups of products are amenable to routine analysis using a standard 4-g or 5.5-g column for clean-up. The fat retention characteristics of the other products listed are much less consistent and need to be determined individually before an adequate single-column clean-up procedure can be applied. In practice this is most readily achieved by applying 0.2 g fat to a standard 4-g column and eluting pesticides with larger volumes of hexane.

When this original method for vegetable oils was expanded to cover all organochlorine insecticides listed in relevant national and international legislation, recoveries of β-BHC, endosulfan A and methoxychlor were found to be low, at 10%, 10% and *ca.* 50%, respectively. Increasing the volume of eluate to 150 ml gave satisfactory recovery of methoxychlor from the 4-g column, but failed to improve the recovery of the other two insecticides.

The precise adsorption properties of an alumina are dependent on the calcining conditions employed. These vary markedly between manufacturers and, in many instances, between different batches from the same manufacturer. The purity of aluminas with respect to eluted material that is responsive to the electron capture detector may also be variable.

Initially we had great difficulty in reproducibly preparing activity-2½ alumina but, latterly, we have standardised on Woelm alumina and have found it to have no significant batch to batch variations in activity or purity.

We have found the traditional procedure for standardising activity using mixed dyestuffs as advocated by Stahl[23] to be insufficiently sensitive. Activity can best be accurately checked with reference to the retention of a standard solution of vegetable oil (*e.g.* a 4-g column of "activity-2½" alumina should retain 0.24 g vegetable oil).

Owing to the hygroscopic nature of alumina, storage presents problems but, provided the prepared material is stored in well sealed containers, in quantities that can be used within a week, no significant decrease in activity should result.

Column of 22 g activity-4 alumina

In view of these various disadvantages we have recently investigated the use of activity-4 alumina. We have found this to be a more versatile material with the following advantages. (1) The precise activity of the material as regards fat retention is far less critical than activity-2½ alumina. Consequently, we have found that reproducible material can be prepared according to the manufacturers' instructions without the need to check the fat retention on each occasion. The material also maintains its activity for longer periods during storage. (2) Small variations in the quantity of excess unsaturated alumina at the foot of the column have only a minimal effect on the elution volume required for complete elution of insecticides. (3) Recoveries of β-BHC and endosulfan A were increased to acceptable levels.

A 10-g column of activity-4 alumina retains approximately 0.28 g sunflower

oil (see Fig. 1). In order to improve sensitivity and to clean-up 0.5 g oil while still retaining an adequate quantity of unsaturated alumina at the foot of the column (approximately 20%), a 22-g column of activity-4 alumina was investigated. To reduce elution volumes/times to an acceptable level a 20-mm I.D. chromatography column was employed. With this system complete elution of a range of organochlorine insecticides was achieved in 150 ml hexane, as shown in Table IV and less than 0.001 g oil was co-eluted in the pesticide fraction. Similar variations in fat retention to those noted with activity-2½ alumina for different types of fat were recorded with this system as shown in Table III. Rather than increase the quantity of alumina we have preferred to decrease the weight of fat from milk and dairy products taken for clean-up to 0.4 g. This maintains the pesticide elution volume at 150 ml and gives adequate sensitivity for residue detection at the low μg/kg level. This procedure has been widely used for the determination of residues in vegetable oils and more recently has been successfully applied to a wider range of foodstuffs. We have found the procedure to offer significant advantages over the 4-g column method in terms of general robustness, sensitivity, ease of preparation and stability of the alumina, and recovery of β-BHC and endosulfan A.

EXPERIMENTAL

A general method for the determination of organochlorine insecticides and HCB in fatty foodstuffs is given below. Modifications to the basic extraction procedure to suit individual product types are discussed later.

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. Alternatively, hexane specially purified for use with the electron-capture detector can be purchased from several sources (*e.g.* Rathburn Chemicals, Walkerburn, Great Britain). Acetone: analytical-reagent grade. Sodium sulphate: analytical-reagent grade, granular, anhydrous and 2% aqueous solution. Alumina: activity-4 grade. Neutral or acidic aluminium oxide (Woelm, Eschwege, G.F.R.) supplied ready activated to grade 1. Deactivate by the addition of 10 ml water to 90 g alumina. Equilibrate for 24 h before use and store in closed vessels at ambient temperature.

Apparatus

Chromatography columns: 30 cm × 20 mm I.D. fitted with sintered discs and PTFE taps. Kuderna Danish evaporators: 250-ml capacity. Exelo tubes: 10 ml graduated, tapered. Gas chromatograph: fitted with an electron-capture detector.

Extraction

(1) Finely mince the sample, if necessary, and weigh sufficient sample to provide approximately 0.7 g fat. (2) Macerate in a high speed macerator with 50 ml acetone for 2 min. (3) Add 200 ml hexane and continue maceration until complete breakdown of sample is achieved. Allow phases to separate. (4) Decant the maximum volume of extract into a 1-l separator through a filter of anhydrous sodium sulphate on glass wool. (5) Wash the extract twice with 500-ml volumes of sodium sulphate

TABLE IV

RECOVERY OF ORGANOCHLORINE PESTICIDES FROM VEGETABLE OILS USING A 22-g COLUMN OF ACTIVITY-4-ALUMINA

Pesticides	*Level of addition (µg/kg)*	*Recovery (%)*
HCB	75	92
	25	98
	5	90
α-BHC	50	96
	25	88
	5	86
β-BHC	15	91
	8	108
γ-BHC	25	87
	15	91
	5	87
Heptachlor	25	93
	5	95
Aldrin	25	90
	5	87
Heptachlor epoxide	80	93
	25	93
	10	95
Endosulfan A	150	98
	80	81
p,p'-DDE	125	88
	80	92
	25	94
Dieldrin	125	99
	80	96
	25	98
Endrin	250	84
	100	91
	50	86
o,p'-DDT	200	105
	125	94
	25	92
	8	110
Methoxychlor	500	95
	400	91
	240	95
p,p'-DDD	160	97
	125	95
	25	124
	10	70
p,p'-DDT	200	104
	150	94
	50	91

solution. (6) Run the lower, aqueous, layers to waste along with a little of the hexane layer. (7) Transfer a 10-ml aliquot of hexane extract to a pre-weighed weighing bottle and determine the fat content (see *Determination of fat content*). (8) Calculate the volume of hexane containing 0.50 g fat and transfer this volume to a Kuderna Danish evaporator fitted with a 10-ml tube. (9) Add a few granules of anhydrous sodium sulphate and evaporate the contents to 5–8 ml over live steam. (10) Disconnect the tube and continue evaporation to 1–2 ml on a water bath at 60–70° under a stream of nitrogen.

Clean-up

(1) Prepare a slurry of 22 g activity-4 alumina in a little hexane and transfer to a chromatographic column. Add a 1-cm layer of anhydrous sodium sulphate to the top of the column and wash with 15–20 ml hexane. Adjust the level of hexane to just below the level of the sodium sulphate layer. (2) Transfer the concentrated hexane extract containing 0.50 fat (0.40 g for dairy products) to the top of the column. (3) Elute the column with hexane collecting the first 150 ml of eluate. (4) Concentrate the eluate in a Kuderna Danish evaporator to approximately 8 ml. Remove the graduated tube and continue the evaporation in a water bath at 50° under a stream of nitrogen to a volume of 2–3 ml. (5) Remove the tube from the water bath and continue evaporation at ambient temperature to 1 ml. (6) Stopper tube and reserve for GLC analysis with an electron caputure detector.

GLC analysis

The following gas chromatographic conditions have been found to be satisfactory, depending upon the instrument used. (1) Glass column: length 200 cm, I.D. 0.2–0.4 cm. (2) Stationary phases and loadings: 11% OV-17 + OV-210 (or QF-1) and 1.5% OV-17 + 1.95% OV-210. (3) Supports (80–100 or 100–120 mesh): Supelcon AW DMCS or Supelcoport; Chromosorb W AW DMCS or W HP: Gas-Chrom Q. (4) Carrier gas flow: *ca.* 20 ml/min for 0.2 cm I.D.; *ca.* 45 ml/min for 0.3 cm I.D.; *ca.* 80 ml/min for 0.4 cm I.D. (5) Temperatures: injection port: 220 ± 20°; detector: 220° for ^{3}H-ECD; 280° for ^{63}Ni-ECD; column: 185–210° isothermal, depending on column length and internal diameter, loading of stationary phase, background current of ECD and required analysis time.

The number of effective plates is calculated for four pesticides using the formula: $N = 5.54\,(t_r/W_{\frac{1}{2}})^2$. As a guideline the column should have at least 2000 plates for early eluting components and 3000 plates from heptachlor epoxide.

Determination of fat content

The fat content of the hexane extract may be determined as follows:

(1) Transfer a 10-ml aliquot of hexane extract to a pre-weighed weighing bottle. (2) Evaporate to dryness at 60° under a stream of nitrogen. (3) Dry in an oven at 105° for 15 min. (4) Cool and re-weigh. (5) Repeat oven drying to constant weight.

RESULTS AND DISCUSSION

One difficulty associated with the evaluation of methods for the low-level

TABLE V

COLLABORATIVE STUDIES ON RECOVERY THROUGH A 4-g COLUMN OF ACTIVITY-2½ ALUMINA OF ORGANOCHLORINE PESTICIDES ADDED TO VEGETABLE OILS

Pesticide	*Level added (μg/kg)*	*Level reported by laboratories (μg/kg)*		
		1	*2*	*3*
HCB	6	5	5	6
	10	9	10	9
α-BHC	6	5	4	6
γ-BHC	10	15	10	10
Heptachlor epoxide	8	5	7	7
Endosulfan A	8	—	—	—
	50	10	11	5
Dieldrin	10	8	11	10
	40	35	41	40
p,p'-DDE	34	39	24	35
Endrin	40	30	31	45
o,p'-DDT	8	10	7	13
	100	70	104	115
p,p'-DDD	10	11	11	8
Methoxychlor	500	600	465	455

determination of insecticide residues is that of ensuring the availability of a pesticide-free substrate to which known amounts of pesticides may be added. Pesticide-free vegetables can be fairly readily obtained, but there are few fatty substrates that can be guaranteed to be free of residues. One such substrate is high-temperature deodorised vegetable oil and this material has been used in a number of collaborative recovery experiments among Unilever laboratories experienced in pesticide residue analysis.

Typical sets of results obtained with the 4-g activity-2½ alumina column

TABLE VI

COLLABORATIVE STUDY ON RECOVERY THROUGH A 22-g COLUMN OF ACTIVITY-4 ALUMINA OF ORGANOCHLORINE PESTICIDES ADDED TO VEGETABLE OIL

Pesticide	*Level added (μg/kg)*	*Level reported by laboratories (μg/kh)*			
		1	*2*	*3*	*4*
HCB	5	5	5	5	7
α-BHC	5	5	4	5	7
γ-BHC	5	9	6	6	4
Heptachlor	5	5	5	4	6
Aldrin	5	4	5	4	9
Heptachlor epoxide	10	10	10	12	11
Dieldrin	25	31	25	19	22
Endrin	50	50	48	42	34
o,p'-DDE	25	27	25	26	22
o,p'-DDT	25	27	25	18	21
p,p'-DDD	25	21	24	28	22
p,p'-DDT	50	41	48	53	51
Toxaphene	1280	1440	1280	1200	1440

TABLE VII

DETERMINATION OF RESIDUE LEVELS IN ANIMAL FEEDS ANALYSED BY DIFFERENT METHODS

Pesticide	*Residue level (μg/kg feed)*								
	DMF partition		*Acetonitrile partition*			*4-g column*			*22-g column*
	3	*5*	*2*	*5*	*6*	*1*	*4*	*6*	*6*
HCB	7	3	5	3	4	4	5	4	4
α-BHC	2	—	2	—	3	1	5	1	2
β-BHC	—	—	3	—	—	—	—	—	—
γ-BHC	6	6	6	5	6	6	7	5	7
Aldrin	—	—	—	—	—	2	—	—	—
Heptachlor epoxide	—	—	—	—	—	—	—	1	1
Dieldrin	—	3	—	3	1	—	—	2	2
p,p'-DDE	4	4	4	5	8	4	5	6	4
o,p'-DDT	10	6	7	7	18	9	10	12	12
p,p'-DDD	2	—	3	—	2	—	5	3	3
p,p'-DDT	36	36	33	33	30	14	25	39	28

method are given in Table V and with the 22-g activity-4 alumina column method in Table VI.

Further collaborative studies have been made on samples of animal feedstuff and extracted ham fat, to which a range of chlorinated insecticides had been added, where participants used methods of their own choice. These methods included the 4-g and 22-g alumina column techniques, the dimethylformamide method of Noren and Westöö[16] and the acetonitrile method of Mills[1] using alumina instead of Florisil for the secondary clean-up step. Results are shown in Tables VII and VIII.

Agreement between determined levels using these procedures are generally excellent in view of the low level of most insecticides present.

TABLE VIII

MEAN DETERMINED RESIDUE LEVELS IN HAM FAT, TO WHICH A RANGE OF ORGANOCHLORINE INSECTICIDE RESIDUES HAD BEEN ADDED, ANALYSED BY FOUR DIFFERENT METHODS

Pesticide	*Mean level (μg/kg) determined*			
	DMF partition	*Acetonitrile partition*	*4-g column*	*22-g column*
HCB	93	48	82	74
α-BHC	60	55	57	61
γ-BHC	56	50	56	53
Heptachlor	42	36	44	37
Heptachlor epoxide	99	64	94	103
Endrin	417	350	402	449
Aldrin	48	31	42	44
Dieldrin	257	212	195	249
p,p'-DDE	277	232	262	271
o,p'-DDT	241	216	245	249
p,p'-DDD	265	218	225	271
p,p'-DDT	518	469	473	464

Separation of polychlorinated biphenyls and organochlorine insecticides

The presence of polychlorinated biphenyls (PCBs) in cleaned-up extracts invariably interferes with the accurate quantitation of organochlorine insecticide levels, and it is necessary to resolve these two species as far as possible prior to estimation. Complete separation has been demonstrated by certain workers using columns of silica[10], silicic acid–celite[17] or charcoal[18], but for a variety of reasons these procedures have been shown to be generally unsuited to routine application[19]. It has been shown, however, that partial separation of PCBs and organochlorine insecticides can be readily obtained using both silica and alumina columns (*e.g.* ref. 10), and that the separation obtained is adequate for the analysis of most products containing PCB residues.

In our laboratories we have used columns of active aluminas in two distinct techniques to achieve satisfactory partial separation.

(i) Cleaned-up extracts that are found by GLC analysis to contain significant residues of PCBs are fractionated on 10 g activity-2½ alumina (standardised as for the 4 g column clean-up procedure) contained in a 10-mm-I.D. column. Fractionation with hexane and 10% acetone in hexane results in the complete separation of PCBs from the majority of organochlorine insecticide residues as shown in Table IX, but, as previously stated, recoveries of β-BHC and endosulfan A are low from activity-2½ alumina.

(ii) Samples expected to contain PCBs can be directly fractionated from the primary clean-up column. This technique can be applied to both the 4-g and 22-g column systems. Table X shows the fractions taken and the separation achieved from these 2 column systems. Resolution of pesticides between the two fractions is not always complete, but satisfactory recoveries of β-BHC and endosulfan A are achieved from the 22-g column system.

TABLE IX

FRACTIONATION OF PCBs AND INSECTICIDES ON 10-g ACTIVITY-2½ ALUMINA COLUMN

Column eluate (ml)	*PCB*	*DDE*	*DDT*	*TDE*	*γ-BHC*	*Heptachlor epoxide*	*Dieldrin*
35 ml hexane	98	93	—	—	—	—	—
5 ml hexane	2	7	2	—	—	—	—
35 ml hexane	—	—	98	—	—	—	—
10 ml } 10% acetone	—	—	—	—	—	—	—
10 ml } in hexane	—	—	—	100	100	100	100
Recovery (%)	102	98	102	100	98	96	100

We are currently investigating the effect of using columns of narrower internal diameter to improve the efficiency of fractionation from the 22-g alumina system. Initial results indicate that optimum separation of species is achieved with columns of 12–15 mm I.D.

In all these procedures the initial fraction contains all the PCBs and any residues of aldrin, heptachlor, HCB, *p,p'*-DDE and *o,p'*-DDT present in the original sample. Of these five pesticides, aldrin and heptachlor are rarely encountered. HCB

TABLE X

FRACTIONATION PATTERN OF ORGANOCHLORINE PESTICIDES AND PCBs ON 4-g AND 22-g COLUMNS OF ALUMINA IN THE PRESENCE OF VEGETABLE OIL

Pesticide	*4 g activity-2½ alumina + 200 mg vegetable oil*		*22 g activity-4 alumina + 500 mg vegetable oil*	
	5 ml	*100 ml*	*10 ml*	*150 ml*
HCB	100	—	100	—
α-BHC	—	100	—	100
γ-BHC	—	100	—	100
β-BHC	—	after 100 ml	—	100
Heptachlor	90–100	0–10	90–100	0–10
Aldrin	100	—	100	—
Heptachlor epoxide	—	100	—	100
DDE	80–100	0–20	90–100	0–10
Dieldrin	—	100	—	100
Endrin	—	100	—	100
o,p'-DDT	80–100	0–20	90–100	0–10
p,p'-DDD	—	100	—	100
p,p'-DDT	0–10	90–100	10–30	90–70
Methoxychlor	—	100	—	100
Aroclor 1242	100	—	100	—
Aroclor 1254	100	—	100	—
Aroclor 1260	100	—	100	—
Toxaphene	—	—	In both fractions	
Endosulfan A	—	Incomplete	—	100
Perthane			30	70
Quintozene			100	
Mirex			100	
Chlordane, Strobane, Toxaphene			Spread over various fractions	

is well resolved on most GLC systems from all PCB components other than the mono- and some dichlorinated species which are normally absent from food and environmental samples. Quantitative determination of *p,p'*-DDE and *o,p'*-DDT residues can present problems if the levels present are low relative to those of PCBs. This is not generally the case and, for the vast majority of routine applications, the separations obtained by fractionation from alumina columns allow satisfactory estimation of all species present.

A further advantage of fractionation is that, even in the absence of PCBs, certain pairs of insecticides which are difficult to resolve by gas chromatography appear in different fractions, *e.g.* dieldrin and DDE, heptachlor and β-BHC, HCB and α-BHC, endrin and *o,p'*-DDT.

In order to test the suitability of fractionation from the 4-g and 22-g columns in the presence of PCBs we have collaboratively analysed a sample of vegetable oil containing a range of organochlorine pesticides and commercial PCB mixtures, Aroclors 1254 and 1260. Results are shown in Table XI. In a further study collaborators were asked to analyse a crude fish oil using method(s) of their own choice.

TABLE XI

COLLABORATIVE STUDY ON THE ANALYSIS OF A MIXTURE OF ORGANOCHLORINE PESTICIDES AND PCBs IN VEGETABLE OIL USING 4-g AND 22-g COLUMNS OF ALUMINA.

Compound	*Level added (μg/kg)*	*Level found (μg/kg)*							
		4-g method				*22-g method*			
		1	*2*	*3*	*4*	*1*	*2*	*3*	*4*
HCB	5	4	4	7	6	4	4	8	4
α-BHC	5	4	4	5	4	4	4	5	4
β-BHC	5	7	—	—	7	6	5	—	6
Heptachlor	5	4	5	5	4	4	4	4	3
p,p'-DDE	24	16	25	9	23	17	24	18	24
Dieldrin	24	19	22	18	25	19	18	19	25
p,p'-DDT	48	35	44	22	43	32	42	29	44
Methoxychlor	240	239	—	220	224	251	—	212	268
Endosulfan A	48	4	—	—	7	35	40	27	38
Aroclor 1260	320	335	260	400	288	363	235	400	248
Aroclor 1254	400	265	260	300	240	298	235	300	275

Results are given in Table XII. PCB recoveries were determined with reference to the major components present in standard solutions of Aroclors.

Agreement between operators and methods is generally satisfactory, but, as in all such methods, the presence of PCBs has considerably complicated the analysis. This has resulted in generally low levels being reported for certain compounds that tend to overlap with PCB components. Some operators also confused PCB and pesticide peaks.

TABLE XII

COLLABORATIVE STUDY ON THE ANALYSIS OF CRUDE FISH OIL USING METHOD OF OWN CHOICE

n = Number of analysis reported; ND = not detected

Reported compound	*Mean levels reported (μg/kg)*			
	Alumina column method		*Acetonitrile partition*	*DMF partition*
	4 g (n = 4)	*22 g (n = 3)*	*(n = 2)*	*(n = 3)*
HCB	75	87	46	104
α-BHC	69	69	61	84
γ-BHC	27	19	20	10
Heptachlor	8	2	3	10
Aldrin	10	ND	ND	ND
Heptachlor epoxide	24	22	15	21
p,p'-DDE	232	174	196	302
Dieldrin	219	220	230	275
Endrin	50	52	100	ND
o,p'-DDT	142	194	120	194
p,p'-DDD	267	272	214	280
p,p'-DDT	249	318	229	306
Total Aroclor	1407	1278	1672	1707

Extraction

The extraction technique given in the section Experimental has been successfully applied to a wide range of products including meat, fish, poultry, cocoa and chocolate, egg powder, mayonnaise and animal feedstuffs. Dried products such as milk powders are allowed to stand with twice their weight of water for 30 min prior to extraction.

When this procedure was applied to milk and a variety of dairy products ranging in fat content from 0.6% in low-fat yogurt to 45% in cream cheese, incomplete extraction of fat from these products was noted. Satisfactory recoveries of fat were, however, obtained following triplicate extractions. This considerably extended the time of the process and resulted in a final extract of 600 ml. For this reason we have preferred to use Samuel's modification[20] of the Rose–Gottlieb procedure, especially for low-fat dairy products. To overcome problems of emulsion formation with this method we have preferred to increase the volume of diethyl ether used and to carry out extractions in a conical flask, transferring the organic layer to a separator only for the washing stage.

As the results in Table XIII show, good agreement was obtained between the modified Rose–Gottlieb and the hexane–acetone procedures both for extracted fat and recoveries of common organochlorine insecticides added at low levels.

TABLE XIII

COMPARISON OF THE ROSE-GOTTLIEB AND HEXANE-ACETONE EXTRACTION PROCEDURES FOR DAIRY PRODUCTS

Dairy product	*Compound determined*	*Level of insecticide added (μg/kg)*	*Recovery (%)*	
			Hexane–acetone	*Rose–Gottlieb*
Milk	Fat		1.8	2.0
	Heptachlor epoxide	80	90	91
	Dieldrin	80	97	104
	p,p'-DDE	80	104	91
	p,p'-DDD	160	92	100
Yogurt	Fat		2.05	1.9
	Heptachlor epoxide	80	88	97
	Dieldrin	80	100	99
	p,p'-DDE	80	92	96
	p,p'-DDD	160	93	90

Vegetable and fish oils and fats such as suet and lard are prepared for clean-up simply by dissolving in hexane. Solid fats may require warming to dissolve completely but 5% solutions are readily prepared.

Gas chromatography

The GLC determination is a critical step in any procedure for organochlorine residue analysis and the advantages of a good extraction and clean-up system can be nullified by poor gas chromatography. It was noted early in the collaborative studies that certain participating laboratories had difficulty on occasions in detecting low levels of certain residues and in distinguishing between certain species. An evaluation

of the various GLC systems employed revealed significant differences in the sensitivity, linearity and stability of the instruments used and in the efficiency and resolution of the different column packings employed. A comparison of different stationary phases showed that those based on mixed OV-17, OV-120 (or equivalent) gave the maximum separation of organochlorine pesticides and that by using suitably deactivated inert support materials good peak shape with negligible decomposition of the more labile species was obtained. Consequently, a further collaborative study was initiated in which each of the participating laboratories used a mixed OV column system to analyse a standard mixture of 14 organochlorine pesticides at the 10–100 μg/kg level after suitable dilution. The inert supports used comprised Gas-Chrom Q, Supelcon AW DMCS, Chromosorb W HP and Varaport 30 in the mesh range 80–100 or 100–120. Columns were all approximately 200 cm in length (I.D. varied from 2 to 4.6 mm) and efficiencies ranged from 1700 to 4800 plates [determined from heptachlor epoxide peak using the formule $N = 5.54\ (t_r/W_{\frac{1}{2}})^2$]. The results from this study indicated satisfactory detection and quantitation with accuracy generally better than $\pm 5\%$ and coefficients of variation between 5–15%.

A typical chromatogram obtained for a high-temperature deodorised bean oil cleaned up on a 22-g alumina column and analysed on 1.5% OV-17–1.95% OV-210 on 100–120-mesh Supelcon AW DMCS, GLC column is shown in Fig. 2. For comparison the response obtained for a range of pesticides at levels equivalent to the low μg/kg level in a sample are shown in Fig. 3. These chromatograms illustrate the low background level obtained through the process for a "clean" oil and the adequate sensitivity of the GLC systems in detecting levels of residues to below those listed in the stringent West German regulations.

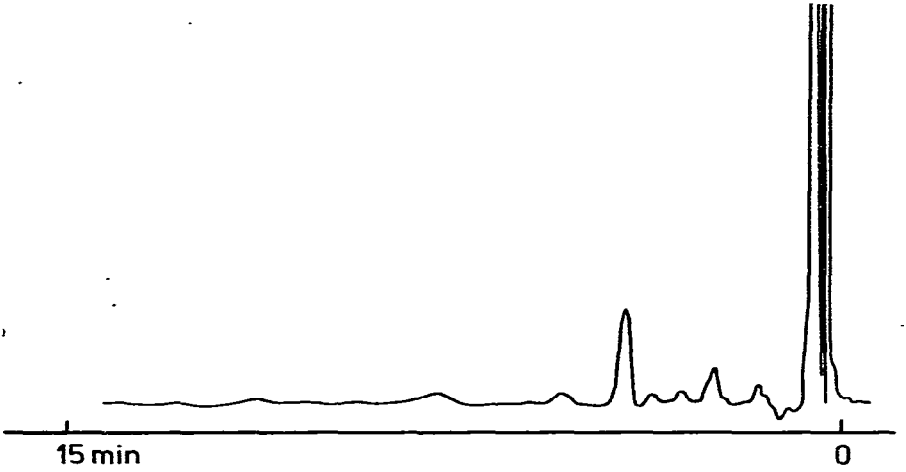

Fig. 2. Chromatogram of final extract from 0.5 g bean oil cleaned-up on a 22-g column of activity-4 alumina.

GENERAL

During the course of this work the micro alumina column method of Greve[11] was published. In this method 50 mg fat is added to the top of a 2 g column of alumina (approximately activity 4) and all common organochlorine insecticices are eluted in

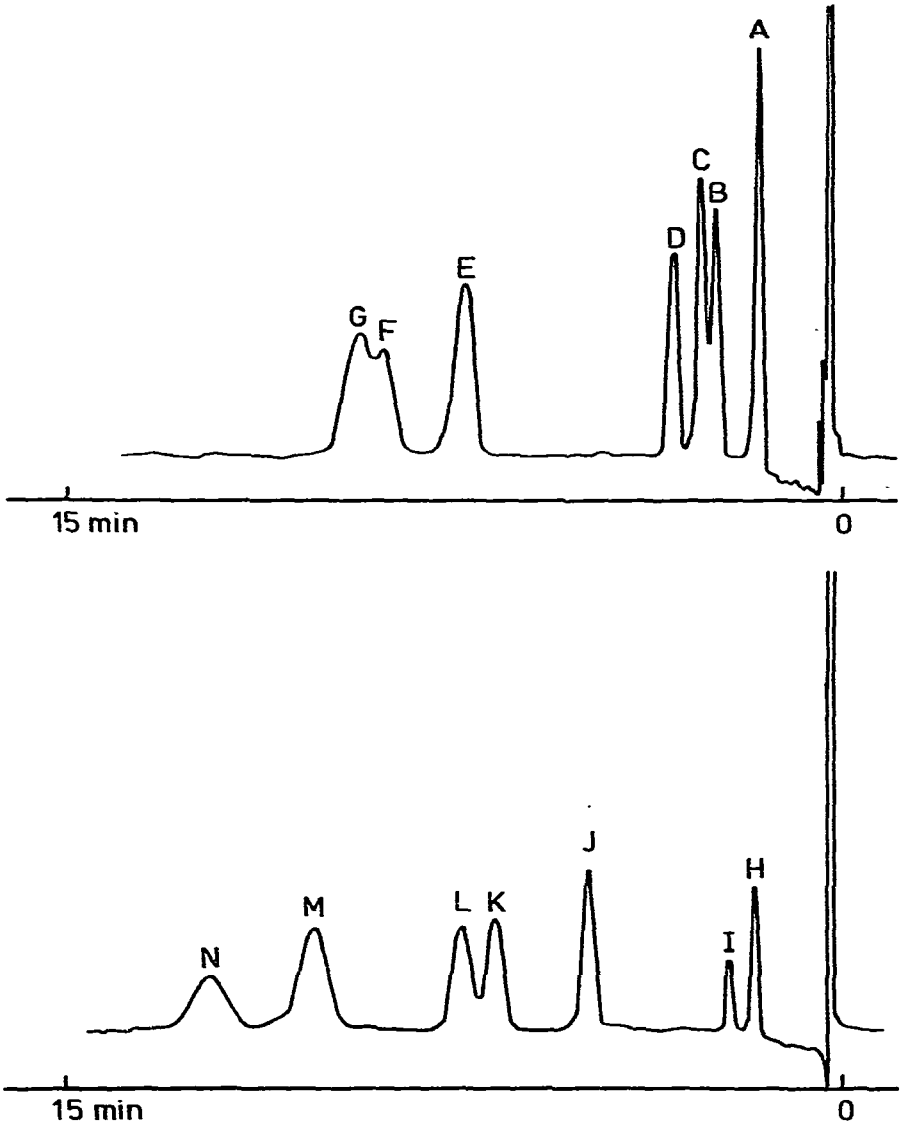

Fig. 3. Chromatograms of residues corresponding to the following levels in samples (μg/kg): A, 6 HCB; B, 40 β-BHC; C, 4 heptachlor; D, 8 aldrin; E, 16 endosulfan A; F, 16 endrin; G, 32 *o,p'*-DDT; H, 4 α-BHC; I, 2 γ-BHC; J, 12 heptachlor epoxide; K, 12 DDE; L, 12 dieldrin; M, 25 *p,p'*-DDD; N, 32 *p,p'*-DDT. For ease of identification, compounds A to G and H to N have been analysed separately under GLC conditions identical to those used to obtain the chromatogram shown in Fig. 2.

13 ml hexane. We have evaluated this method and found it superior to our micro-column method described in this paper in that good recoveries of β-BHC are obtained with the less active alumina. The technique, however, lacks sensitivity and is therefore unsuitable as a general screening technique, especially for vegetable oils.

In discussions with Holmes[21], we learned that the Laboratory of the Government Chemist (London) has been experimenting with dry-filled columns of basic alumina and have found an increased capacity for certain types of fat. When applied to our 22-g method, this increase was found to be marginal (0.69 *versus* 0.65 g).

We have also examined the replacement of the Florisil column used for secondary clean-up in the acetonitrile partition procedure[1] with an alumina column. For most products a 10-g column of activity-5 alumina removes all residual fat from an initial 5-g and all common organochlorine insecticides are eluted in 50 ml hexane.

Although the nominal fat capacity for 10 g activity-5 alumina is approximately 0.1 g (see Fig. 1), up to 0.4 g of residual fat could be removed on this column. This suggests that fractionation of the fat occurs during the acetonitrile partition steps and that the column has a much higher capacity for the residual, apparently more polar, fat components. In a few instances, *e.g.* raw and crude coconut oil and raw palm kernel oil, a 10-g column of activity-4 alumina was required to remove all residual fat with an elution volume of 70 ml hexane. These elution volumes compare with the 400 ml solvent required for elution from standard Florisil columns.

CONCLUSIONS

Activated alumina is an excellent adsorbent for fat and can be used in a single stage column clean-up method for the determination of organochlorine insecticides and related compounds in fatty foodstuffs.

If offers a number of advantages over many other published techniques:

(1) The method is robust enough for use by junior staff and is equivalent in performance to the official techniques bases on partition between hexane or light petroleum and acetonitrile or dimethylformamide. (2) It offers advantages in routine screening in terms of speed of analysis and cost of reagents and apparatus. (3) It eliminates the use of potentially hazardous reagents such as acetonitrile and dimethylformamide.

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NOTE ADDED IN PROOF

Recently it has been drawn to our attention that, contrary to our own experience, some other laboratories have reported difficulties in preparing alumina of the correct activity by the procedure given above. Therefore, it may be necessary to check the activity of the final prepared alumina before use. This is best done by determining its fat retention capacity in the following manner. Add 1 g refined vegetable oil in hexane to the top of a standard 22 g column of activity-4 alumina and elute with 150 ml hexane. Determine the weight of fat eluted from the column. A 22 g column of correct activity-4 alumina should return 0.62 ± 0.02 g oil.

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