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THIN-LAYER CHROMATOGRAPHY OF DDT AND SOME RELATED COMPOUNDS ON ALUMINUM OXIDE CHROMATOPLATES

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SUMMARY

The chromatographic behavior of DDT and fourteen related compounds, metabolites, analogs, and degradation products on commercially available pre-coated aluminum oxide thin-layer chromatoplates was investigated using thirty-three solvent systems. The color and/or fluorescence response of the DDT-type compounds and their sensitivity to a chromogenic reagent consisting of ammoniacal silver nitrate and 2-phenoxyethanol in acetone is discussed. A total of eleven compounds of the mixture were separated by two-dimensional thin-layer chromatography on aluminum oxide plates.

INTRODUCTION

The resolution of chlorinated pesticides by thin-layer chromatography (TLC) has been performed using microslides[1] and two-dimensional TLC[2]. Identification and estimation of the compounds was done by a thin-layer chromatographic–enzymatic system[3], comparison of spot size[4], chromogenic reagents[5] and UV irradiation[6].

Laboratory humidity[6,7] and temperature[8] were found to influence the TLC of chlorinated pesticides. For this reason, many workers preferred relative migration data, *e.g.* R_{DDD}[9] and R_{TDE}[10] for single and multiple development[11]. Silica gel[12–14], alumina[8–10] and silica gel or alumina incorporated with silver nitrate[4,6,15–17] are the most frequently used adsorbents for the TLC of chlorinated pesticides. Kieselguhr[8] was used to a lesser extent.

Incorporation of silver nitrate into alumina was found to be a better adsorbent for the TLC of organochlorine pesticides than silica gel incorporated with silver nitrate[4,18]. However, spraying with mixtures of silver nitrate and 2-phenoxyethanol followed by exposure to short wavelength UV light has also been used[1,9,19,20]. MORLEY AND CHIBA[12] substituted ammonia for 2-phenoxyethanol with no loss of sensitivity.

Diphenylamine[2], diphenylamine–zinc chloride[21,22], Brilliant Green[23], a combination of pH indicators such as Bromcresol Green or Bromphenol Blue with silver nitrate[8], *o*-toluidine[14] and exposure to iodine vapor[5] were among other chromogenic

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reagents used for the detection of organochlorine pesticides on thin-layer chromatograms.

The TLC work on DDT-type compounds as reported by SIEWIERSKI AND HELRICH[13] and ABOU-DONIA AND MENZEL[14] was performed on silica gel. In this study we were interested in investigating the chromatographic behavior of DDT and fourteen of its closely related — readily available — compounds on aluminum oxide to attempt to find a better and more sensitive TLC system which would improve the resolution of these compounds. In addition, the fluorescence quenching of the compounds on the TLC plates was used as a means of detection which was confirmed by spraying with a modified ammoniacal silver nitrate chromogenic reagent containing 10 % 2-phenoxyethanol.

Previous experience in the fractionation and separation of mixtures of closely related prednisolones[24], fluorinated corticosteroids[25] and estrogens[26] on commercially available pre-coated TLC plates proved their efficacy with regard to uniformity of layer thickness, homogeneity, superior resolution they provide and layer stability. Such characteristics are necessary for reproducible results when dealing with mixtures of closely related compounds. Hence, commercially available pre-coated aluminum oxide TLC plates were used.

EXPERIMENTAL

Chemicals

All chemicals were reagent grade and were used without further purification.

DDT and related compounds

1,1,1-Trichloro-2,2-bis(*p*-chlorophenyl)ethane (*p,p'*-DDT), 1,1,1-trichloro-2-(*o*-chlorophenyl)-2-(*p*-chlorophenyl)ethane (*o,p'*-DDT), 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene (*p,p'*-DDE), 1,1-dichloro-2-(*o*-chlorophenyl)-2-(*p*-chlorophenyl)ethylene (*o,p'*-DDE), 1,1-dichloro-2-(*m*-chlorophenyl)-2-(*p*-chlorophenyl)ethane (*m,p'*-DDD), 1,1-dichloro-2-(*o*-chlorophenyl)-2-(*p*-chlorophenyl)ethane (*o,p'*-DDD), bis (*p*-chlorophenyl) acetic acid (DDA), 1,1,1-trichloro-2,2-diphenylethane (DPE), and 4,4'-dichlorobenzhydrol (DBH) were obtained from Aldrich Chemical Co., Inc., Milwaukee, Wisc.; 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane (*p,p'*-DDD), 4,4'-dichlorobenzophenone (DBP), and 2,2,2-trichloro-1,1-bis(*p*-chlorophenyl)ethanol (Kelthane) from Rohm and Haas Co., Philadelphia, Pa.; 1-chloro-2,2-bis(*p*-chlorophenyl) ethylene(DDMU) from PHS, Perrine, Fla.; and 1,1-bis(*p*-chlorophenyl)ethanol (BPE) and bis(*p*-chlorophenyl)methane (DDM) from Regis Chemical Co., Chicago, Ill. Structures are shown in Fig. 1.

Solvent systems

All solvents were freshly mixed on a v/v basis immediately before being used. Table I shows a list of the solvents and their composition.

Chromogenic reagent

Ammoniacal silver nitrate[12] was prepared by dissolving 1.7 g of silver nitrate in 10 ml of water and 5 ml of ammonium hydroxide (sp. gr. 0.897) was added. The solution was diluted to 200 ml with acetone. To 45 ml of the ammoniacal silver nitrate,

5 ml of 2-phenoxyethanol was added, mixed well and used to spray the chromatograms.

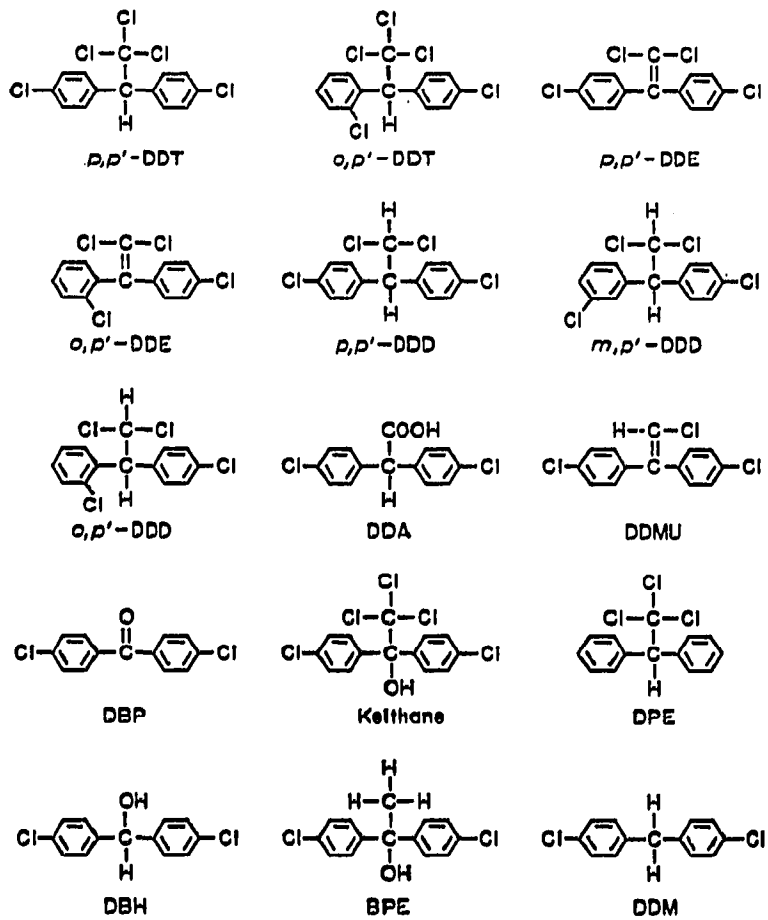

Fig. 1. Structures of DDT and related compounds.

Equipment

Pre-coated 250-μ Aluminum Oxide (type E) F_{254} plates were obtained from Brinkmann Instrument, Inc., Westbury, N.Y.; micro-pipettes from Drummond Scientific Co., Broomall, Pa.; and a Chromato-vue Cabinet Model CC-20 equipped with visible, short and long wavelength UV light from Ultra-Violet Products, Inc., San Gabriel, Calif.

Procedure

The TLC chamber, lined with filter paper, was loaded with 120 ml of the appropriate solvent system and allowed to equilibrate for at least 30–60 min to achieve complete saturation. A sample of each of the fifteen DDT-type compounds was dissolved in benzene at a concentration of 2 mg/ml. Using micropipettes, a 20 × 20 cm Aluminum Oxide (type E) F_{254} plate was spotted with 1 μl of each compound (equivalent to 2 μg) at points 2.5 cm from the bottom of the plate and 1 cm apart. The plate was developed, at room temperature(22°) and 50% relative humidity, until the solvent front had traveled 15 cm above the point of application of the spots and was then removed from the chamber. The developing solvent was allowed to evaporate for

about 3 min at room temperature. The plate was then activated under short wavelength UV light for 15 min in the chromato-vue cabinet and the quenched spots were marked. For further detection and confirmation of the spots the plate was sprayed with the chromogenic reagent and re-exposed for 15 min to short wavelength UV light.

RESULTS AND DISCUSSION

Thirty-three solvent systems (Table I) were screened to obtain optimum separation of the fifteen DDT-type compounds. *n*-Hexane, cyclohexane and *n*-pentane constituted the "background" solvent of twenty, four and seven solvent systems, respectively. The remaining two systems were absolute ethanol and methanol. Since most of these solvent systems were mixtures in different proportions, they should be prepared fresh before use to reduce any change of concentration due to evaporation. Differing R_F values result from changes in composition of the solvent systems used. The chromatographic mobilities (R_F values) were calculated by dividing the distance from the point of application (POA) to the center of the spot by the distance the solvent front traveled (15 cm). The chromatographic mobilities ($\times$ 100) of DDT and

TABLE I

SOLVENT SYSTEMS AND THEIR DEVELOPMENT TIMES ON ALUMINUM OXIDE (TYPE E) F_{254} PLATES

Number	*Composition*	*Time (min)*
I	*n*-Hexane	31
II	*n*-Hexane–benzene (90:10)	31
III	*n*-Hexane–chloroform (90:10)	36
IV	*n*-Hexane–chloroform (80:20)	36
V	*n*-Hexane–chloroform–acetic acid (90:10:1)	36
VI	*n*-Hexane–ethyl ether–acetic acid (90:10:1)	34
VII	*n*-Hexane–ethyl acetate (90:10)	26
VIII	*n*-Hexane–ethyl acetate–acetic acid (80:20:2)	36
IX	*n*-Hexane–ethyl propionate (90:10)	31
X	*n*-Hexane–carbon disulfide (90:10)	29
XI	*n*-Hexane–ethylene dichloride (90:10)	32
XII	*n*-Hexane–acetone (95:5)	34
XIII	*n*-Hexane–acetone–acetic acid (95:5:1)	32
XIV	*n*-Hexane–acetone–ethyl ether (80:10:10)	27
XV	*n*-Hexane–ethanol–acetic acid (90:10:1)	35
XVI	*n*-Hexane–ethanol (80:20)	40
XVII	*n*-Hexane–ethanol–acetic acid (80:20:2)	50
XVIII	*n*-Hexane–propionic acid (95:5)	46
XIX	*n*-Hexane–propionic acid (90:10)	60
XX	*n*-Hexane–acetic acid (90:10)	34
XXI	Ethanol (absolute)	106
XXII	Methanol (absolute)	55
XXIII	Cyclohexane	72
XXIV	Cyclohexane–carbon tetrachloride (90:10)	65
XXV	Cyclohexane–acetone (95:5)	70
XXVI	Cyclohexane–acetic acid (90:10)	70
XXVII	*n*-Pentane	34
XXVIII	*n*-Pentane–carbon disulfide–acetic acid (80:20:2)	35
XXIX	*n*-Pentane–acetone (95:5)	29
XXX	*n*-Pentane–methanol (50:50)	60
XXXI	*n*-Pentane–propionic acid (99:1)	45
XXXII	*n*-Pentane–propionic acid (90:10)	64
XXXIII	*n*-Pentane–ethanol–acetic acid (90:10:1)	42

its fourteen related compounds developed by thirty-three solvent systems are reported in Table II.

Depending on the general chromatographic mobilities and separation of the fifteen DDT-type compounds on aluminum oxide plates, they could be classified into three groups, *viz.* the non-polar compounds, exhibiting higher mobilities (R_F values) — *p,p'*-DDT, *o,p,'*-DDT, *p,p'*-DDE, *o,p,'*-DDE, DDMU, DPE and DDM; the polar compounds, having lower mobilities — DDA, Kelthane, DBH and BPE; and an intermediate group consisting of one polar, DBP, and three non-polar, *p,p'*-DDD, *m,p'*-DDD, *o,p'*-DDD, compounds with mobilities in between the preceding two groups. Substitution of the less polar chlorine atom with a more polar hydrogen[27] atom on the dichloromethyl group of the three DDD isomers increased their polarity compared with the rest of the non-polar compounds. This resulted in lower mobilities. On the other hand, DBP, with a less polar carbonyl group[28], showed higher mobilities than the rest of the polar compounds. Ethanol or methanol (solvents XXI and XXII, respectively) separated the fifteen DDT-type compounds into two groups. DDA was left at the POA in solvent XXI or slightly above the POA in solvent XXII, and the rest of the compounds had almost the same chromatographic mobilities, close to the solvent front. Similar behavior to that of solvent XXII was also observed with solvent XXX.

In the non-polar group of compounds *p,p'*-DDE showed higher chromatographic mobility than *o,p'*-DDE and was also the fastest moving among the fifteen compounds used in this study. On the other hand, *o,p'*-DDT showed higher mobility than *p,p'*-DDT. DDMU migrated less then *p,p'*-DDE because of substituting a non-polar chlorine atom with a more polar hydrogen atom[27]. DPE showed the same mobilities as *p,p'*-DDT on aluminum oxide TLC plates. Resolution of the two compounds is discussed later. In most of the solvent systems, DDM showed similar R_F values as *o,p'*-DDT, *o,p'*-DDE and DDMU. However, systems III, XXIII, XXIV and XXXI will separate DDM from *o,p'*-DDT and *o,p'*-DDE. Solvents VII and X will separate DDM from DDMU.

DDA was the only carboxylic acid in the polar group and, due to its high polarity, did not migrate easily from the POA. Twelve solvent systems of the thirty-three screened moved DDA. These contained acid (acetic or propionic) and/or alcohol (methanol or ethanol) in their composition. Kelthane, DBH and BPE contained a polar hydroxyl group and did not move in several solvent systems. However, when migrated, the chromatographic mobilities were in an ascending order: Kelthane > BPE > DBH. The trichloromethyl group of Kelthane increased the non-polar characteristics relative to the unsubstituted, more polar methyl group of BPE and to DBH, which contains no methyl group. No single pattern of R_F values for the three isomers of DDD was observed. The chromatographic mobilities were in ascending order, *viz p,p'*-DDD>*m,p'*-DDD>*o,p'*-DDD, in some solvents (*e.g.* I, II, V and X), in descending order in some other solvents (*e.g.* VII, VIII and XII), and equal in some other solvents (*e.g.* III, IV and XVII). The mobility of DBP was usually intermediate between those of the polar and non-polar compounds. Benzene was found to be a better solvent than acetone for dissolving the fifteen DDT-type compounds because of a less volatile nature. Benzene solutions of the compounds were stable (*cf.* ABOU-DONIA AND MENZEL[14], who reported that an old solution of Kelthane in acetone gave an extra spot which corresponded to DBP).

TABLE II

CHROMATOGRAPHIC MOBILITIES ($R_F \times 100$) OF DDT AND SOME RELATED COMPOUNDS[a] DEVELOPED BY ASCENDING ONE-DIMENSIONAL TLC ON ALUMINUM OXIDE (TYPE E) F_{254} PLATES

Solvent[b] *systems*	*p,p'-DDT*	*o,p'-DDT*	*p,p'-DDE*	*o,p'-DDE*	*p,p'-DDD*	*m,p'-DDD*	*o,p'-DDD*	*DDA*	*DDMU*	*DBP*	*Kelthane*	*DPE*	*DBH*	*BPE*	*DDM*
I	25	35	41	36	10	11	12	0[c]	32	2	0	27	0	0	35
II	48	55	61	56	28	29	30	0	55	8	2	44	0	0	54
III	69	72	75	72	52	52	52	0	67	24	3	63	0	0	67
IV	76	76	77	75	64	64	64	0	74	45	8	72	2	4	75
V	67	69	72	69	52	53	54	0	70	69	7	66	1	2	71
VI	67	70	75	68	51	51	50	0	72	45	10	66	2	4	70
VII	70	70	75	70	63	61	58	0	74	61	23	66	6	13	69
VIII	78	79	83	79	77	76	74	6	83	75	53	77	27	39	80
IX	69	71	76	69	60	60	57	0	73	56	19	67	4	9	71
X	35	44	49	45	16	18	19	0	48	4	0	35	0	0	42
XI	58	63	65	62	42	43	46	0	62	18	3	55	0	0	61
XII	60	64	71	64	43	41	39	0	69	50	8	60	2	5	67
XIII	63	66	71	64	48	47	46	0	68	55	16	62	4	9	67
XIV	73	74	77	72	65	64	61	0	75	68	48	77	24	36	76
XV	83	84	85	83	78	78	79	5	83	81	58	82	46	55	83
XVI	84	84	84	83	80	79	80	0	84	82	75	84	71	74	83
XVII	87	88	89	87	81	81	81	20	87	83	64	86	59	63	86
XVIII	59	68	73	69	39	39	40	8	67	34	34	60	33	34	67
XIX	92	94	96	93	83	83	84	32	92	82	72	91	61	70	93
XX	78	80	80	77	69	70	71	17	78	67	43	74	24	35	78
XXI	82	82	82	80	81	81	81	0	81	81	81	81	80	81	82
XXII	80	80	80	77	79	79	79	5	78	78	79	79	79	79	80
XXIII	35	46	50	45	13	15	16	0	40	2	0	36	0	0	39
XXIV	40	51	52	48	16	18	20	0	44	3	0	42	0	0	42
XXV	71	74	77	72	59	57	56	0	74	64	21	70	5	13	72
XXVI	77	77	78	77	72	72	74	30	78	72	56	78	34	47	78
XXVII	27	40	43	42	10	10	11	0	35	2	0	32	0	0	35
XXVIII	70	75	77	75	51	52	53	2	74	20	4	69	4	4	75
XXIX	65	69	76	68	48	46	44	0	72	57	7	64	2	5	70
XXX	85	85	85	84	84	84	84	4	80	83	84	84	83	84	84
XXXI	54	67	74	69	22	24	26	0	61	6	6	54	6	6	62
XXXII	sf[d]	sf	sf	sf	94	94	94	35	sf	94	92	sf	83	92	sf
XXXIII	93	94	96	94	90	90	90	7	96	93	75	94	67	70	92

[a] See EXPERIMENTAL for identification of compounds.
[b] The numbering of solvent systems corresponds to Table I.
[c] Migration from point of application could not be detected.
[d] Compound migrated with solvent front.

The inclusion of silver nitrate in the adsorbent layer affected the R_F values, showed a rather "grainy" background[22], and tended to darken during storage[4]; therefore spraying of the chromatograms with a silver nitrate–chromogenic reagent was preferred. Three chromogenic reagents[12–14] were tried to detect DDT and the fourteen related compounds on commercially available Aluminum Oxide (type E) F_{254} plates. None of the compounds studied were clearly detected by the 2 % *o*-toluidine reported by ABOU-DONIA AND MENZEL[14]. Application of MITCHELL's silver nitrate reagent according to SIEWIERSKI AND HELRICH[13] gave poor to very poor detection. The ammoniacal silver nitrate of MORLEY AND CHIBA[12] detected all compounds except DDA, DBP, DBH, and BPE. The chromogenic reagent used in this work (see EXPERIMENTAL) may be considered a modification of the preceeding reagent. *p,p'*-DDD, *m,p'*-DDD, *o,p'*-DDD, DBH, BPE and DDM showed better sensitivity to our reagent. A characteristic dark blue fluorescence of the three isomers of DDD and a yellow fluorescence of DBP were only observed with the plates sprayed with the chromogenic reagent reported here when viewed under long wavelength UV light.

The preliminary method of detecting the spots was to observe them under short wavelength UV light, where they quenched the fluorescence of the UV indicator incorporated with the adsorbent layers. The compounds appeared as light to dark purple spots on a yellowish green fluorescing background. Activation of the plate for 15 min under short wavelength UV light, before viewing or spraying, was found to increase the quenching of the spots as well as their response to the chromogenic reagent, hence improving their detection. Therefore, the significance of the UV preactivation should not be overlooked.

A spot test, to study the color and/or fluorescence response of DDT and its fourteen related compounds to the chromogenic reagent when applied to the aluminum oxide plate and examined under various conditions (visible, short and long wavelength UV light) is summarized in Table III. Under long wavelength UV light, *p,p'*-DDD, *m,p'*-DDD and *o,p'*-DDD showed a characteristic dark blue fluorescence. DDMU and DBP showed bright blue and yellow fluorescence, respectively, under the same conditions. Generally, prolonged exposure to UV light[4] or laboratory light will intensify the spots and will also darken the background. In case of weakly detected spots, spraying lightly with the chromogenic reagent twice is better than a single heavy spraying since it will be harder to detect the spots on a dark background.

Aluminum oxide plates developed with solvent systems containing up to 10 % propionic acid (solvent XIX) or acetic acid (solvent XX) showed light to dark brown spots when sprayed with the chromogenic reagent and exposed to short UV light. This differed from other plates developed with non-acidic solvents where the compounds appeared as light to dark grayish blue spots on a white background.

The lower limits of detecting the fifteen DDT-type compounds developed with *n*-pentane–methanol (50:50) (solvent XXX) on aluminum oxide plates, with the chromogenic reagent, are shown in Table IV. The values reported for DDMU and DBP are 0.5 μg, but these two compounds could be detected at a lower level of 0.1 μg by observing the TLC plate under short wavelength UV light where they will quench the fluorescing indicator incorporated with the adsorbent.

None of the solvent systems mentioned in Table I separated all compounds tested. However, a mixture of *p,p'*-DDT, *o,p'*-DDT, *p,p'*-DDE, *p,p'*-DDD, *o,p'*-DDD, DDA, DDMU, DBP, Kelthane, DPE, DBH and BPE was resolved when applied on

TABLE III

COLOR AND/OR FLUORESCENCE RESPONSE OF DDT AND SOME RELATED COMPOUNDS ON ALUMINUM OXIDE (TYPE E) F_{254} TLC PLATES (2 μg PER COMPOUND)

(A) Viewing in short wavelength UV light; (B) viewing in long wavelength UV light; (C) spraying with chromogenic reagent and viewing like (A); (D) Spraying with chromogenic reagent and viewing like (B); (E) spraying with chromogenic reagent followed by exposing to short wavelength UV light for 5 min and viewing like (A); (F) like (E) except viewing as (B); (G) like (E) except viewing in visible light; (H) spraying with chromogenic reagent followed by exposing to short wavelength UV light for 15 min and viewing like (A); (I) like (H) except viewing as (B); (J) like (H) except viewing in visible light; (K) spraying with chromogenic reagent followed by exposing to short wavelength UV light for 30 min and viewing like (A); (L) like (K) except viewing as (B); (M) like (K) except viewing in visible light; (N) expose to short wavelength UV light for 30 min, then like (K); (O) exposing to short wavelength UV light for 30 min, then like (L); (P) exposing to short wavelength UV light for 30 min then like (M). Abbreviations: B = blue, F = fluorescence, G = gray, N = negative, P = purple, Y = yellow, dk = dark, ft = faint, lt = light.

No.	*Compound*	*Method of detection*															
		A	*B*	*C*	*D*	*E*	*F*	*G*	*H*	*I*	*J*	*K*	*L*	*M*	*N*	*O*	*P*
1	*p,p'*-DDT	lt P	N	N	ft Y	P	ft YBF	dk G	dk P	ft BF	dk G	dk P	P	dk G	dk P	dk P	dk G
2	*o,p'*-DDT	lt P	N	N	ft Y	P	ft YBF	dk G	dk P	ft BF	dk G	dk P	P	dk G	dk P	dk P	dk G
3	*p,p'*-DDE	dk P	N	ft P	ft Y	P	ft YBF	dk G	P	ft YBF	dk G	dk P	YBF	dk G	dk P	P	dk G
4	*o,p'*-DDE	dk P	N	ft P	ft Y	P	ft YBF	dk G	P	ft YBF	dk G	dk P	YBF	dk G	dk P	P	dk G
5	*p,p'*-DDD	lt P	N	N	ft Y	BF	dk BF	G	BF	dk BF	G	BF	dk BF	G	dk PF	dk BF	dk G
6	*m,p'*-DDD	lt P	N	N	ft Y	BF	dk BF	G	BF	dk BF	G	BF	dk BF	G	dk PF	dk BF	dk G
7	*o,p'*-DDD	lt P	N	N	ft Y	BF	dk BF	G	BF	dk BF	G	BF	dk BF	G	dk PF	dk BF	dk G
8	DDA	lt P	N	N	ft Y	ft B	ft BF	ft G	ft P	ft YBF	lt G	lt P	YBF	lt G	P	YBF	G
9	DDMU	dk P	ft Y	ft P	YF	BF	BF	lt G	BF	BF	lt G	BF	BF	lt G	BP	BF	dk G
10	DBP	dk P	ft Y	ft P	YF	lt P	YF	N	lt P	YF	N[a]	P	YF	N	P	YF	G
11	Kelthane	lt P	N	N	ft Y	P	P	dk G	dk P	P	dk G	dk P	P	dk G	dk P	dk P	dkG
12	DPE	lt P	N	N	ft Y	P	ft YBF	dk G	P	P	dk G	dk P	P	dk G	dk P	dk P	dk G
13	DBH	lt P	ft Y	N	ft Y	ft B	ft YBF	ft G	lt P	ft YBF	lt G	P	lt YBF	lt G	P	YBF	lt G
14	BPE	lt P	ft Y	N	ft Y	ft B	ft YBF	ft G	lt P	ft YBF	lt G	lt P	lt YBF	lt G	lt P	lt YBF	lt G
15	DDM	lt P	N	N	ft Y	P	ft YBF	G	P	ft YBF	G	P	lt YBF	G	dk P	lt YBF	dk G

[a] Spot starts to appear as light gray within 1–2 h.

TABLE IV

LIMIT OF DETECTABILITY (μg) OF DDT AND ITS FOURTEEN RELATED COMPOUNDS ON ALUMINUM OXIDE (TYPE E) F_{254} TLC PLATES DEVELOPED WITH *n*-PENTANE–METHANOL (50:50)

No.	*Compound*	*Concentration (μg)*
1	*p,p'*-DDT	0.05
2	*o,p'*-DDT	0.05
3	*p,p'*-DDE	0.05
4	*o,p'*-DDE	0.05
5	*p,p'*-DDD	0.05
6	*m,p'*-DDD	0.05
7	*o,p'*-DDD	0.05
8	DDA	0.20
9	DDMU	0.50
10	DBP	0.50
11	Kelthane	0.05
12	DPE	0.05
13	DBH	0.10
14	BPE	0.25
15	DDM	0.25

aluminum oxide plate and developed in two dimensions using *n*-hexane, three times, in direction 1 and *n*-hexane–ethyl ether–acetic acid (90:10:1) in direction 2. Fig. 2a shows the separated spots. As previously recommended[29], it is advisable in case of the multiple development of this class of compounds, not to expose the aluminum oxide TLC plates to short wavelength UV light in between the developments to prevent the phenomena of "spot per development". Several degradation products were also found as a result of UV irradiation of chlorinated hydrocarbon insecticides on silica gel plates[3]. As shown in Fig. 2a, *p,p'*-DDT and DPE were overlapped. For their complete resolution the same two-dimensional TLC system was used to develop the mixture on a Silica Gel F_{254} plate (also obtained from Brinkmann Instrument, Inc.). Fig. 2b shows the separation achieved. Thus the combination of two-dimensional TLC on aluminum oxide and silica gel plates separated a mixture of DDT and eleven related compounds.

For the TLC of these DDT-type compounds we found that Aluminum Oxide (type E) F_{254} was superior to Silica Gel F_{254} plates because (1) the solvent systems will travel faster (shorter developing time), (2) the background does not darken since it contains a binder which will permit spraying with silver nitrate[30], (3) it allows increased sensitivity of detection, and (4) the spot will show greater quenching properties. Chromatographic mobility data from Table II in combination with two-dimensional TLC will help to find appropriate solvent system(s) to separate any of the DDT-type compounds. Several systems reported here are currently being investigated for the separation and detection of DDT and its related compounds in animal tissues and vegetable products.

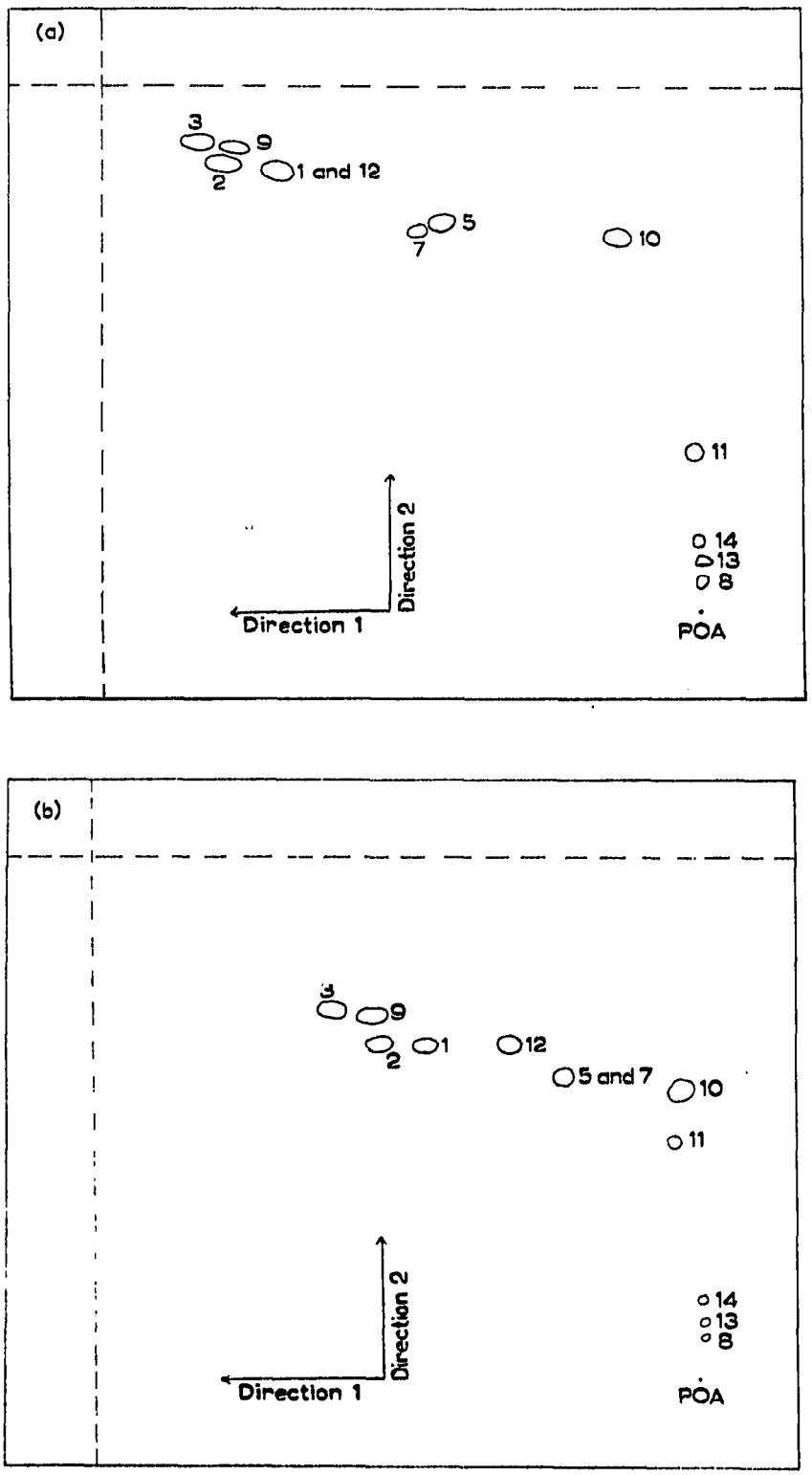

Fig. 2. Two-dimensional thin-layer chromatograms of DDT and eleven related compounds. The plates were developed three times with *n*-hexane in direction 1 and twice with *n*-hexane–ethyl ether acetic acid (90:10:1) in direction 2 (130 ml per solvent system). The numbers of the spots correspond to Table IV. (a) Aluminum Oxide (type E) F_{254} thin-layer plate; the mixture spotted at the point of application (POA) contained 1 μg of each compound. (b) Silca Gel F_{254} thin-layer plate; 4 μg of each compound were spotted.

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