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THE SEPARATION OF FIFTY PESTICIDES AND RELATED COMPOUNDS AND POLYCHLOROBIPHENYLS INTO FOUR GROUPS BY SILICA GEL MICROCOLUMN CHROMATOGRAPHY

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SUMMARY

An analytical scheme used in the study of the pollution of surface water by pesticides in Italy is described, and the separation of pesticides and polychlorobiphenyls into four groups by chromatography on a silica gel microcolumn is explained in detail.

The advantages of the more accurate gas-chromatographic identification of pesticides present in the fractions eluted from silica gel are shown. Several organophosphorus pesticides were also identified in the waters by the technique described, the persistence of which in the environment had not previously been suspected.

INTRODUCTION

The need for standardised techniques to separate complex mixtures of pesticides into groups is felt especially when investigations are conducted on environmental pollution, particularly pollution of surface waters. In such investigations, the analyses are usually made using gas chromatographic (GC) methods, but the interpretation of gas chromatograms is very difficult due either to the high number of constituents and their metabolites that have to be determined in amounts of fractions of a part per billion (μg/l), or to the simultaneous presence of substances that may interfere in the determinations because their retention volumes are very similar to those of certain pesticides, *e.g.* polychlorobiphenyls[1].

KADOUM[2,3] suggested the use of microcolumns of ultra-pure silica gel for the purification of extracts from different origins (food, soil, waters). Small alumina and silica gel columns had been tested by HOLDEN AND MARSDEN[4] to purify animal tissue extracts containing chlorinated pesticides, and recently LAW AND GOERLITZ[5] and JOHNSON[6] have proposed the use of alumina, Florisil or silica gel microcolumns to purify extracts for the analysis of pesticides in waters.

In 1967–1968 we had to establish a scheme of analysis for a research programme on pollution of pesticides in the main Italian drainage basins: preliminary tests showed that a rigorous clean-up of the water samples extracts was necessary and that the pesticides had to be separated into groups before the GC determination. The type of silica gel proposed by KADOUM[2] was chosen to complete the purification process and

to standardise the separation of the pesticides into four groups by successive elutions from the same column with solvents of increasing polarity. Some aspects of the analytical method used have already been published, such as the GC separation of pesticides recoverable in elution groups from the silica gel[7] and the use of thin-layer chromatography (TLC) to evaluate the anti-cholinesterase activity (anti-ChE) of the samples and to confirm the presence of organophosphorus pesticides in waters[8,7]. The complete results of the investigation on pollution of the main Italian drainage basins by pesticides during 1969 have also been published[9].

In this study, the previous analytical scheme was used in the research on pesticides in samples of superficial or drinking waters, and the separation of the pesticides into groups by chromatography on a silica gel microcolumn is described in detail. This technique may also be usefully applied to the analysis of pesticides in samples other than waters (*e.g.* the determination of residues in food).

EXPERIMENTAL

Analytical method

All solvents and other materials used were of "pesticide grade" and were concentrated and analysed by gas–liquid chromatography (GLC) using an electron-capture detector; all precautions commonly taken in these analyses were respected, above all those concerning the washing of the glassware with solvents, the technique of concentrating the solution and the pre-extraction of reagents and materials to be used.

A sample (10–15 l for surface water, 20–25 l for drinking water) was taken and stored, where possible, in glass containers, filtered if necessary through wire gauze to remove the largest suspended particles, and was acidified with concentrated hydrochloric acid to pH 1–3 and then subjected to continuous liquid–liquid extraction using an apparatus similar to that of KAHN AND WAYMAN[10] made up of three extraction stages in series. In the first and second stages, petroleum ether (b.p. 40–60) was used as solvent, and in the third stage benzene. At the end of the extraction, the benzene solution from the third stage was evaporated, the residue taken up in hot petroleum ether and the solution combined with those of the other two stages. The combined solutions were concentrated to 15 ml and partly purified by repartition with acetonitrile saturated with petroleum ether (4 × 30 ml) according to the commonly used technique of purifying food extracts from fats for determinations of pesticide residues[11]; this technique, when applied to pesticides in waters, was found to remove more than 50 % of the material that interfered in GC analyses with the electron-capture detector. The solutions of acetonitrile were combined and diluted with 700 ml of a 2 % solution of sodium chloride; the mixture was extracted twice with petroleum ether (2 × 100 ml). The mixed acetonitrile/aqueous sodium chloride phase was rejected and the combined solutions of petroleum ether were washed twice with distilled water (2 × 100 ml). The petroleum ether solution, filtered on a cotton-wool plug, was concentrated to a small volume and poured into a special small container, then completely evaporated and treated with a known volume (usually 1 ml) of *n*-hexane. All the processes described were quantitative.

In our laboratories, preliminary tests for the identification of organophosphorus pesticide groups and semi-quantitative evaluations of the anti-ChE activity of the samples[8] were carried out by TLC on a portion of this solution (200–300 μl); with the

remaining portion of the solution, the purifying process was completed and the simultaneous separation of pesticides into groups was achieved by chromatography on a silica gel microcolumn.

Chromatography on a silica gel microcolumn

Reagents and materials. Standard solution of pesticides (Table I) and of various polychlorobiphenyls were prepared in *n*-hexane (to a final concentration of 20 μg/ml). The polychlorobiphenyls (Fenclor, Caffaro Co. S.p.A., Milan, Italy) were four different compounds containing an average of *ca.* 40, 52, 60 and 65 % of chlorine, respectively, and were made up of mixtures of tri-chloro- to hepta- or octa-chlorobiphenyl isomers. To standardise the chromatographic separation, it was necessary to prepare a cumulative standard of pesticides having different concentrations (with reference to the sensitivity of the electron-capture detector for the different constituents) so that it contained *ca.* 5 μg of *p,p'*-DDT, 1.5 μg of dieldrin, 5 μg of methylparathion and 10 μg of malathion per ml of solution.

The absorbent used was Silica Gel grade 950, 60–200 mesh, Davison Code 950-08-08-220 (Grace Davison Chemical, Baltimore, Md. 21220, U.S.A., distributed in Europe by Fisher Scientific Corp.). The microcolumn used (Fig. 1) was made of Pyrex glass by Tecnochimica Moderna (Rome, Italy). The lower section has I.D. 4.2 mm, so that 1 g of Silica Gel grade 950 fills it to a height of 10 cm (the height is important for the reproducibility of the separation); the upper section acts as a reservoir for the eluents. The four eluents were *n*-hexane, 60 % benzene in *n*-hexane, benzene and 50 % ethyl acetate in benzene, all of them being pesticide-grade solvents.

Silica gel standardisation. The silica gel was activated before use for 2 h at 130° in an oven with pre-warmed air, using a large porcelain crucible; after activation, the silica gel, still warm, was put into a weighed erlenmeyer flask with a ground-glass stopper. After cooling, the product was weighed and partially deactivated by adding distilled water (5 % by weight). If *a* is the weighed amount of silica gel, the following calculation can be made, $a:95 = b:100$ and $b - a =$ the amount of water in g (ml) to be added to the silica gel.

The water must be added dropwise, down the walls of the flask, which was swirled continuously. The flask was then vigorously shaken for about 20 min and left to stand overnight; it was always stored in a hermetically sealed container, but *without* drying agents ($CaCl_2$). Silica gel thus prepared usually maintained its characteristics for 8–10 days.

The chromatographic technique was as follows: the lower section of the microcolumn (Fig. 1) was closed with a quartz wool plug, then 1 g (± 20 mg) of the partially deactivated silica gel was added, which filled the column to a height of 10 cm; more quartz wool was added, then a layer of anhydrous sodium sulphate, 1 cm high. Without pre-washing of the column, 1 ml of the cumulative standard of *p,p'*-DDT, dieldrin, methylparathion and malathion in *n*-hexane was added, and after absorption, 1 ml of *n*-hexane was poured in (*n*-hexane was necessary to simulate the washing of the sample container). After absorption, the upper portion of the column was joined on and it was eluted consecutively with the following mixtures, changing the collecting vessel whenever the level of eluent reached the upper portion of silica gel: I, *n*-hexane, 20 ml; II, 60 % benzene in *n*-hexane, 8 ml; III, benzene, 8 ml; IV, 50 % ethyl acetate in benzene, 14 ml. The flow speed must be *ca.* 1 ml/min and could be obtained by

TABLE I

SEPARATION OF PESTICIDES AND POLYCHLORINATED BIPHENYLS IN GROUPS BY SILICA GEL MICROCOLUMN CHROMATOGRAPHY
— = absent; + = present.

Pesticides	*Solvent system*													
	n-Hexane, 20 ml											*60% Benzene in n-hexane, 8 ml*		
	0	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*
Hexachlorobenzene (HCB)	83.4	6.0	—	—	—									
Aldrin	—	+	57.5	40.5	2.0	—	—	—						
p,p'-DDE	—	+	2.2	98.5	47.2	2.1	—	—	—					
Heptachlor	—	—	—	10.2	10.1	10.1	—	—	—					
o,p'-DDT	—	—	+	6.0	94.0	38.4	6.0	—	—	—				
Polychlorinated biphenyls[a]	—	—	—	—	—	+	+	+	+	+	—	—	+	
o,p'-DDT		—	—	—	10.7	51.2	27.1	9.7	—	—	—			
p,p'-DDT			—	—	—	3.1	30.5	30.5	24.0	9.3	1.7	—	—	—
p,p'-MDE						—	—	—	12.3	18.7	50.2	12.8	+	—
γ-Chlordane						—	—	—	11.7	18.0	22.2	47.1	+	—
α-Chlordane						—	—	—	11.1	18.2	21.0	48.8	+	—
p,p'-DCBP								—	—	—	5.1	88.3	6.3	—
o,p'-TDE									—	—	—	65.0	1.1	—
p,p'-TDE									—	+	—	91.7	5.3	—
γ-BHC									—	—	—	93.7	6.3	—
β-BHC									—	—	—	86.0	14.0	—
α-BHC									—	—	—	93.0	3.1	—
δ-BHC									—	—	—	62.5	37.5	+
Perthane									—	—	—	56.0	43.0	—
Trithion									—	—	—	47.1	52.0	+
Bromophos[b]									—	+	—	+	+	—
Dursban									—	—	—	40.8	54.2	+
Kelthane									—	—	—	34.8	57.3	
Heptachlor epoxide									—	—	+	31.0	9.3	
Endosulfan I									—	—	—	30.5	62.5	
Ronnel									—	—	—	25.0	73.8	
Methyl trithion									—	—	—	23.0	7.1	—
Endrin									—	—	—	4.2	5.3	3
Nogadex									—	—	—	1.0	10.5	3
Dieldrin										—	—	—	15.9	4
Silvex (methyl ester)										—	—	—	6.1	3
Endosulfan II											—	—	—	3
Ethion											—	—	—	1
Methoxychlor												—	—	+
2,4,5-T (methyl ester)												—	—	+
Dursban[c]												+	—	—
Parathion													+	—
Methyl parathion													—	—
Photodieldrin													—	—
2,4-D (methyl ester)														—
Ethyl guthion														
Captan														
Malathion[d]														
Diazinon														
Paraoxon														
Methyl paraoxon														
Methyl guthion[b]														
Dimethoate[e]														

[a] Evaluation by GLC with electron-capture detection.
[b] Evaluation by TLC with anti-cholinesterase detection.
[c] Recovery *ca.* 55%.
[d] Recovery *ca.* 90–95%.
[e] Recovery *ca.* 52%.

	100% Benzene, 8 ml				50% Ethyl acetate in benzene, 14 ml						
14	15	16	17	18	19	20	21	22	23	24	25
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7.0	–	–	–								
–	–	–									
0.0	–	–	–								
30.0	19.1	–	–	–							
41.5	18.5	–	–	–							
31.7	31.0	13.0	–	–	–						
38.0	50.0	11.1	–	–	–	–					
0.0	52.0	37.5	3.0	–	–	–	–				
–	–	–	–	–							
–	04.1	20.0	0.0	–	–	–					
–	11.0	45.0	13.1	–	–	–	–				
–	15.0	11.0	27.1	11.8	–	–	–				
–	–	50.0	31.8	8.3	–	–	–				
	–	–	–	8.2	87.7	4.1	–	–	–		
		–	–	–	95.0	5.0	–	–	–		
		–	–	–	92.0	8.0	–	–	–		
		–	–	–	73.0	26.1	–	–	–		
		–	–	–	38.2	49.1	0.7	2.7	–	–	–
			–	–	–	75.3	10.3	4.0	1.1	–	–
			–	–	–	+	+	+	+	–	–
				–	–	–	+	+	+	–	–

putting a slight pressure on the column. At the end of the chromatography, the various fractions were almost dry by the use of a rotary evaporator, then they were treated with 1 ml of *n*-hexane and examined by GLC in comparison with the same cumulative standard added to the column.

For the correct use of the microcolumn, the separation and the recovery to be obtained is: 100% *p,p'*-DDT in the first fraction, 100% dieldrin in the second,

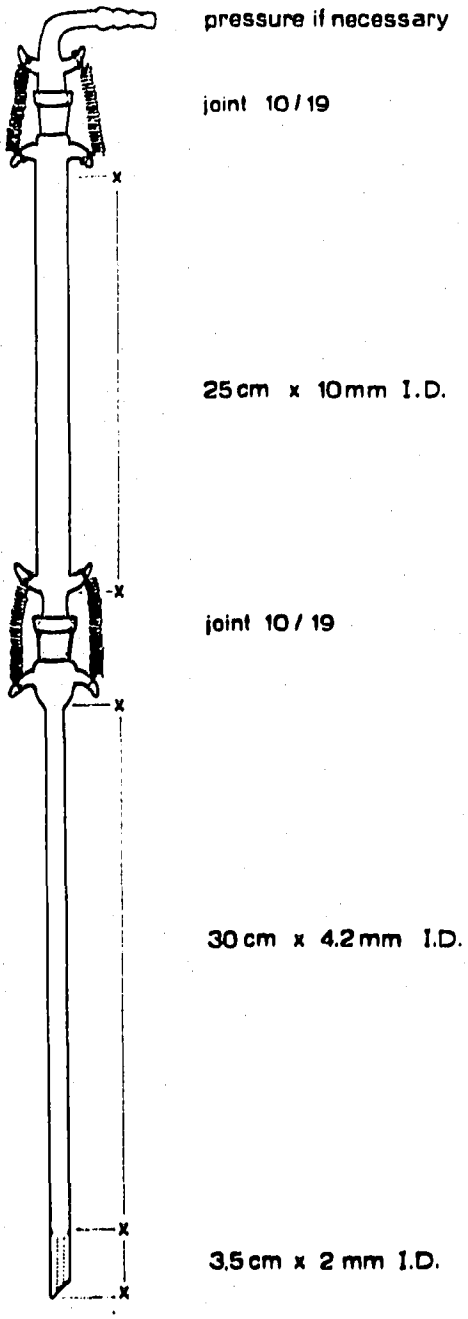

Fig. 1. The microcolumn.

100% methylparathion in the third, and malathion not less than 90% in the fourth fraction (the elution of some phosphorus esters may be critical). If the analysis involves only organochlorine pesticides, the separative ability of the silica gel may be checked by chromatography of a standard of *p,p'*-DDT + *p,p'*-TDE; the two compounds have to be separated perfectly (the *p,p'*-DDT in the first group, the *p,p'*-TDE in the second).

Usually, variations of ± 5% in the elution volumes do not cause significant changes in the separation.

The various samples of silica gel tested showed constant characteristics but if the above separation is not achieved at once, it may be obtained more easily by changing the proportions of the eluents than by changing the silica gel activity.

Using the same method as for the standardisation of the adsorbents, the extracts of water samples were chromatographed, being concentrated to 1 ml in *n*-hexane; the various fractions were then suitably concentrated and separately analysed by GLC.

RESULTS

The types of elution from the silica gel column of the pesticides examined and the polychlorobiphenyls are reported in Table I, obtained by analysing 2-ml fractions (eluted from the column after introducing 20 μg of each product) by GLC (with the electron-capture and/or a phosphor detector) or by TLC (with anti-ChE detection). The first "zero" fraction is formally made up of the solution of the sample (1 ml) and the washings (another 1 ml in all) of the container.

Except when otherwise indicated, the recovery of the various pesticides from the column was quantitative (95–100%) within experimental error, and the reported values indicate the percentages found in the different fractions with respect to the total amount eluted. Anomalies in the elution were observed for malathion (mean recovery *ca.* 90–95%), di-syston (mean recovery 55%), dimethoate (recovery of *ca.* 50%) and for thimet, which was not eluted as such.

In practice, the silica gel used was not very suitable for the chromatography of phosphorodithioic esters with short side chains: these products were in fact degraded completely (as thimet) or to a great extent (as di-syston, dimethoate) during the chromatography, giving products eluted in the fourth group.

In contrast, pesticides such as methyl- or ethyl-guthion and phosphorothioic esters (parathion, ronnel, etc.) were recovered from the column without degradation.

Some pesticides may be present in two successive elution groups, *e.g.*, α- and γ-chlordane were divided between the first and second groups and ethion, methoxychlor and others between the second and the third groups; this fact may represent a positive factor for the identification of these products.

Though the general type of separation might be changed quite easily, the scheme in Table I is nevertheless the most satisfactory for the subsequent identification of the pesticides by GLC[7]; in fact, pairs of pesticides which are difficult to separate in the usual chromatographic stationary phases (HCB/α-BHC; dieldrin/*p,p'*-DDE; *o,p*-DDT/*p,p'*-TDE; heptachlor/BHC; parathion/malathion; aldrin/ronnel, etc.) have been found in different elution groups of silica gel.

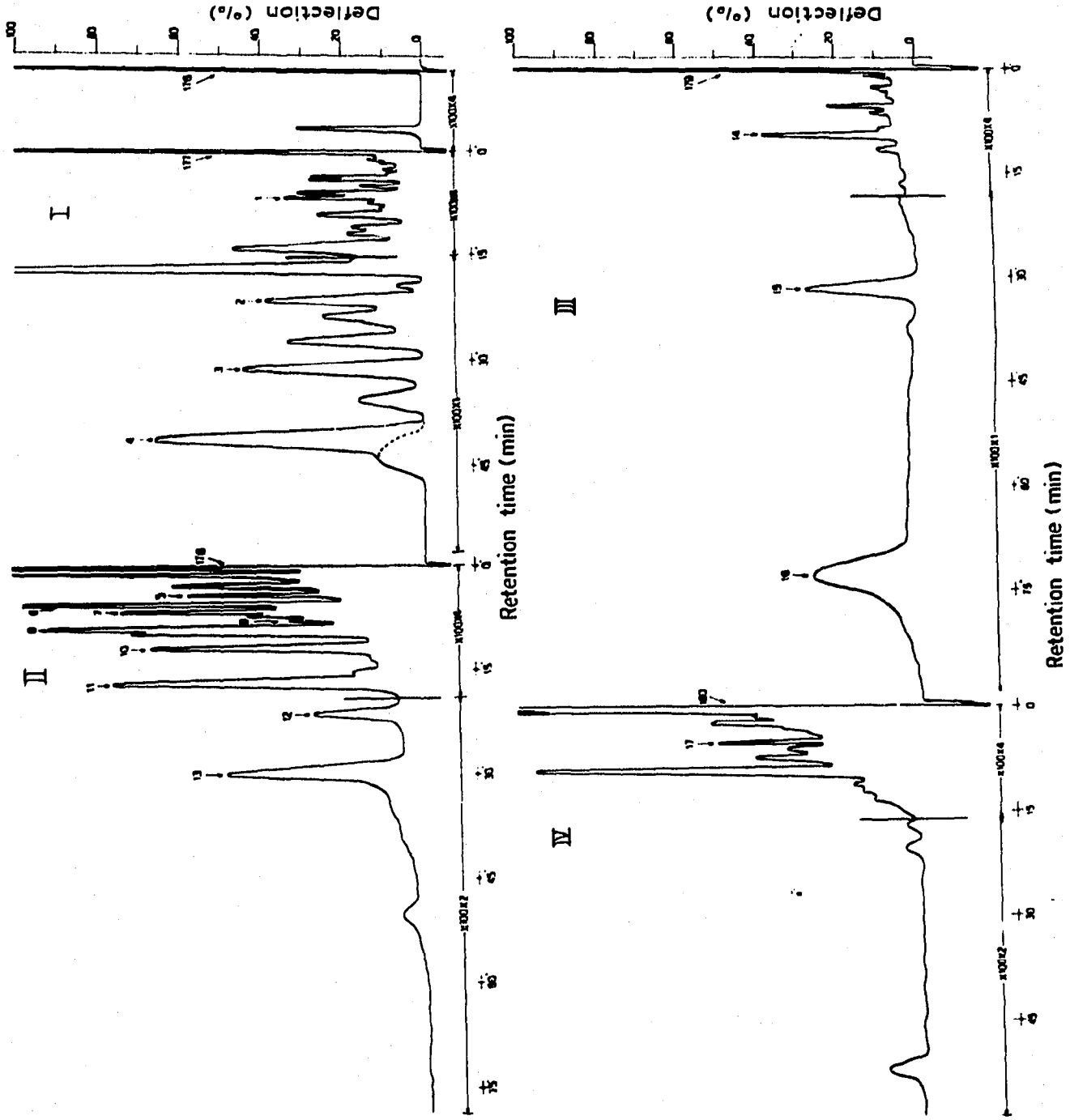

Fig. 2. Gas chromatogram, obtained using OV-17 as stationary phase and an electron-capture detector, of the four fractions eluted from silica gel of a sample of water polluted by pesticides (River Tirso, Sardinia, winter 1969). Injection 170: standard of aldrin. Injection 177: first elution group from silica gel, 1 = heptachlor; 2 = *p,p'*-DDE; 3 = *o,p'*-DDT; 4 = *p,p'*-DDT (the dotted line is an unidentified compound, interfering occasionally). Injection 178; second group, 5 = α-BHC; 6 = lindane; 7 = β-BHC; 8 = δ-BHC; 9 = ronnel; 10 = dursban; 11 = endosulfan I; 12 = dieldrin; 13 = endosulfan II. Injection 179, third group, 14 = methylparathion; 15 = endosulfan II; 16 = silica gel impurity. Injection 180, fourth group, 17 = diazinon. The presence of the four phosphorus esters (ronnel, dursban, methylparathion, diazinon) has been confirmed by TLC and then by GC on an SE-30 column with a phosphor detector in parallel with a usual flame-ionisation detector.

DISCUSSION AND CONCLUSIONS

With respect to the fate of thimet during the chromatography, our results do not agree with those of KADOUM[12], who obtained quantitative recovery by elution with benzene from the same type of silica gel; we observed that thimet was completely transformed during GC into a product which was eluted from silica gel in the fourth fraction (the thimet oxygen analogue?). Similarly, BOWMAN *et al.*[13] used silica gel columns (of the type made by Baker Chemical Corp.) to separate quantitatively di-syston from some of its metabolites, while we found that about 50 % of the di-syston was changed in the column. At present we are trying to improve the recovery of these compounds and their derivatives, because they may be involved in water pollution in Italy.

For more than two years we applied the process described for the purification of extracts and the separation of pesticides into groups to a study of surface water pollution. In Fig. 2, a gas chromatogram is shown, relative to a sample of water particularly polluted with pesticides (River Tirso, Sardinia, winter 1969); from this we discovered the need to have at our disposal a standard technique for the separation of the pesticides into groups. In fact, with this method, pollution of Italian waters was proved[9] to be caused not only by organochlorine pesticides, which are notoriously persistent, but also by organophosphorus pesticides (such as ronnel, parathion, dursban, malathion and diazinon), the persistence of which in the environment was not exactly known.

We are now also studying the suspected pollution of waters by polychlorobiphenyls with a method similar to that described, but using mass spectrometry combined with gas chromatography.

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