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Reverse Phase Thin Layer Chromatography of Some Aroclors, Halowaxes, and Pesticides

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The components of Aroclors® 1232, 1248, 1254, and 1260; Halowaxes® 1099, 1013, and 1014; and several chlorinated pesticides are resolved by reverse phase thin layer chromatography (RPTLC), which permits component separation by partition between a nonpolar stationary phase and a polar mobile phase. R_f values of resulting spots were calculated for 2 of 4 new solvent systems (mobile phases). RPTLC patterns were reproducible and characteristic of each material examined. The spots were recovered from the plates and characterized by gas-liquid chromatography (GLC) and/or GLC-mass spectrometry. In some cases, single GLC peaks of Aroclor standards were resolved into more than one component by RPTLC, whereas some RPTLC spots of Halowaxes were resolved into as many as 4 GLC peaks. The analysis of environmental residues of chlorinated compounds was facilitated by this technique.

Polychlorinated biphenyls (PCBs) are widespread and persistent industrial pollutants of the environment (1). Chlorinated naphthalenes (Halowaxes®) are industrial compounds with a variety of uses similar to PCBs (2).

GLC of PCBs and chlorinated naphthalenes results in multicomponent chromatograms and until recently only GLC or GLC-mass spectrometry (MS) was available for PCB component resolution. PCB components have been separated by 2-dimensional thin layer chromatography (TLC) (3), although resolving *p,p'*-DDE from PCBs was the primary object of these experiments. Also reverse phase thin layer chromatography (RPTLC) has been used to resolve PCB components of Phenochlor® DP6 or Aroclor® 1260 (4) but, in our experience, several components of Aroclors 1254, 1248, and 1232 were very near the solvent front and were not clearly resolved. Nevertheless, RPTLC appeared to offer potential as an auxiliary analytical method for examining complex residues of PCBs and other multicomponent chemicals. We modified the existing RPTLC method (4) to permit separations of Aroclors 1254, 1248, and 1232; Halowaxes 1099, 1013, and 1014; and several chlorinated pesticides. In addition, we characterized these components by GLC and/or GLC-MS.

Experimental

Apparatus

(a) *Reverse phase thin layer chromatographic plates.*—Prescored (20 × 20 cm) glass plates coated with kieselguhr G ($CaSO_4$ binder) to thickness of 250 μm (Analtech, Inc., 100 S. Justison St., Wilmington, Del. 19801).

(b) *Chromatographic tank.*—Closed glass developing tank lined with filter paper.

(c) *Irradiation lamp.*—General Electric G8T5 germicidal UV lamp.

(d) *Gas chromatograph.*—Perkin-Elmer Model 880 with ^{63}Ni-electron capture detector. Operational parameters: column—7′ × 2 mm id coiled glass packed with 0.3% (w/w) OV-7 on glass beads; nitrogen carrier gas at 40 ml/min; temperatures (°C)—injection port 220, detector 350, column 160 or 190.

(e) *Mass spectrometer.*—Perkin-Elmer Model 270 double-focusing, low-resolution mass spectrometer coupled through Watson-Biemann molecular separator to temperature-programmed gas chromatograph.

Reagents and Standards

(a) *Standards.*—*Pesticides.*—*p,p'*-DDT, *p,p'*-DDE, aldrin, dieldrin, and technical chlordane (obtained from Pesticide Repository Section, Perrine Primate Research Laboratory, Environmental Protection Agency, Perrine, Fla. 33157). *PCBs.*—Technical Aroclors 1232, 1248, 1254, and 1260 (provided by Monsanto Chemical Co., 800 N. Lindbergh Blvd., St. Louis, Mo. 63166). *Chlorinated naphthalenes.*—Halowaxes 1000, 1013, 1014, 1031, 1051, and 1099 (secured from Koppers Co., Inc., Bridgeville, Pa. 15017).

(b) *Solvents.*—Pesticide grade, redistilled in glass.

(c) *Paraffin oil.*—Liquid petrolatum (Matheson, Coleman & Bell).

(d) *Silver nitrate.*—Reagent grade (Fisher Scientific Co.).

Preparation of Reverse Phase Thin Layer Chromatographic Plates

Enough paraffin oil was dissolved in a shallow tray filled with petroleum ether to yield a solution of 8% by volume of the oil in the ether. Next, a precoated plate was carefully immersed in the paraffin-petroleum ether solution until the adsorbent layer was saturated. Then the plate was removed and allowed to air-dry overnight. Plates thus prepared can be stored for several weeks without any loss of separation efficiency.

Solvent Systems

Four solvent systems were employed to obtain maximum resolution of the various components of the Aroclors, Halowaxes, and chlorinated pesticides (Table 1). All developing solvents were saturated with paraffin oil by shaking the solvent and about 5 ml paraffin oil in a separatory funnel.

Sample Application and Development

All reference standards were dissolved in benzene (1 mg/ml) and 2–50 µl was spotted at points 2 cm above the bottom edge of the plate. Paraffin-saturated developing solvent (200 ml of 1 of the 4 solvent systems listed in Table 1) was added to a filter paper-lined chromatographic tank, the tank was covered with a glass plate, and the atmosphere was allowed to become saturated with the solvent. Each plate was developed to a point 15 cm above the sample origin, removed from the tank, air-dried in a hood, and redeveloped and air-dried twice in a similar manner. All development was done at ambient temperature (ca 25°C).

Visualization

After final development and drying, the plate was moved to a holder for spraying with a chromogenic spray consisting of 1.7 g $AgNO_3$ in 200 ml EtOH (00%) with 1 ml NH_4OH added just before application. A uniform spray was applied to the plate; then the plate was held over a steam bath for a few sec to allow the steam to play over the adsorbent layer. Finally, the plate was placed under a UV lamp for several min. All chlorinated components appeared as dark spots on a light background.

Environmental Analysis

RPTLC was used to separate PCB residues in a goldfish, *Carassius auratus* L., which was collected for the National Pesticide Monitoring Program from the Hudson River at Poughkeepsie, N.Y. The PCB fraction of a petroleum ether extract from this fish (Sample F012) was obtained by using silicic acid column chromatography (5). Approximately 25 µg PCBs from the extract and standard Aroclor 1248 were spotted on an RPTLC plate and the plate was developed and visualized (Fig. 1). This fish contained a PCB residue in excess of 200 µg/g whole fish as determined by GLC (6).

Results and Discussion

Visualization sensitivities for the resolved components of Aroclor and Halowax mixtures and for selected pesticides were determined by using a silver nitrate chromogenic spray (the fluorescent indicators tried were too insensitive for the determination of PCBs and Halowaxes). There appeared to be some correlation of sensitivity with degree of chlorination, particularly for the PCBs and Halowaxes. Approximately 15 µg Aroclor 1232 or Halowax 1099 was required to achieve visualization of major components, while only 8 µg Aroclors 1248 and 1254 and Halowaxes 1013 and 1014 was needed for detection. Sensitivity for some other compounds or mixtures are as follows: Aroclor 1260, 2 µg; *p,p'*-DDT, <2 µg; *p,p'*-DDE, <2 µg; and technical chlordane, 5 µg. Because chlordane is a multicomponent pesticide, a lower detection sensitivity is reasonable.

We found that 2 solvent systems (II and IV, Table 1) were adequate for resolving components of the compounds tested (*see* Figs. 2 and 3). Components (spots) of the resolved Aroclors and Halowaxes were numbered in ascending order. Standards of Aroclors and Halowaxes were analyzed by GLC at 2 different column temperatures, depending on GLC retention time (Figs. 4–6).

Table 1. Developing solvents for reverse phase thin layer chromatography of PCBs, chlorinated naphthalenes, and chlorinated pesticides

Solvent system[a]	Composition (v/v)	Compounds[b]
I	water-acetonitrile-MeOH (20 + 40 + 40)	Aroclor 1232[c]
II	water-acetonitrile-MeOH (15 + 40 + 45)	Aroclor 1248 chlordane dieldrin DDT aldrin
III	water-acetonitrile-MeOH (30 + 45 + 45)	Aroclor 1254
IV	water-acetone-acetonitrile-MeOH (5 + 15 + 40 + 40)	Aroclor 1260 Halowax 1013 Halowax 1014 Halowax 1099

[a] Each paraffin-coated kieselguhr G TLC plate is developed 3 times.
[b] *p,p'*-DDE may be resolved by using any of the 4 developers.
[c] Mixture of Aroclors 1221 and 1242.

Table 2. Chromatographic characterization of polychlorinated biphenyls (Aroclors): correlation of reverse phase thin layer chromatographic (RPTLC) spots and GLC peaks[a]

RPTLC			GLC[d]		GLC-MS	
Solvent system	Spot no.[b]	R_f[c]	Peak no.[b]	R_t relative to p,p′-DDE	No. of chlorines	Molecular weight
Aroclor 1232						
II	1	0.35	13	1.46	6	358
	2	0.40	11	1.07	5	324
	3	0.46	12	1.29	6	358
	4	0.55	8	0.64	5	324
	5	0.60	1	0.07	1	188
	6	0.66	10	0.97	5	324
	7	0.72	9,6	0.76,0.40	5,4	324,290
	8	0.75	5,7[f]	0.30,0.52	3,4	256,290
	9	0.78	7A	0.48	4	290
	10	0.84	7,5	0.48,0.30	4,3	290,256
	11	0.87	3	0.21	2	222
	12	0.89	2	0.15	2	222
Aroclor 1248						
II	1	0.37	11	1.06	5	324
	2	0.42	12,9	1.28,0.76	6,5	358,324
	3	0.47	8	0.63	8	324
	4	0.51	10	0.99	5	324
	5	0.57	9	0.76	5	324
	6	0.63	5,6	0.30,0.40	3,4	256,290
	7	0.68	7A&B[e]	0.47,0.51	4	290
	8	0.76	3,2	0.21,0.15	2	222
	9	0.79	4	0.24	3	256
Aroclor 1254						
II	1	0.21	16	2.36	6	358
	2	0.27	13	1.55	6	358
	3	0.35	12,15	1.28,1.95	6	358
	4	0.40	14	1.61	6	358
	5	0.45	9	0.79	5	324
	6	0.55	11	1.05	5	324
	7	0.69	8,6	0.66,0.40	5,4	324,290
	8	0.76	7A	0.48	4	290
Aroclor 1260						
IV	1	0.50	18	5.23	7	392
	2	0.60	16	3.10	7	392
	3	0.68	12A,14,17	1.51,2.12,3.96	6,7	358,392
	4	0.76	12B,13,15	1.67,1.69,2.58	6,7	358,392
	5	0.87	9,11	0.82,1.28	5,6	324,358
	6	0.92	10,12A	1.22,1.51	6	358
	7	0.96	8,10	0.72,1.12	5,6	324,358

[a] Conditions for RPTLC and GLC are those described in text.
[b] See Figs. 2 and 3 for spot numbers and Figs. 5 and 6 for peak numbers.
[c] R_f values of p,p′-DDE for solvent systems II and IV were 0.60 and 0.92, respectively.
[d] A column temperature of 160°C was used, except for GLC of Aroclor 1260 which was resolved at 190°C.
[e] Two components under the same peak.

GLC peaks were numbered in order of increasing retention time.

In order to relate the retention time of GLC peaks obtained from RPTLC separations, 3 standards of the same material were spotted approximately 1 cm apart and 2 cm above the lower edge of a plate. The plate was developed as previously described and a narrow strip of foil was placed over the center area of the developed plate, covering the area of the middle sample. The chromogenic agent was carefully applied and the plate was irradiated sufficiently to reveal the 2 outside rows of spots. The foil was removed and horizontal zones of the unreacted middle standard, corresponding to spots of the 2 outside standards, were scraped and eluted with 5% diethyl ether in

Table 3. Chromatographic characterization of chlorinated naphthalenes (Halowaxes): correlation of reverse phase thin layer chromatographic (RPTLC) spots and GLC peaks[a]

RPTLC			GLC[d]		GLC-MS	
Solvent system	Spot no.[b]	R_f[c]	Peak no.[b]	R_f relative to aldrin[e]	No. of chlorines	Molecular weight
Halowax 1099						
IV	1	0.52	11	1.97	5	298
	2	0.67	6,7, 12,13	0.83,1.03, 2.48,2.82	4 5	264 298
	3	0.77	8	1.18	4	264
	4	0.81	4,9	0.44,1.28	3,4	230,264
	5	0.85	10,5	1.53,0.62	4,3	264,230
	6	0.89	2	0.23	2	196
Halowax 1013						
IV	1	0.46	11,15	1.67,4.64	5	298
	2	0.50	16,17	4.98,5.70	6	332
	3	0.58	5B,12	0.70,1.97	4,5	264,298
	4	0.66	6,7, 13,14	0.82,1.02, 2.48,2.82	4 5,6	264 298,332
	5	0.76	9	1.27	4	264
	6	0.81	10,4	1.52,0.43	4,3	264,230
	7	0.85	5A	0.60	3	230
Halowax 1014						
IV	1	0.35	15,16,20	1.60,1.89,4.21	6,7	332,366
	2	0.44	11,12, 17,18	0.72,0.84 1.98,2.28	5 6	298 332
	3	0.56	5,19	0.30,2.53	3,6	230,332
	4	0.61	6,13,14	0.38,1.05,1.19	4,5	264,298
	5	0.81	9,4	0.23,0.58	4,3	264,230

[a] Conditions for RPTLC and GLC are those described in text.
[b] See Fig. 3 for spot numbers and Figs. 4 and 6 for peak numbers.
[c] R_f of p,p'-DDE = 0.92.
[d] A column temperature of 160°C was used, except for GLC of Halowax 1014 which was resolved at 190°C.
[e] Except for Halowax 1014; R_f values for this compound are reported relative to p,p'-DDE.

FIG. 1—Reverse phase thin layer chromatogram of PCB fraction of goldfish extract (F012), standard DDE, and standard Aroclor 1248. TLC plate was prepared as described in text and developed 3 times in solvent system II (Table 1).

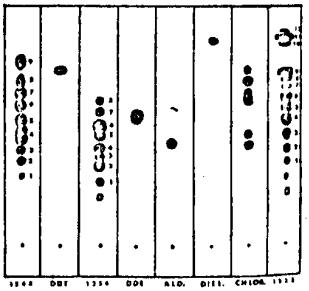

FIG. 2—Reverse phase thin layer chromatograms (solvent system II) of Aroclors 1232, 1248, and 1254, p,p'-DDT, p,p'-DDE, aldrin, dieldrin, and technical chlordane.

95% petroleum ether. The resulting solutions were analyzed by GLC.

Temperature-programmed GLC-MS was used to characterize Halowax standards as to chlorine content of individual GLC peaks. In a previous

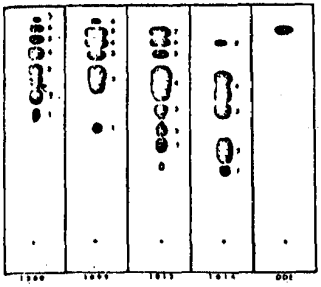

FIG.—3. Reverse phase thin layer chromatograms (solvent system IV) of Aroclor 1260, Halowaxes 1013, 1014, and 1099, and p,p'-DDE.

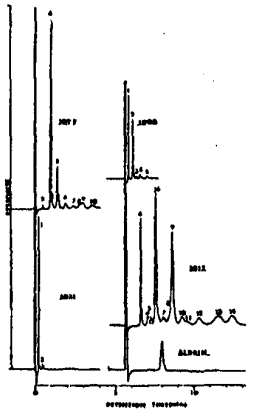

FIG. 4—Gas chromatograms of Halowax standards: 8 ng 1031, 6 ng 1000, 5 ng each of 1031 and 1099, and 4 ng 1013. See text for instrumental conditions. Column temperature—160°C. Numbers above peaks are assigned in order of increasing retention time.

study, we used GLC-MS to determine the chlorine content of GLC peaks representing components of Aroclor standards (5).

The R_f values of RPTLC spots and their corresponding GLC retention times (R_t) were determined for the various Aroclors and Halowaxes resolved by RPTLC (Tables 2 and 3). R_f and R_t values were based on ≥ 2 determinations and were reproducible in subsequent separations. From MS data, the number of chlorines and component molecular weight of corresponding GLC peaks and RPTLC spots were determined.

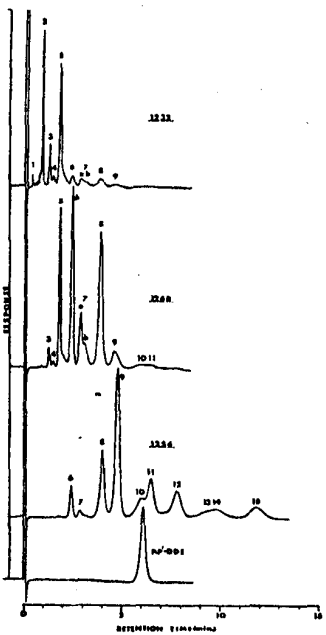

FIG. 5—Gas chromatograms of Aroclor standards: 6 ng 1232, 8 ng 1248, and 4 ng 1254. See Fig. 4 for GLC conditions and numbering of peaks.

Components of the Aroclors were resolved better by RPTLC than were Halowax components. In some cases, RPTLC separated Aroclor components which eluted as a single GLC peak. However, RPTLC separations of Halowaxes resulted in several spots which were resolved by GLC into

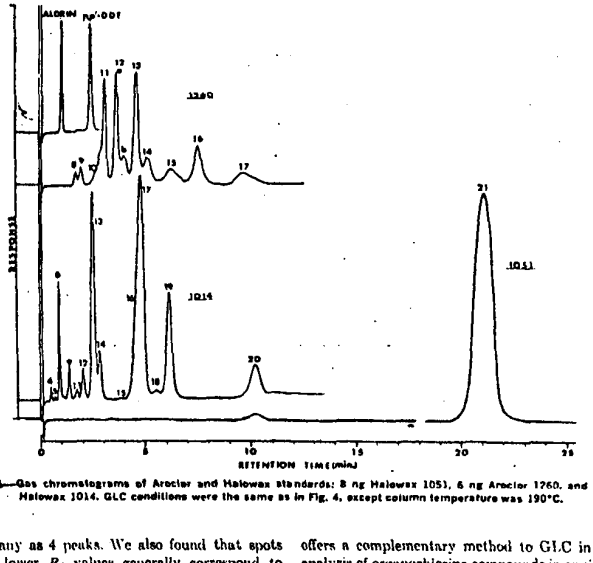

FIG. 6.—Gas chromatograms of Aroclor and Halowax standards: 8 ng Halowax 1051, 6 ng Aroclor 1260, and 5 ng Halowax 1014. GLC conditions were the same as in Fig. 4, except column temperature was 190°C.

as many as 4 peaks. We also found that spots with lower R_f values generally correspond to PCB or Halowax components substituted with more chlorine atoms and that GLC peaks with longer retention times correspond to spots with lower R_f values (4). Spot patterns of individual Aroclors or Halowaxes were reproducible and characteristic of the material they represented.

Aroclor 1232 consists of a mixture of Aroclors 1221 and 1242 and it is not a well defined PCB formulation (7). Thus, RPTLC spot pattern intensities may vary somewhat for different samples of this Aroclor.

Separation of PCB residues in fish with RPTLC provides effective resolution of PCB homologs. The technique in combination with GLC can provide information on the isomer and homolog composition of PCB residues when MS is not available for confirmation. RPTLC separation of Aroclors, Halowaxes, and chlorinated pesticides offers a complementary method to GLC in the analysis of organochlorine compounds in environmental samples.

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