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Diphenylamine-Zinc Chloride as a Chromogenic Agent for the Detection of a Mixture of DDT, Chlordane, and Toxaphene on Thin Layer Chromatograms

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Diphenylamine-ZnCl₂ has been evaluated as a chromogenic agent for detection of toxaphene, DDT, and chlordane on Al₂O₃ thin layer plates. Characteristic colors were obtained for these pesticides; a lower level of about 5 μg was detectable. Color reactions of 34 pesticides at the 20 μg level are presented.

Within the past few years thin layer chromatography has become a valuable tool for the residue analyst who must detect, identify, and perhaps quantitate multiple residues in a sample.

In this laboratory mixed toxaphene and DDT residues are frequently encountered in a variety of samples; toxaphene, DDT, and chlordane have been found together in grains and feeding materials. The thin layer procedure of Kovacs (1), which we use routinely, does not provide satisfactory identification for DDT or chlordane in the presence of toxaphene. Identification depends on direct comparison with standards chromatographed with the sample. The absorbents and solvent systems do not separate toxaphene, DDT, and chlordane cleanly: toxaphene and Strobane® chromatograph as streaks; technical DDT gives two spots; and chlordane gives two or three spots, as well as many indistinct spots (1). The R_f values overlap to a greater or lesser degree depending on the combination present. A need exists for a test which distinguishes between toxaphene, DDT, and chlordane.

Various chromogenic agents have been used to visualize chlorinated pesticides on thin layer plates. Baumler and Rippstein (2) used *N,N*-dimethyl-*p*-phenylenediamine hydrochloride in sodium methylate followed by ultraviolet radiation; Petrowitz and Pastuschka (3) used methanolamine-AgNO₃ and ultraviolet radiation. Yamamura and Niwaguchi (4) sprayed with Marquis reagent and

detected spots under ultraviolet light. Salo, *et al.* (5) developed plates successively with AgNO₃, CH₂O, 2*N* KOH, and HNO₃-H₂O₂. Yamamura, *et al.* (6) used concentrated and fuming H₂SO₄ (4:1) or ammoniacal AgNO₃ and exposure to ultraviolet light. Walker and Beroza (7) reported the reactions of 62 pesticides, including chlorinated ones, to iodine and to bromofluorescein-AgNO₃, with and without ultraviolet radiation. Kovacs (1) and Morley and Chiba (8) employed modifications of the 2-phenoxyethanol-AgNO₃ reagent introduced by Mitchell (9) for paper chromatography; Morley and Chiba also reported detection of DDE and DDT without spraying when the silica plate was viewed under ultraviolet light. Abbott, *et al.* (10) used an ethanolic solution of AgNO₃ as a spray reagent, followed by irradiation with ultraviolet light. These authors also investigated the effect of indicators combined with AgNO₃ and reported best results with bromphenol blue. The pesticides were detected as bright yellow spots on a deep blue background.

Katz (11) applied diphenylamine-ZnCl₂ to pesticides chromatographed on Kieselgel G and reported characteristic colors for seven pesticide compounds, including DDT and toxaphene. He provided an iodine test for DDE, which did not react.

McKinley and Graham (12) used diphenylamine-ZnCl₂ to identify captan on paper chromatograms. Cueto (13) employed this reagent for the colorimetric determination of dieldrin, and Graupner and Dunn (14) used it for the colorimetric determination of toxaphene. These authors also reported on colors given by pesticides other than those for which they developed methods.

The objective of the present study was to find the chromogenic agent that would best distinguish toxaphene, DDT, and chlordane in combination when they are chromato-

graphed on thin layer plates. The literature cited above (11-14) showed that diphenylamine-ZnCl₂ gave various colors with a number of chlorinated pesticides; therefore, this reagent was investigated. The results reported here were obtained by applying diphenylamine-ZnCl₂ to chlorinated pesticides on Al₂O₃ plates.

METHOD

Apparatus

(a) *Desaga/Brinkmann standard model applicator.*

(b) *Desaga/Brinkmann standard mounting board.*

(c) *Window glass.*—8 × 8" double strength (Pittsburgh Plate Glass Co.).

(d) *Desaga/Brinkmann drying rack.*—Accommodates ten 8 × 8" plates.

(e) *Desiccating cabinet.*—Boekel #1340, stainless steel or equivalent.

(f) *Chromatographic tank and accessories.*—Described in *This Journal*, **36**, 1187 (1953); **40**, 999 (1957) (Arthur H. Thomas Co., Cat. No. 3106-F05 or equivalent). *Note:* Use metal instead of glass trough.

(g) *Spotting pipets.*—1 and 5 μl.

(h) *Chromatography spray flask.*—250 ml.

(i) *Tank liners.*—Two pieces, 12¼ × 8¾", cut from desk blotters and bent into an L-shape to fit the tank.

(j) *Ultraviolet light source.*—Long wave.

Reagents

(a) *Absorbent.*—Aluminum Oxide G (Research Specialties Co., Richmond, Calif.) or Aluminum Oxide G (neutral) with CaSO₄ (manufactured by E. Merck, Darmstadt, West Germany, distributed by Brinkmann Instruments, Inc., Great Neck, N.Y.).

(b) *Solvent system.*—Mobile: *n*-heptane, commercial grade (Phillips Petroleum Co., Bartlesville, Okla.).

(c) *Chromogenic spray.*—(1) Diphenylamine (stabilized with ammonium carbonate, Matheson, Coleman and Bell), 20% solution in acetone, AR grade, prepared fresh daily; (2) zinc chloride, AR grade, 10% solution in acetone, AR grade, filtered, prepared fresh daily. For use, mix 1 part of (1) and 2 parts of (2).

Procedure

Preparation of chromatographic plates.—Prepare chromatographic plates as described by Kovacs (1) with modifications of the slurry concentration and heating time of the prepared

plate (15). Weigh out 30 g Al₂O₃ G, add 50 ml distilled water, swirl moderately for 1 min., and pour into the applicator chamber. Air-dry the prepared plates for about 15 min. and then dry at 80°C for 45 min. in a forced draft oven. Remove, cool, and place in desiccator cabinet.

Sample spotting.—Spot sample as described by Kovacs (1).

Development.—Place the two liners and metal trough in the chromatography tank. Pour 50 ml *n*-heptane into the trough and 50 ml into the bottom of the tank. Spot samples and standards, and place the plate in the metal trough. Cover tank with glass cover and seal with masking tape. When the solvent front reaches the 10 cm line, remove the plate and allow to air-dry for about 3-4 min.

Detection of pesticides.—Spray the plate with chromogenic reagent perpendicular to the direction of solvent flow, being careful not to spray the plate too heavily. Allow plate to stand ca 1 min. while the layer becomes saturated with the reagent. Place the plate in a draft oven, preheated to 250 ± 5°C, for ca 5-8 min., or until the background begins turning blue. Remove plate from oven and evaluate the chromatogram within 5 min. (Some spots change color or fade.) Also observe plate under long wave ultraviolet light.

Results and Discussion

The reagent was used to test 20 μg each of 34 pesticides, plus isomers and reaction products. Results are shown in Table 1.

Toxaphene and Strobane appear as grayish-green streaks. They could not be distinguished from each other. Technical chlordane showed up as a purple streak with three distinct spots having *R*_{DDD} values of 1.62, 1.52, and 1.40. One of these isomers had a higher *R*_f value than toxaphene. No spots were detected for the α- and β-chlordane isomers. Separately, a minimum of 3 μg toxaphene and 5 μg chlordane could be detected; when they were combined, 5 μg of each could be detected. When in combination they could be distinguished more easily when the plate was observed from its reverse side and/or held in front of a fluorescent light. Under long wave UV light, chlordane fluoresced slightly, whereas toxaphene did not.

Technical DDT produced two reddish-orange spots, the *o,p'* and *p,p'* isomers. The

Table 1. Colors obtained with chlorinated and other pesticides and diphenylamine-ZnCl

Pesticide (20 μ g)	Color
Toxaphene	grayish-green
Toxaphene (saponified)	grayish-green
Chlordane (tech.)	purple
Chlordane (tech., saponified)	purple
α -Chlordane	—
α -Chlordane (saponified)	purple
β -Chlordane	—
β -Chlordane (saponified)	purple
DDT (tech.) ^a	reddish-orange
DDT (tech., saponified)	—
<i>o,p'</i> -DDT ^a	reddish-orange
<i>p,p'</i> -DDT ^a	reddish-orange
DDE	—
TDE	bright green
Aldrin	—
BHC ^a	—
Captan ^b	purple
Carbaryl	—
Carbophenothion	green
Chlorbenside (tech.)	—
Chlorobenzilate ^b	purple
Diazinon	—
Dieldrin	purple
DPA (tech.)	—
Endosulfan	—
Endrin	dark green
Heptachlor	dark gray
Heptachlor epoxide	light purple
Hexachlorobenzene	—
Kelthane ^b	purple
Lindane	—
Methoxychlor	bluish-gray
Methyl parathion ^b	dark green
α -Naphthol	—
Ovex	—
Parathion ^b	dark green
PCNB	—
Perthane	blue-green
Ronnel ^a	light blue
Strobane [@]	grayish-green
TCNB	—
Tetradifon	—
Vegedex	—

^a Fluorescent spot(s) under long wave ultraviolet light.
^b Spot remains on baseline (Al₂O₃ plate, *n*-heptane mobile solvent).

minimum sensitivity for each was about 5 μ g. These spots fluoresced a bright pink under long-wave UV light, and the sensitivity was increased. In the presence of 5 μ g toxa-

phene, 5 μ g of *o,p'*- or *p,p'*-DDT could be detected under visible light. TDE produced a bright green spot under visible and UV lights; as little as 1 μ g was detectable. On standing, the spot changes to blue.

The behavior of toxaphene, chlordane, and DDT after alkali hydrolysis, as described by Mills, *et al.* (16), was explored as a means of confirming the identity of these compounds. Toxaphene underwent no detectable color change as a result of saponification. DDT gave no reaction due to its conversion to DDE. The α - and β -chlordane isomers did not react before alkali hydrolysis. After alkali hydrolysis the α -isomer produced three grayish-purple spots; the β -isomer produced one grayish-purple spot with the same *R_f* as one of the α -isomer spots. Technical chlordane underwent a change. Two spots differed in *R_f* and intensity, one of which had an *R_f* value equal to that of one of the saponified α -isomer spots.

Plates coated with silica gel and Al₂O₃ were used in the experimental work. The Al₂O₃ plate proved to be the more sensitive of the two. The chromogenic agent was varied in concentration; acetone and methanol were tried as solvents. Acetone was finally used because it gave a whiter background than methanol. Incorporation of the chromogenic reagent into the Al₂O₃ plate did not produce as good a chromatogram as when the reagent was sprayed, nor did the pesticides separate as well.

Quantitative aspects of the test have not been explored for all pesticides which give colors reported in Table 1. Experiments with DDT, toxaphene, technical chlordane, and TDE show that semiquantitative estimation is possible for these pesticides when they are present individually and for DDT and TDE when the four are combined.

A mixture of 50 μ g each of toxaphene, technical chlordane, technical DDT, and TDE were added to 100 g of sweet potatoes and carrots and to 4 g of cottonseed oil. The extracts were prepared and cleaned up according to Mills, *et al.* (16) and Mills (17), respectively; aliquots representing 10 g of the vegetables and 0.5 g of the cotton-

seed oil sample were spotted and chromatographed. DDT and TDE were easily detected. Chlordane overlapped toxaphene but the high R_f purple spot of chlordane was prominent over the toxaphene streak.

The test has been applied qualitatively to sample extracts and has been successful when thoroughly cleaned up samples were chromatographed.

The diphenylamine-ZnCl₂ test described gives characteristic colors for several common chlorinated pesticides but lacks the sensitivity of the reduced silver test (1). When the residue levels fall within its range and it is run in conjunction with the reduced silver test, the thin layer chromatogram for the identification of 1 μ g of DDT and 5 μ g each of chlordane and toxaphene can be made with greater confidence than before.

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