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Comparative study of clean-up and fractionation methods for the determination of organochlorine pesticides in lipids by gas chromatography

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ABSTRACT

Three of the methods most often used for the clean-up and fractionation of organochlorine pesticides in lipid residue analysis by gas chromatography with electron-capture detection were compared. The overall recoveries of twenty pesticides from spiked samples were higher than 88%, the relative standard deviation being in the range 3–11% ($n = 6$) at the 36–80 ppb (10^9) level. The three methods were compared by analysis of variance, with no differences in precision at the 0.05 significance level. Differences in recoveries appeared in only two instances. None of the three methods seems to be significantly better than the others for the determination of the pesticides studied.

INTRODUCTION

The well known persistence of organochlorine pesticides due to their low biodegradability, together with their biosolubility in lipid tissues and high toxicity, require the analysis of this kind of sample in routine pollution studies [1]. Owing to the complexity of these lipid samples and their low pesticide concentrations, the analytical technique most often used is gas chromatography (GC) with electron-capture detection (ECD) after extraction of the pesticides in organic solvents. In order to prevent deterioration of the detector, extracts must be clean, particularly regarding the absence of fatty matter; consequently, clean-up is necessary. Fractionation of pesticides in groups by differential elution should simplify chromatograms and signal processing.

Extracts containing lipid residues may be purified by both chemical or physical methods. The main reagents used for purification include concentrated sulphuric acid, chromium trioxide and potassium hydroxide in ethanol [2–8]; however, the organochlorine pesticides are decomposed in different ways. Hence physical purification is preferred; this involves the use of adsorption columns containing silica gel [9], alumina [1,10] or Florisil [11–15]. The use of these adsorbents involves particular problems. Thus, silica contains large amounts of impurities, which are extremely difficult to remove [16], and the fractionation ability of alumina is limited [17]. The main problem with Florisil is the repeatability, but the standardization proposed by Mills [18] seems to have overcome this problem, and the use of Florisil has become well accepted [19]. However, some disagreement still remains regarding the fractionation of pesticides when different eluents are used.

This paper reports the results of a comparative study of the three elution systems most often used with Florisil to purify extracts from lipid samples for the determination of organochlorine pesticide residues.

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EXPERIMENTAL

Apparatus

A Hewlett-Packard Model 5890A gas chromatograph equipped with a packed-column injection port, a Hewlett-Packard cross-linked 5% phenylmethylsilicone (2.65 μm film thickness) fused-silica capillary column (30 m × 0.53 mm I.D.) and an electron-capture detector (^{63}Ni) was used. Data from the detector were processed using an HP Vectra ES/12 computer and were reported with an HP 3365 ChemStation system. The injection port and detector temperatures were 230 and 300°C, respectively. The oven temperature was programmed with an initial hold for 6 min at 180°C, followed by an increase at 7°C/min to 220°C, a hold for 6 min, then an increase at 10°C/min to 260°C, with a final hold for 3 min. Nitrogen flow-rates were carrier gas 10 ml/min and make-up gas 50 ml/min.

Soxhlet extractors (125 ml) were used, equipped with extraction cartridges with No. 1 fritted plate bottoms. A Heidolph VV 2000 rotary evaporator, a P-Selecta hot water-bath and a Waring blender were employed. Chromatographic columns with a borosilicate stopcock and a coarse fritted plate (No. 0), 40 × 1.6 cm I.D., were used. Volumetric glassware of Class A was used, all glassware being washed with soapy water, rinsed with tap water, immersed in chromic acid mixture for about 5 h, rinsed with distilled water and acetone and stored with openings covered with aluminium foil previously heated at 350°C for 12 h.

Reagents and standards

The solvents benzene, *n*-hexane, light petroleum (b.p. 40–60°C), diethyl ether, dichloromethane, acetonitrile and acetone were of pesticide grade from Carlo Erba. Lauric acid and absolute ethanol (analytical-reagent grade) were obtained from Merck and phenolphthalein (PRS) and sodium hydroxide (PRS, 97%) from Panreac. Anhydrous sodium sulfate (analytical-reagent grade) from Merck was used as a drying agent.

Florisil (60–100 mesh) was purchased from Carlo Erba (RS) and was activated at 676°C and stored in the dark in a glass container with a glass stopper or foil-lined screw-cap. The amount of Florisil used in each column was calculated by standardization [18]. Before use, each portion was activated overnight at 130°C in a foil-covered glass container and cooled in a desiccator at room temperature. The three elution procedures compared use a glass chromatographic column loaded with 8.5 g of Florisil, determined from the lauric acid value and column inside diameter. The column was topped with glass-wool, 2 cm of anhydrous sodium sulphate and finally glass-wool.

Pesticide standards were obtained from Riedel-de Häen: aldrin, captan, chlorfenson, *p,p'*-DDD, *o,p'*-DDE, *p,p'*-DDE, *o,p'*-DDT, *p,p'*-DDT, dicofol, dieldrin, endosulfan-I, endosulfan-II, endrin, hexachlorobenzene (HCB), heptachlor, heptachlor epoxide, lindane, methoxychlor, mirex, tetradifon and trifluralin. Stock solutions containing 80–150 ppm were prepared by dissolving the analytical reference standards in benzene and diluting to 50 ml in a volumetric flask. These solutions were stored in glass bottles under refrigeration (0°C). Suitable dilutions were made to obtain more dilute standard solutions and mixtures in *n*-hexane.

Sample

About 1.1 kg of bonito fish (*Thynnus pelamys*) sample, taken as recomended by Aminot and Chaussepied [20], was cut, blended and spiked with pesticides as indicated in Table I. Subsequently the sample was freeze dried.

Extraction

A 5-g amount of sample was weighed and extracted in a Soxhlet extractor with 150 ml of *n*-hex-

TABLE I

FORTIFICATION LEVELS IN UNLYOPHILIZED SAMPLE

Pesticide	ppb[a] (w/w)	Pesticide	ppb (w/w)
Captan	37	Endosulfan-II	42
Chlorfenson	49	Endrin	51
p,p'-DDD	64	HCB	42
o,p'-DDE	53	Heptachlor	36
p,p'-DDE	56	Heptachlor epoxide	42
o,p'-DDT	60	Lindane	43
p,p'-DDT	56	Methoxychlor	57
Dicofol	80	Mirex	52
Dieldrin	49	Tetradifon	69
Endosulfan-I	51	Trifluralin	42

[a] Throughout this article, the American billion (10^9) is meant.

ane for 4 h. The extract was concentrated to 10 ml in the rotary evaporator under vacuum.

Clean-up and fractionation

The elution volumes were 100 ml as required by the use of columns of 1.6 cm I.D. The three procedures are given in the form of instructions.

Standard procedure [19]. Pre-elute the column with 25–30 ml of light petroleum. Discard the eluted solutions until just before the sodium sulphate layer is exposed to air; quantitatively transfer 3 ml of sample extract into the column by careful decantation and subsequently wash with light petroleum ether (2 ml maximum). Adjust the elution rate to about 5 ml/min. Collect three fractions in 125-ml flasks using the following eluents: first, diethyl ether–light petroleum (6:94, v/v); second, diethyl ether–light petroleum (15:85, v/v); and third, diethyl ether–light petroleum (50:50, v/v). Alternatively, separate polychlorinated biphenyls (PCBs) by eluting first with 100 ml of light petroleum.

Stimac procedure [21]. This is a modification of the previous procedure. Pre-wash the column with 50 ml of diethyl ether–light petroleum (30:70, v/v) followed by 25 ml of light petroleum. Let the light petroleum elute down to 1–2 mm above the packing. Transfer 3.0 ml of the sample extract to the column. Rinse the walls of the column with 1 ml of light petroleum and elute at *ca.* 5 ml/min with 100 ml of diethyl ether–light petroleum (6:94, v/v).

Mills et al. procedure [22]. Pre-wet column with 40–50 ml of *n*-hexane. Transfer 3 ml of the sample extract solution to the column, allowing it to pass through at *ca.* 5 ml/min. Rinse the walls of the column with 1 ml of *n*-hexane. Elute the column with 100 ml of each of the following solvent mixtures: first, eluent A, dichloromethane–*n*-hexane (20:80, v/v), second, eluent B, dichloromethane–*n*-hexane (50:50, v/v) containing 0.35% (v/v) of acetonitrile; and third, eluent C, dichloromethane–*n*-hexane (50:50, v/v) containing 1.5% (v/v) of acetonitrile.

In all procedures, the sample extract must be dry and free from polar solvents when placed in the column.

Determination

Each eluted sample was evaporated to dryness in a rotary evaporator at 40°C under vacuum. The residue was dissolved in 1–2 ml of *n*-hexane, 40 ppb of aldrin as internal standard were added and the volume was made up to 5 ml with *n*-hexane. A 1-μl volume of this solution was injected into a gas chromatograph. The chromatographic peaks were identified by comparing their relative retention times with respect to the aldrin peak with those of the respective pesticide standards. Quantification was by internal standard calibration, measuring peak heights.

RESULTS AND DISCUSSION

Studies on standard mixtures

First, the experimental chromatographic conditions, mainly regarding the oven temperature programme, were optimized to separate the twenty pesticides studied in the 45–95 ppb range. The best results were given by the temperature programme specified under Experimental. The resulting chromatogram is shown in Fig. 1. It shows that the three pairs dieldrin–*p,p'*-DDE, *p,p'*-DDD–*o,p'*-DDT and dicofol–methoxychlor completely overlap (the first pair can be separated by changing chromatographic conditions, but not the other two pairs) and that the pair heptachlor epoxide–captan does not permit more than 98% separation; however, the other pesticides are clearly separated (>98%); the criterion used to judge this was a resolution higher than 1. Individual determination of the above overlapping pairs requires previous fractionation by one of the three procedures specified under Experimental.

The analytical characteristics obtained by injecting pesticide standard mixtures in which no peak overlap appeared are shown in Table II. Internal calibration graphs were prepared in all instances using 1.8 ppm of aldrin; this concentration was useful for relative comparison purposes over the whole pesticide concentration range studied. The detection limit was defined as $2N/S$ [23], where S is the slope of the calibration graph and N is the noise; it was determined for low attenuation and then the software was used to convert it into the attenuation employed in the above expression. Under these conditions, the detection limits ranged between 24 and 80 ppt (10^{12}). The calibration graphs were linear over five orders of magnitude. The relative standard deviations for eight determinations at a concentration level of about 40 ppb, using 40 ppb of aldrin as internal standard, were 0.8–2.2%. The pesticides

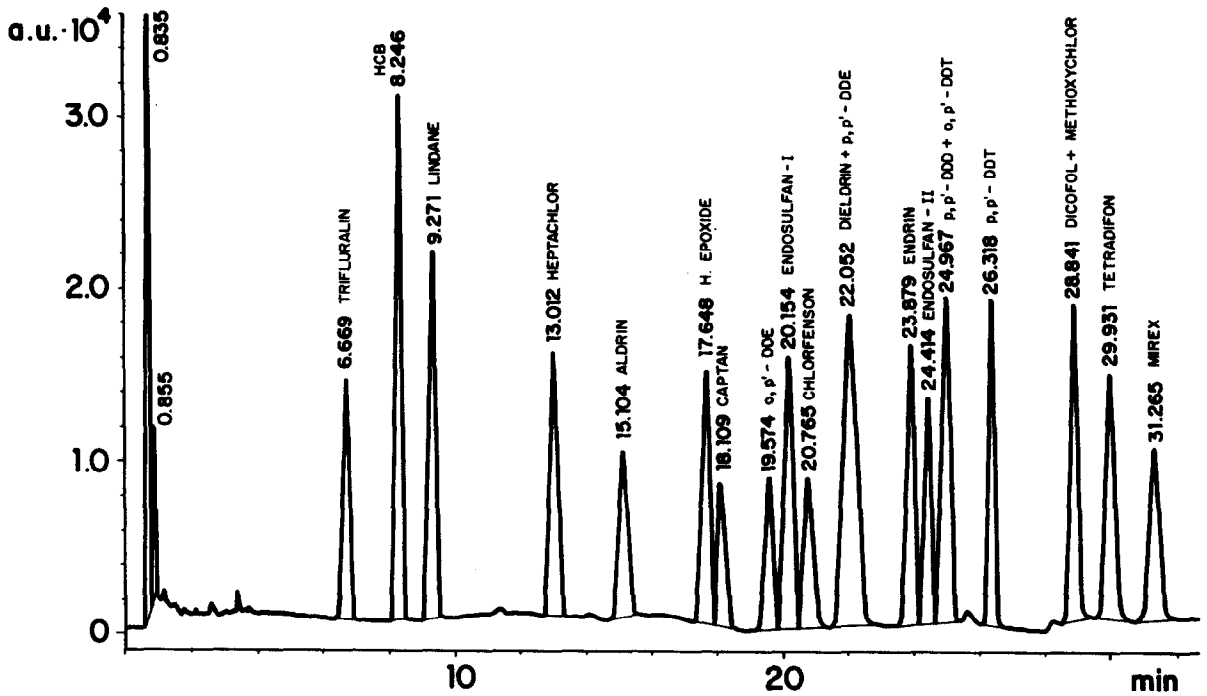

Fig. 1. Gas chromatogram of pesticides. For conditions of analysis, see Experimental.

TABLE II

ANALYTICAL CHARACTERISTICS

Conditions as in Results and Discussion section. S = sensitivity; D.L. = detection limit ($2N/S$); Detn.L. = determination limit ($10N/S$); L.U.L. = linear upper limit; R.S.D. = Relative standard deviation for eight determinations.

Pesticide	S [μV (ppb)$^{-1}$]	D.L. (ppb)	Detn.L. (ppb)	L.U.L. (ppb × 10^4)	R.S.D. (%)
Captan	85	0.025	0.126	1.22	1.69
Chlorfenson	33	0.064	0.322	3.11	0.85
p,p'-DDD	41	0.053	0.263	2.54	1.62
o,p'-DDE	33	0.065	0.325	2.91	1.87
p,p'-DDE	47	0.046	0.230	2.10	2.20
o,p'-DDT	27	0.080	0.401	3.84	1.24
p,p'-DDT	40	0.053	0.267	2.56	2.17
Dicofol	44	0.049	0.243	2.59	1.93
Dieldrin	51	0.042	0.212	2.01	1.32
Endolfulfan-I	61	0.035	0.177	1.67	1.30
Endosulfan-II	55	0.039	0.197	1.91	2.17
Endrin	54	0.040	0.198	1.87	1.18
HCB	67	0.032	0.161	1.50	0.79
Heptachlor	71	0.031	0.153	1.43	0.93
Heptachlor epoxide	85	0.025	0.126	1.14	1.53
Lindane	91	0.024	0.118	1.14	1.58
Methoxychlor	28	0.076	0.380	3.60	2.21
Mirex	39	0.055	0.276	2.63	1.98
Tetradifon	35	0.061	0.305	2.15	2.11
Trifluralin	27	0.081	0.404	1.84	1.67

TABLE III

RETENTION TIMES

Conditions as in Experimental. t_R = absolute retention time; RRT = relative retention time with respect to aldrin as internal standard.

Pesticide	t_R (min)	RRT
Trifluralin	6.669	0.442
HCB	8.246	0.546
Lindane	9.271	0.614
Heptachlor	13.012	0.862
Aldrin	15.104	1.000
Heptachlor epoxide	17.648	1.168
Captan	18.109	1.199
o,p'-DDE	19.574	1.296
Endosulfan-I	20.154	1.334
Chlorfenson	20.765	1.375
p,p'-DDE	22.052	1.460
Dieldrin	22.052	1.460
Endrin	23.879	1.581
Endosulfan-II	24.414	1.616
p,p'-DDD	24.967	1.653
o,p'-DDT	24.967	1.653
p,p'-DDT	26.318	1.742
Dicofol	28.841	1.909
Methoxychlor	28.841	1.909
Tetradifon	29.931	1.982
Mirex	31.265	2.070

were identified by comparing their retention times with those of aldrin. The results are given in Table III.

Studies on spiked lipid samples

Clean-up. The *n*-hexane extracts obtained from spiked lipid samples were fractionated by following the three procedures specified under Experimental; the non-spiked sample did not give significant peaks. Regarding the removal of lipids from the extracts, which is necessary for the use of ECD, the blanks obtained from the three procedures (first-eluted fraction) are shown in Fig. 2. The standard and Stimac procedures gave similar, fairly clean chromatograms with no significant peaks at the retention times at which pesticides are eluted. This was also so with the Mills *et al.* procedure, although the noise in the blank was clearly higher; in fact, the eluted fractions showed slight turbidity.

Fractionation and recoveries. The results of fractionation, after passing the extracts through the Florisil column and eluting as detailed under Experimental, are given in Tables IV–VI. Recoveries were calculated from internal calibration graphs. Different standard pesticide mixtures in the concentration range 5–140 ppb, also containing 40 ppb of aldrin as internal standard, were used to prepare these calibration graphs. In the standard procedure, no pes-

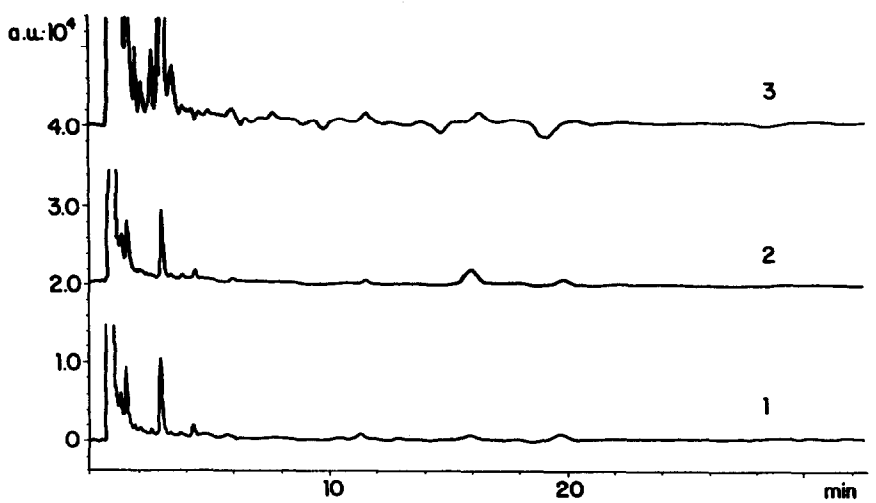

Fig. 2. Gas chromatograms from unfortified sample extracts. For conditions of analysis, see Experimental. Procedures: 1 = standard [19]; 2 = Stimac [21]; 3 = Mills *et al.* [22].

TABLE IV

STANDARD PROCEDURE [19]: FRACTIONATION AND RECOVERIES

Conditions as in Experimental. DE–LP = diethyl ether–light petroleum; Rec. = recovery and R.S.D. = relative standard deviation for six determinations.

Pesticide	Eluent					
	DE–LP (6:94, v/v)		DE–LP (15:85, v/v)		DE–LP (50:50, v/v)	
	Rec. (%)	R.S.D. (%)	Rec. (%)	R.S.D. (%)	Rec. (%)	R.S.D. (%)
Captan	–		–		90.2	5.12
Chlorfenson	–		101.3	8.07	–	
p,p'-DDD	93.5	6.98	–		–	
o,p'-DDE	94.8	6.97	–		–	
p,p'-DDE	95.3	5.74	–		–	
o,p'-DDT	99.2	7.25	–		–	
p,p'-DDT	100.5	9.20	–		–	
Dicofol	87.8	6.58	–		–	
Dieldrin	–		92.8	9.35	–	
Endosulfan-I	–		91.7	5.80	–	
Endosulfan-II	–		–		99.7	8.83
Endrin	–		89.8	7.26	–	
HCB	98.2	7.16	–		–	
Heptachlor	99.7	7.14	–		–	
Heptachlor epoxide	92.7	4.99	–		–	
Lindane	96.5	6.93	–		–	
Methoxychlor	98.2	6.22	–		–	
Mirex	99.3	10.06	–		–	
Tetradifon	–		–		97.0	6.71
Trifluralin	90.8	4.54	–		–	

ticide appeared in significant amounts in more than one eluted fraction. Further, no disagreements appeared with respect to the data from the literature.

The Stimac procedure involves partial Florisil deactivation by running 50 ml of diethyl ether–light petroleum (30:70, v/v) before the sample; this may explain differences from the standard procedure, although both of them use diethyl ether–light petroleum (6:94, v/v) as the first eluent. When the Stimac procedure was used, two pesticides, dieldrin and endrin, were distributed over two eluted fractions, which was not in agreement with the literature. The reproducibility, expressed as the relative standard deviation, confirmed these results. An eluent consisting of diethyl ether-light petroleum (35:65, v/v) was necessary to elute these pesticides completely in addition to captan, chlorfenson, endosulfan-II and tetradifon.

When the Mills *et al.* procedure was used, only heptachlor epoxide was distributed over two eluted fractions, and dicofol was limited to eluted fraction B; these results are in disagreement with those reported in the original paper by Mills *et al.* [22]. According to Mills *et al.* [22], heptachlor epoxide should appear in eluted fraction B and dicofol should be distributed over eluted fractions A and B. This disagreement was also found using hexane pesticide standard solutions, so it cannot be attributed to the polarity of the sample. No explanation has been found.

The results obtained and the relative standard deviations are given in Tables IV–VI. They show that total recoveries are close to 100%. It must be emphasized that the relative standard deviations are usually lower than 10–11% except for those pesticides which are distributed over two eluted fractions such as dieldrin and endrin in the Stimac procedure. These results are compared in Table VII

TABLE V

STIMAC PROCEDURE [21]: FRACTIONATION AND RECOVERIES

Conditions as in Experimental. Abbreviations as in Table IV.

Pesticide	Eluent			
	DE–LP (6:94, v/v)		DE–LP (35:65, v/v)[a]	
	Rec. (%)	R.S.D. (%)	Rec. (%)	R.S.D. (%)
Captan	–		88.8	6.66
Chlorfenson	–		99.5	8.45
p,p'-DDD	94.5	4.95	–	
o,p'-DDE	94.7	4.80	–	
p,p'-DDE	96.5	4.76	–	
o,p'-DDT	97.3	4.89	–	
p,p'-DDT	101.2	7.51	–	
Dicofol	88.7	5.28	–	
Dieldrin	22.3	44.98	78.0	10.09
Endosulfan-I	107.7	5.17	–	
Endosulfan-II	–		97.8	3.00
Endrin	14.5	24.97	77.7	10.09
HCB	98.2	7.16	–	
Heptachlor	102.8	8.81	–	
Heptachlor epoxide	94.2	5.94	–	
Lindane	96.0	9.61	–	
Methoxychlor	101.2	7.19	–	
Mirex	102.0	7.86	–	
Tetradifon	–		94.7	5.33
Trifluralin	88.5	7.56	–	

[a] This fraction was not used in the original Stimac method.

and Fig. 3, in which these aspects are apparent. For pesticides distributed over two eluted fractions the total percentage was used for calculation purposes.

Precision and accuracy. The precision and accuracy of the three methods were compared by analysis of variance (ANOVA) [24,25]. The relative standard deviations were not different at the 0.05 significance level. Regarding accuracy, significant differences at the 0.05 significance level were observed only for dicofol and captan. For these compounds, the best procedure seems to be that proposed by Mills *et al.* [22], as its precision is similar to that of the other procedures studied but its recoveries are closer to 100%.

CONCLUSIONS

We conclude that the three methods studied give good and similar results for the determination of eighteen of the twenty organochlorine pesticides studied. However, the method of Mills *et al.* [22] seems to be superior for the determination of dicofol and captan because its precision is similar to that of the other two methods but the recoveries are closer to 100%; the standard method seems the most suitable with regard to lipid removal from the *n*-hexane extracts and pesticide distribution in a single eluted fraction.

TABLE VI

MILLS *et al.* PROCEDURE [22]: FRACTIONATION AND RECOVERIES

Conditions as in Experimental. For eluents A, B and C, see procedure; Rec. and R.S.D. as in Table IV.

Pesticide	Eluent					
	A		B		C	
	Rec. (%)	R.S.D. (%)	Rec. (%)	R.S.D. (%)	Rec. (%)	R.S.D. (%)
Captan	–		–		105.8	5.46
Chlorfenson	–		106.3	6.11	–	
p,p'-DDD	88.7	4.60	–		–	
o,p'-DDE	92.0	3.76	–		–	
p,p'-DDE	95.3	6.00	–		–	
o,p'-DDT	96.7	5.31	–		–	
p,p'-DDT	99.7	3.57	–		–	
Dicofol	–		106.8	3.31	–	
Dieldrin	–		96.7	3.68	–	
Endosulfan-I	–		98.3	3.26	–	
Endosulfan-II	–		101.7	5.48	–	
Endrin	–		96.0	4.97	–	
HCB	91.3	6.27	–		–	
Heptachlor	95.7	4.61	–		–	
Heptachlor epoxide	13.8	11.59	87.3	4.08	–	
Lindane	91.3	10.77	–		–	
Methoxychlor	–		99.2	3.22	–	
Mirex	95.8	5.34	–		–	
Tetradifon	–		98.2	4.88	–	
Trifluralin	–		95.0	10.32	–	

TABLE VII

TOTAL RECOVERIES OF PESTICIDES BY THE THREE METHODS STUDIED

No.	Pesticide	Recovery (%)		
		Standard [19]	Stimac [21]	Mills *et al.* [22]
1	Captan	90.2	88.8	105.8
2	Chlorfenson	101.3	99.5	106.3
3	*p,p'*-DDD	93.5	94.5	88.7
4	*o,p'*-DDE	94.8	94.7	92.0
5	*p,p'*-DDE	95.3	96.5	95.3
6	*o,p'*-DDT	99.2	97.3	96.7
7	*p,p'*-DDT	100.5	101.2	99.7
8	Dicofol	87.8	88.7	106.8
9	Dieldrin	92.8	100.3	96.7
10	Endosulfan-I	91.7	107.7	98.3
11	Endosulfan-II	99.7	97.8	101.7
12	Endrin	89.8	92.2	96.0
13	HCB	98.2	100.2	91.3
14	Heptachlor	99.7	102.8	95.7
15	Heptachlor epoxide	92.7	94.2	101.2
16	Lindane	96.5	96.0	91.3
17	Methoxychlor	98.2	101.2	99.2
18	Mirex	99.3	102.0	95.8
19	Tetradifon	97.0	94.7	98.2
20	Trifluralin	90.8	88.5	95.0

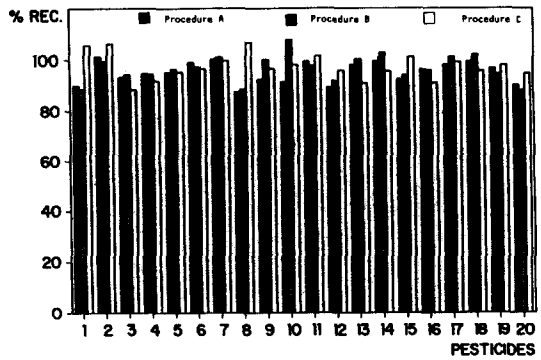

Fig. 3. Comparison of recoveries. Procedures: A = standard [19]; B = Stimac [21]; C = Mills *et al.* [22]. Compound numbers as in Table VII.

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