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An observation on the multiple development of DDT and some metabolites on aluminum oxide thin-layer chromatograms

Thin-layer chromatography (TLC) has proved to be particularly useful in the field of pesticide residue analysis. Workers have used aluminum oxide as a chromatographic substrate for the separation of organochlorine pesticides[1–13]. None of the previously reported work indicated any interaction or decomposition between this class of chlorinated pesticides and the aluminum oxide thin layers. This note deals with an observation on the behavior of DDT and four of its well known metabolites[14] on commercially available pre-coated aluminum oxide TLC plates that were developed by the one-dimensional, multiple-development technique.

Experimental

Reagents. All solvents and chemicals used were reagent grade.

Pesticides. 1,1,1-Trichloro-2,2-bis(*p*-chlorophenyl)ethane (DDT), 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene (DDE), 1,1-dichloro-2,2-bis-(*p*-chlorophenyl)ethane (DDD), bis(*p*-chlorophenyl)acetic acid (DDA) and 1-chloro-2,2-bis(*p*-chlorophenyl) ethylene (DDMU). DDD was received as a gift from Rohm and Haas Company, Philadelphia, Pa. DDMU was kindly supplied by the Department of Health, Education and Welfare, PHS, Perrine, Fla. The other pesticides were obtained from Aldrich Chemical Company, Inc., Milwaukee, Wisc.

Solvent systems. (A) *n*-pentane; (B) *n*-hexane; (C) *n*-hexane–acetic acid (9:1); (D) *n*-hexane–ethyl ether–acetic acid (100:1:1)[15]; (E) chloroform–methanol (67:33)[16]; (F) chloroform–acetic acid (9:1).

Chromogenic reagent. Ammonical silver nitrate was prepared according to MORLEY AND CHIBA[17] 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.

Equipment. Pre-coated 250-μ TLC plates aluminum oxide (type E) F_{254}, Brinkmann Instruments, Inc., Westbury, N.Y. Micro-pipettes, Drummond Scientific Co., Broomall, Pa. Chromato-vue cabinet model CC-20 equipped with shortwave UV light, Ultra-Violet Products, Inc., San Gabriel, Calif.

Methods. Solutions of DDT and its four metabolites made up in benzene (2.5 mg/ml) were spotted on the plates using 1 μl micropipettes. Plates 10 × 20 cm size, with an approximately 5 mm wide strip of the layer removed from the right and the left sides[18], and 10 μg of each compound were used. These were developed at room temperature (25°) in a lined TLC chamber that was equilibrated with the solvent for at least 30 min to achieve complete saturation. The solvent front was allowed to move 15 cm from the point of application (about 30 min for solvents A–D and 40 min for solvents E and F). The plate was removed from the chamber and left at room temperature for 4 min to allow for the evaporation of the solvent. The developed chromatogram was then exposed to shortwave UV light, in the chromato-vue cabinet for 2 min. The same procedure was repeated once more for plates developed with solvents C, E and F. Plates developed with solvents D, A and B were redeveloped 2, 3 and 4 more times, respectively. At the end of each multiple-development scheme mentioned above,

the zones of DDT and its metabolites were visualized by spraying the plates with the chromogenic reagent and exposing them to shortwave UV light for about 15 min.

Results and discussion

When the developed plate was examined under shortwave UV light, each compound showed a single dark spot in a brightly yellowish-green fluorescing field. This resulted from quenching the fluorescence of the UV indicator incorporated with the aluminum oxide thin layers[19]. However, on reexamining the plate after being sprayed with the chromogenic reagent and exposed to shortwave UV light, each compound showed the main spot plus other fainter spots with lower R_F values. The number of extra spots were equal to the number of the developments minus one. That is to say, plates developed with solvent B showed 4 extra spots, with solvent A and D showed 3 and 2 extra spots respectively, and plates developed with solvents C, E and F showed one extra spot. Measurements were made from the point of application to the center of the spot and the R_F values of DDT and its metabolites were calculated according to GALLETTI[20] as the observed movement of the spot after several runs (2, 3, 4, or 5, see above) divided by the distance of a single development (15 cm). The average values, of four determinations, of the R_F ($\times$ 100) for the main spot of each compound as well as the extra spots are given in Table I. The highest figure of each solvent system represents the R_F value of the main spot.

No extra spots were observed if the plate was developed under the light conditions of the laboratory (fluorescent light and no windows) and was not exposed to shortwave UV light between the multiple developments. A residual spot was observed at the point of application in addition to the other extra spots mentioned above, when the plate was exposed to the shortwave UV light before development. When the plate was developed in the dark no extra spots were observed unless exposure to shortwave UV light had occurred between developments.

This led to the conclusion that a change occurred in DDT and its four metabolites resulting from their interaction and/or decomposition on the aluminum oxide thin-layer chromatograms under the influence of the shortwave UV light.

To study the stability of DDT on the aluminum oxide thin layers after exposure to shortwave UV light, the SRS technique (separation, reaction, separation) suggested by STAHL[21] was employed. On a 20 $\times$ 20 cm TLC plate, 20 μg of DDT was spotted 2.5 cm from the lower left corner. The plate was developed in direction 1 with *n*-hexane

TABLE I

R_F ($\times$ 100) VALUES FOR DDT AND ITS FOUR METABOLITES

The highest figure of each solvent represents the R_F value of the main spot.

Compound	*Solvent system number*																			
	A				*B*					*C*		*D*			*E*		*F*	
DDT	67	56	43	24	74	68	57	44	24	85	67	92	80	53	94	77	99	96
DDE	85	75	62	38	89	85	75	63	39	94	73	95	84	64	94	79	99	96
DDD	39	33	24	12	47	40	31	23	12	85	57	75	57	35	94	78	99	95
DDA	0	0	0	0	0	0	0	0	0	32	16	3	0	0	0	0	87	66
DDMU	80	71	56	33	85	80	69	55	32	95	75	91	79	57	94	79	95	99

and was exposed to shortwave UV light as mentioned above for 5 times. The chromatogram was then turned through 90° and similarly developed for 5 more times in direction 2. Fig. 1 shows the developed chromatogram after visualization. Only the DDT main spot (solid circle line) lies on the diagonal line between the two development directions. The dotted circle lines represent the extra spots that were formed as a result of exposing the plate to shortwave UV light. Since none of these extra spots moved from the place they were formed, and they were not scattered around the diagonal line, it appears that an interaction has resulted between the DDT and the components of the aluminum oxide type-E plates. On trying solvents with different polarity from the eluotropic series of solvents[22], the more polar methanol could cause the migrations of the extra spots while the moderately polar ethyl acetate and the least polar *n*-hexane did not elute them. This gave evidence to support the fact that the extra spots are very polar.

When pre-coated 250 μ thin-layer plates (Silica Gel F_{254}) also obtained from Brinkmann Instruments, Inc., were treated in a manner similar to the aluminum oxide plates, only one main spot per compound was detected. The phenomena of "a spot per development" was not observed.

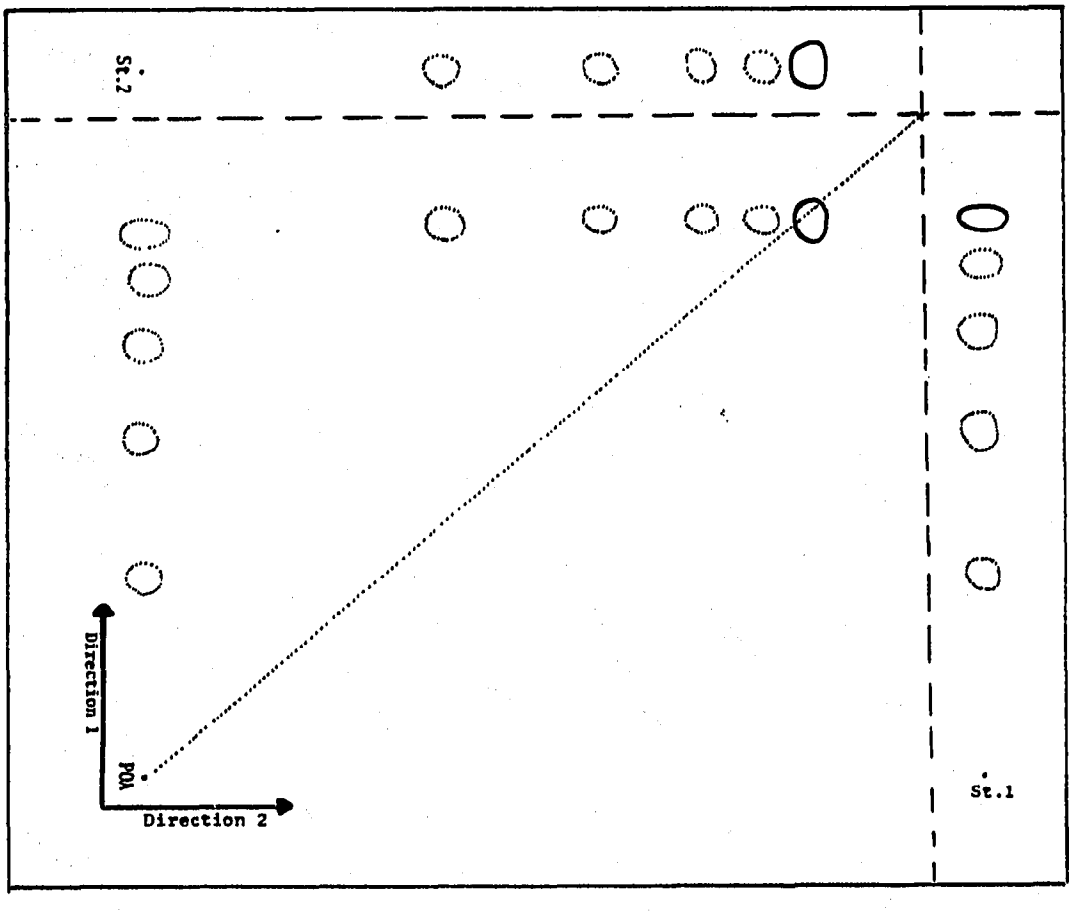

Fig. 1. Thin-layer chromatogram (SRS-technique)[21] of DDT on aluminum oxide type-E TLC plate. 20 μg of DDT were spotted at the point of application (POA). The chromatogram was developed 5 × in *n*-hexane in direction 1 and 5 × in direction 2. St. 1, standard developed in direction 1; St. 2, standard developed in direction 2. Solid line is the main spot of DDT, dotted lines are the extra spots.

The reported observation may be the result of aluminum oxide being a more reactive adsorbent than silica gel. Alumina catalyzes several reactions such as isomerization of double bonds and ester hydrolysis[23]. Another difference between silica gel and alumina is that the former is acidic and the latter is basic[18] with a somewhat lower adsorption activity[24].

Although the aluminum oxide type E layers are especially recommended for pesticide TLC[19] and although we are having satisfactory results in resolving a mixture of DDT-type compounds on them, it would be advisable in case of multiple-development not to expose the plates to shortwave UV light in between developments. Identification of the extra spots is in progress and results will be reported in the future.

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