SOME OBSERVATIONS ON THE THIN-LAYER CHROMATOGRAPHY OF ORGANO-CHLORINE PESTICIDES

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Thin-layer chromatography has grown rapidly in importance in recent years and is now accepted as a quick and efficient technique for the detection and determination of compounds of many differing types, both organic and inorganic. However, its use in the field of pesticide residue analysis appears to have been rather limited. Starch-bound silica gel plates have been used[1] for the separation of aldrin, dieldrin and endrin, while more detailed studies have been made by PETROWITZ[2] and KOVACS[3]. WALKER AND BEROZA[4] included 16 organo-chlorine compounds among the 61 pesticides they examined by 19 solvent systems on silica-gel plates but few of them were sufficiently resolved to be of any positive assistance in the identification of residues, particularly where several pesticides occur together. Quite good separation of six organo-chlorine insecticides has been claimed[5], using activated-alumina (200–220°) plates with hexane as mobile solvent. More recently loose-layer chromatography has been used[6] to separate organo-chlorine pesticides into two groups prior to gas–liquid chromatographic determination.

In this laboratory, thin-layer chromatography has been used for the separation and identification of some herbicides[7]; this work has now been extended to cover several organo-chlorine insecticides and some of the metabolites of DDT. Several separatory systems have been devised which are intended for use for the confirmation of the provisional identifications provided by gas–liquid chromatography[8,9], or paper chromatography[10,11], of pesticide residues present in animal or vegetable tissue.

EXPERIMENTAL

Apparatus

A Desaga thin-layer spreading apparatus (obtainable from Camlab Ltd., Cambridge) was used.

Materials

Silica gel G, alumina G, and kieselguhr G (all obtainable from E. Merck, Darmstadt) were used throughout, either singly or in admixture.

Pesticide solutions

Solutions, 5 mg per ml, of the pesticides examined were made in ethyl acetate.

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Chromatographic technique

The carrier plates (20 cm × 20 cm) were layered (250 μ thick) in the usual way[3], dried and activated by heating in an air oven at 120° for 2 h. Activated plates were then stored over silica gel until used, preferably within 36 h of preparation. The pesticide solutions were spotted with a 1 μl micro-pipette at 1 cm intervals along an origin line 1.5 cm from one edge of the plate and parallel to it; a second line was marked 15 cm from the origin. Each plate was developed by ascending chromatography in a suitable tank[10], under conditions of equilibrium, until the solvent front reached the second line, generally between 45 min and 2 h from commencement. The plate was then removed from the tank and dried at 100° for 10 min prior to spot development by one of the systems described below.

Spot development systems

Several types of spot indicators have been investigated. The "chromogenic reagent" used by MITCHELL[11], silver nitrate with 2-phenoxyethanol in ethanol, has been found to be less satisfactory for use with thin-layer plates than it is for paper chromatograms[10]. Owing to the rapid darkening of the background, the shapes of the spots were rather poorly defined and small amounts of pesticides were readily overlooked owing to the faintness of the spot.

The use of a simple ethanolic solution of silver nitrate (0.5 % w/v) as a spray reagent, followed by irradiation with germicidal ultraviolet light for 10 min, has proved very satisfactory, well-defined black-brown spots being obtained on an off-white background which is slow to darken. Because of its simplicity, this system has been used for most of the work described here, although several other promising indicator systems have also been examined as shown below. This silver nitrate indicator may also be used semi-quantitatively; 0.5 μg or less of most of the pesticides was readily observed although α- and γ-BHC required longer irradiation (30 min).

The combination of a pH indicator material with silver nitrate, both with and without ultraviolet irradiation, has given some colourful plates; in each case the developed plates were sprayed with 0.5 % ethanolic silver nitrate, dried, sprayed with indicator (0.2 % ethanolic) and dried again before irradiation. Bromocresol green gave yellow spots on a green-blue background after irradiation, the spots becoming a deep orange colour on heating. Yellow spots on blue were shown by bromocresol purple but the spots faded to off-white on irradiation and gave poor definition. Spraying with bromothymol blue yielded yellow spots on a white basis, the spots darkening on irradiation. Methyl orange was not very suitable, giving pale-pink spots on a white background.

The best indicator of this type that we have examined comprises a mixture of bromophenol blue with silver nitrate; a similar system has been used by WOOD[12] for the marking of chloride and bromide spots on paper. Following spraying with 0.5 % ethanolic silver nitrate, the plates were dried for 5 min at 100° and then sprayed with a solution of bromophenol blue (0.2 %) and silver nitrate (0.15 %) in a mixture of equal volumes of ethanol and ethyl acetate. On further drying at 100° for 10 min the pesticides were marked as bright yellow well-defined spots superimposed upon a deep blue background; irradiation with ultraviolet light had no effect on this coloration, which was stable for at least a week.

Some thin-layer plates have also been prepared in which silver nitrate has been

"built-in" either during layering or by spray application after layering in the usual way. Addition of silver nitrate to the layer-mix gives rise to rapid gelation; this has been offset by reducing the mixing-time, prior to pouring the mix into the spreader, by one quarter. No alteration of R_F values was observed on using these plates.

Separatory systems investigated

The bulk of the work described here has been carried out on silica gel, with some use of alumina and silica gel–alumina mixtures. Preliminary studies showed that kieselguhr was unlikely to be satisfactory, most of the pesticides migrating with the solvent front or else streaking badly. In general the mobile solvents used were of one of three types: (a) light hydrocarbon solvents; (b) light hydrocarbon solvent together with liquid paraffin or silicone oil; (c) solvents similar to (b) together with dioxane. The R_F values obtained in this study are listed in Table I.

TABLE I

R_F VALUES OF PESTICIDES USING VARIOUS SEPARATORY SYSTEMS

Compound	$R_F \times 100$ in system														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Aldrin	88	98	73	58	69	67	70	79	64	62	67	98	82	95	70
α-BHC	—	69	—	—	43	37	—	59	28	29	52	92	63	87	—
γ-BHC	—	58	—	—	37	27	—	47	18	19	46	94	55	78	—
p,p'-DDE	87	98	87	74	62	61	68	73	57	56	65	98	78	95	65
o,p'-DDT	71	90	74	50	58	54	62	71	46	48	59	97	73	89	50
p,p'-DDT	72	91	78	52	54	49	60	69	39	40	57	98	69	89	42
de-HCl-*p,p'*-TDE	85	98	88	67	62	61	72	76	53	51	49	98	75	93	53
p,p'-Dichlorobenzophenone	—	—	—	—	48	45	53	67	27	26	59	92	55	31	14
Dieldrin	69	58	53	30	48	41	46	63	48	54	65	88	52	37	12
Endosulfan A	—	—	—	—	52	47	63	61	35	31	58	94	64	65	17
Endosulfan B	—	—	—	—	—	—	—	—	—	—	12	86	9	4	2
Endrin	—	—	—	—	52	42	58	65	26	26	49	88	61	51	13
Heptachlor	82	98	69	48	62	61	65	73	53	52	65	88	78	95	58
Heptachlor epoxide	—	—	—	—	—	—	—	—	—	—	39	88	57	49	17
Methoxychlor	—	—	—	28	36	27	30	45	10	13	—	—	—	—	—
p,p'-TDE	66	77	58	67	46	33	45	59	26	28	52	92	57	71	25

Key to systems

System No.	Plate	Mobile solvent
1	Silica gel–alumina (1:1)	Cyclohexane–liq. paraffin (20%)
2	Silica gel–alumina (1:1)	Cyclohexane–silicone oil (8%)
3	Silica gel	Cyclohexane–*n*-hexane (1:1)
4	Silica gel	Cyclohexane–benzene (1:1)–liq. paraffin (10%)
5	Silica gel	Cyclohexane–liq. paraffin (20%)–dioxane (10%)
6	Silica gel	Cyclohexane–liq. paraffin (20%)–dioxane (5%)
7	Silica gel	Cyclohexane–liq. paraffin (10%)–dioxane (3.5%)
8	Silica gel	Cyclohexane–liq. paraffin (5%)–dioxane (2%)
9	Silica gel	Pet. ether (40–60°)–liq. paraffin (20%)
10	Silica gel	Pet. ether–liq. paraffin (10%)
11	Silica gel	Pet. ether–liq. paraffin (5%)–dioxane (1%)
12	Kieselguhr	as 11
13	Alumina	as 11
14	Alumina	*n*-Hexane
15	Silica gel	*n*-Hexane

Development of 1:1, silica gel–alumina plates with cyclohexane containing 20 % v/v of liquid paraffin showed some promise. Replacement of the paraffin with a silicone oil (8 %) gave good separation of *p,p'*-DDE, dieldrin and *p,p'*-TDE from each other, but did not resolve aldrin, *p,p'*-DDE, dehydrochlorinated *p,p'*-TDE and heptachlor, all of which travelled very close to the solvent front. Better separations were obtained when silica gel plates were developed in cyclohexane–*n*-hexane or cyclohexane–benzene–liquid paraffin; this system gave particularly good separation of a mixture containing aldrin, *p,p'*-DDE, dehydrochlorinated *p,p'*-TDE, dieldrin and heptachlor.

The conjunction of various proportions of liquid paraffin and dioxane with cyclohexane as the mobile phase has been studied with silica gel plates. From Table I it may be seen that by suitable choice of the appropriate mixture of the three components of the mobile phase separations, of α- and γ-BHC or of dieldrin and *p,p'*-TDE for example, may be improved. Replacement of cyclohexane with petroleum ether (40–60°) in systems of this type has given other useful separations, *e.g.*, aldrin–heptachlor (system 9), *o,p'*-DDT–*p,p'*-DDT (system 10) and endosulfan A–endosulfan B (system 11).

Kieselguhr was again shown to be of little use with solvents of this type but alumina showed more promise than had previously been achieved with this material. The use of an alumina plate with *n*-hexane alone as mobile phase was found to be very suitable for the separation of endrin from *p,p'*-DDT and of dieldrin from *p,p'*-DDE; using this system up to nine separate spots could be obtained from a single mixture. Application of the same solvent to silica gel plates proved similarly useful, good resolution being obtained of those compounds whose R_F values were rather high on the alumina plate, *i.e.*, aldrin, heptachlor, *p,p'*-DDE, *o,p'*-DDT, *p,p'*-DDT and endosulfan A.

TABLE II

SYSTEM OF CHOICE FOR A GIVEN SEPARATION

Numbers refer to systems detailed in the key to Table I.

	Aldrin											
α-BHC	9	α-BHC										
γ-BHC	9	8	γ-BHC									
p,p'-DDE	4	9	9	*p,p'*-DDE								
o,p'-DDT	9	2	2	4	*o,p'*-DDT							
p,p'-DDT	9	2	2	15	15	*p,p'*-DDT						
de-HCl-*p,p'*-TDE	15	9	2	11	4	9	de-HCl-*p,p'*-TDE					
Dieldrin	15	10	14	14	14	14	14	Dieldrin				
Endosulfan A	15	14	9	15	15	15	15	14	Endosulfan A			
Endrin	15	14	14	15	15	14	14	10	14	Endrin		
Heptachlor	15	2	2	15	1	15	4	14	15	15	Heptachlor	
Heptachlor epoxide	15	14	14	15	14	14	14	11	11	11	14	Heptachlor epoxide
p,p'-TDE	15	14	2	15	15	3	3	14	7	14	15	14

From the results given in Table I the system for the best separation of any given pair of the compounds may be chosen. Table II lists the selected systems. From Table II it is obvious that a few systems are of general utility. Six such systems are listed in Table III, together with a selection of resolvable pesticides.

As can be seen from these tables, by choice of the appropriate thin-layer system,

TABLE III

COMPOUNDS RESOLVED BY PREFERRED SYSTEMS

System No. (Table I)	*Resolved compounds*
2	α-BHC, γ-BHC, *p,p'*-DDE, *p,p'*-DDT, *p,p'*-TDE
4	Aldrin, *p,p'*-DDE, dehydrochlorinated *p,p'*-TDE, dieldrin, heptachlor
9	Aldrin, α-BHC, γ-BHC, *p,p'*-DDT, heptachlor, methoxychlor
11	Dieldrin, *p,p'*-DDT, endrin, heptachlor epoxide, endosulfan B
14	α-BHC, γ-BHC, dichlorobenzophenone, dieldrin, endosulfan A, endosulfan B, heptachlor, heptachlor epoxide, *p,p'*-TDE
15	Aldrin, *p,p'*-DDE, *o,p'*-DDT, *p,p'*-DDT, dieldrin, endosulfan B, heptachlor, heptachlor epoxide, *p,p'*-TDE

it is possible to separate all of the pesticides examined one from another. The resolution and identification of the components of mixtures of pesticide residues is also possible. The procedures described have been successfully applied to extracts of vegetable and animal origin which have been "cleaned-up" by a N,N-dimethylformamide partition process[13].

Effect of temperature of development on R_F value

Differing views have been expressed in the literature as to the extent of the effect of temperature upon development times and R_F values in thin-layer chromatography. STAHL *et al.*[14], studying essential oils on silica gel plates developed in a hexane–acetic acid mixture, found no alteration in running time on changing the temperature from 20° to either 4° or 28°. The effects of insecure closure of the tank and variation in the depth of immersion in the mobile phase were much more important. Similarly BRENNER *et al.*[15] found that raising the temperature from 18° to 38° had virtually no effect on the R_F values of a number of amino acids developed with a phenol–water mixture, although the reproducibility of these values became poorer.

MULLER AND HONERLAGEN[16], however, in their study of the chromatography of cinchona bark alkaloids with a mixed kerosine–diethylamine–acetone solvent, found that the R_F values were strongly temperature dependent; they advised the use of 25° as being the most convenient for their purpose. HARTHON[17] also found that the control of the development temperature was critical if constant R_F values of nitramine-explosives were to be obtained. To guard against small variations in R_F he used a reference compound on every silica gel plate developed in a petroleum ether–acetone mixture.

The R_F values of the pesticides given in Table I were obtained at room temperatures (15–20°). Attempts have now been made to show the effects of development at different temperatures ranging from —20° (deep-freeze cabinet) to 40° (warm water-bath). Silica gel plates were developed in *n*-hexane for 40 min, a metal tank being used for the highest and lowest temperatures. Spots were marked by the silver nitrate–ultraviolet irradiation process described. For results obtained see Table IV.

It is clear that the R_F values of all of these compounds were temperature dependent to some extent. Compounds with R_F values above 0.40 at room temperature showed the greatest variation. Endrin, dieldrin and endosulfan A, with R_F values

TABLE IV

VARIATION IN R_F WITH DEVELOPMENT TEMPERATURE

Compound	$R_F \times 100$ at temperature					
	—20°	0°	10°	20°	30°	40°
Aldrin	33	55	68	77	85	90
p,p'-DDE	27	49	60	70	80	90
o,p'-DDT	20	37	50	58	68	77
p,p'-DDT	17	31	40	48	58	70
de-HCl-*p,p'*-TDE	21	42	48	60	70	79
Dieldrin	1	7	10	12	12	12
Endosulfan A	6	14	16	17	17	17
Endosulfan B	2	2	3	4	4	4
Endrin	2	9	10	11	12	12
Heptachlor	22	45	55	65	75	86
Heptachlor epoxide	4	13	15	17	18	19
p,p'-TDE	12	21	25	32	38	42

below 0.20 at room temperature, showed almost no variation at higher temperatures but gave a decided drop in R_F value at —20°. *p,p'*-TDE held an intermediate position in all respects.

For practical purposes chromatography at 0° has some uses. Development is more rapid and consequently more compact spots are obtained, thus improving the resolution obtained between pesticides of close R_F values, *e.g.* dieldrin and heptachlor epoxide or heptachlor and *p,p'*-DDE. For development at the higher temperature it was essential to pre-heat the prepared plate to the required temperature before placing it in the chromatographic tank; without this precaution immediate condensation of the solvent occurred on to the plate.

Documentation of thin-layer plates

During this study the need arose for a simple and rapid method for recording the chromatograms obtained in a permanent form. Preservation of the actual chromatogram either on or off[18] the plate was discounted on the grounds of the cost of the carrier plates, the fragile nature of the layer and the impermanence of the developed image. Photographic methods[19], while excellent for coloured compounds and the preparation of slides for projection, involve expensive equipment and lengthy processing; commercial photo-copying apparatus is also expensive although suitable[20].

Attention has recently been given to the use of photosensitive papers[21,22] for documentation purposes. A dry process has been advocated by EISENBERG[23], using a direct positive Diazo blueprint paper to produce positive replicates of chromatograms. Diazo papers readily available to us appeared to be somewhat slower than that used by EISENBERG and his exposure time, 10 min, was already inconveniently long. However, a study of this method involving several grades of ammonia-process paper and different lighting systems has led to the evolution of the procedure detailed below. The papers may be handled openly under normal laboratory lighting conditions without loss in sensitivity. The final prints are permanent and are readily filed for storage. Although the use of overrun photoflood bulbs is advised, ordinary 60 W or 100 W bulbs can be used provided the exposure time is increased accordingly.

Documentation method

Circumscribe the spots on the developed plate down to the glass with a soft-lead pencil or scrape them completely from the plate. (Fluorescent spots may be so marked under suitable irradiation and weak spots are suitably defined against an opal illuminated background.) Similarly mark the spot origin and the solvent front, remove the excised material by blowing gently. Place the marked plate face downward on a sheet of the Diazo paper slightly longer than the carrier plate. "Ammonax" 8.M.13 Positive Diazo Paper, Hall-Harding Ltd., London and "Densblack" Ammonia Process Paper, Type 45, 9X, E. N. Mason and Sons Ltd., Colchester, have been found suitable. The former blue paper is about three times as fast as the "Densblack" but the greater contrast given by the black background of this paper gives better definition.

Illuminate from above with two 275 W photoflood bulbs mounted in a 25 cm × 25 cm × 20 cm reflector placed centrally over the plate, the bulbs being about 10 cm above the glass surface. After 5 sec ("Ammonax") or 15 sec ("Densblack") remove the illumination source and suspend the exposed paper in an atmosphere of ammonia; a paper-chromatographic tank[10] is very suitable for this purpose. The marked spots appear as white rings or circles on the blue or black background almost immediately.

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SUMMARY

The thin-layer chromatography of several organo-chlorine insecticides and related compounds has been studied using fifteen separatory systems. Various indicators have been used and the effect of temperature upon R_F value is described. The separations obtained support other methods in establishing the identity of pesticide residues. A simple method for the documentation of thin-layer plates is also included.

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