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HIGH-SPEED LIQUID AND THIN-LAYER CHROMATOGRAPHY OF POLYCHLORINATED BIPHENYLS

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SUMMARY

High-speed liquid chromatography in the system silica gel/dry *n*-hexane and ultraviolet spectrometry have been used to study the composition of various types of commercially available mixtures of chlorinated biphenyls. Special attention has been paid to the analysis of highly chlorinated products. In addition to data previously published, retention times are recorded for 11 individual polychlorinated biphenyls.

The results of high-speed liquid chromatography are compared with those obtained in several normal and reversed-phase thin-layer chromatographic systems.

INTRODUCTION

Recently, high-speed liquid chromatography (HSLC) of polychlorinated biphenyls (PCBs) and several common pesticides has been extensively discussed[1]. Chromatography in the system silica gel/dry *n*-hexane permits rapid qualitative analysis of such compounds, as has been amply demonstrated for the lower members of the Aroclor series. Two further aspects of the chromatographic analysis of PCBs are discussed in the present paper. First, detailed information is given concerning the composition of the more highly chlorinated Aroclors, and the HSLC chromatograms of the Aroclors are compared with those of PCB mixtures from other manufacturers. Secondly, the usefulness of thin-layer chromatography (TLC), in the normal and reversed-phase modes, is demonstrated for the qualitative analysis of the PCB mixtures, and the results so obtained are compared with those from HSLC.

MATERIALS AND METHODS

The PCB samples investigated included the Aroclors 1221, 1232, 1242, 1248, 1254, 1260 and 1268 (Monsanto, St. Louis, Mo., U.S.A.), Phenoclor DP3, DP4, DP5 and DP6 and Pyralene 2000, 1500 and 3010 (Prodelec, Paris, France) and Clophen A30, A40, A50 and A60 (Bayer, Leverkusen, G.F.R.). Small samples of pure 2,3,2',4'- and 2,5,2',5'-tetrachlorobiphenyl were obtained from Dr. R. G. Webb (Southeast Environmental Research Laboratory, Athens, Ga., U.S.A.); samples of 2,4,5,2',4',5'-, 3,4,5,3',4',5'- and 2,3,6,2',4',5'-hexachlorobiphenyl and 2,3,4,5,2',3',6'-heptachlorobiphenyl were gifts from Dr. A. C. Tas and Dr. R. J. C. Kleipool (CIVO, Zeist, The Netherlands), who also placed at our disposal samples containing 2,4,5,2′,4′,6′-hexachlorobiphenyl and 2,3,4,5,2′,3′,4′-, 2,3,4,5,2′,4′,5′-, 2,3,4,5,2′,4′,6′- and 2,3,4,5,3′,4′,5′-heptachlorobiphenyl. All of the other chemicals were of reagent-grade quality, with the exception of *n*-hexane (see below).

For HSLC, a Siemens S100 liquid chromatograph equipped with a 10-μl automatic injection syringe and a Zeiss PM2 DLC UV-detector was used. Baseline noise was eliminated by inserting an RC-filter (τ_{RC} 0.9 sec) at the output of the recorder; long-term drift was decreased by using a home-made thermostatted detector cell (volume, 8 μl; path length, 1 cm). The column was a stainless-steel tube (25 cm × 3 mm I.D.), pre-packed with 5-μm Lichrosorb SI 60 silica gel (Merck, Darmstadt, G.F.R.). The system was thermostatted at 27 ± 1°.

TLC in the normal mode was carried out on pre-coated silica gel plates (Kieselgel$_{254}$; Merck) as the stationary phase, with dry *n*-hexane as the mobile phase. Reversed-phase TLC was carried out on Kieselguhr G (Merck) impregnated with paraffin oil, using mixtures of acetonitrile, methanol, acetone and water as solvent systems. Details of the various procedures and the method of detection are presented in the next section.

UV spectra were recorded on a Beckman Acta CIII spectrometer using cells with quartz windows.

RESULTS AND DISCUSSION

High-speed liquid chromatography

During the HSLC work, problems arose concerning the quality of the *n*-hexane used as the mobile phase. Samples of *n*-hexane obtained from several firms were found to contain impurities which showed UV absorption in the 200–250 nm range. This causes interference in the detection of the PCBs, which have absorption maxima between 195 and 225 nm. Further study showed that benzene is often the main impurity. In order to avoid such complications, we recommend the use of ChromAR *n*-hexane (Mallinckrodt, St. Louis, Mo., U.S.A.) which has a very low absorbance down to 200 nm.

HSLC chromatograms of the Aroclor 1221–1268 series in the system silica gel/dry *n*-hexane have been published in a recent paper[1]. In the present study, chromatograms were run of other commercial PCB mixtures. All of the Phenoclors DP3–DP6 and the Clophens A30–A60 displayed UV spectra and HSLC chromatograms which are essentially the same as those of the Aroclors 1242–1260 (Table I). A typical HSLC chromatogram is presented in Fig. 1d. Small differences between the various series do occur. For example, in Clophen A30, no peak due to 2-chlorobiphenyl could be found, although this compound is present in both Aroclor 1242 and Phenoclor DP3. Further, the characteristic peaks with t_R 2.30–2.60 min previously observed[1] in Aroclor 1248 also occurred in Phenoclor DP4, but they were virtually absent from Clophen A40.

More striking differences are found when taking into consideration the Pyralene series. As is evident from Fig. 1a, Pyralene 2000 contains a much higher proportion of 2,4′- and of 2,2′-dichlorobiphenyl than does Aroclor 1232, whereas the peaks due to biphenyl and 2-chlorobiphenyl were more prominent in the Aroclor mixture. Ac-

TABLE I

CHARACTERISTICS OF COMMERCIAL PCB MIXTURES

PCB mixture		Average no. of Cl per molecule	$\lambda_{max.}$ in nm ($\varepsilon \cdot 10^{-3}$ in l/mole·cm)*		Corresponding Aroclor
			Main band	κ band	
Aroclor	1221	1.15	202 (39)	245 (12)	
	1232	2.04	202 (38)	244 (11)	
	1242	3.10	204 (41)	240–245	
	1248	3.90	205 (45)	240–250	
	1254	4.96	208 (49)		
	1260	6.30	209 (58)		
	1268	8.70	213 (80)		
Clophen	A30		204 (42)	240–245	1242
	A40		206 (46)	240–250	1248
	A50		209 (51)		1254
	A60		209 (64)		1260
Phenoclor	DP3		204 (40)	240–245	1242
	DP4		206 (47)	240–250	1248
	DP5		209 (61)		1254
	DP6		209 (73)		1260
Pyralene	2000	2	202 (41)	245 (11)	1232
	1500	2.5	203 (43)	240–245	—
	3010	3	204 (43)	240–245	1242

* Values of $\varepsilon \cdot 10^{-3}$ for Clophens and Phenoclors were calculated on the basis of the assumption that the molecular weights of the various mixtures are equal to those of the corresponding Aroclors.

cording to expectation, Pyralene 1500 displayed a chromatogram (Fig. 1b) intermediate between those of Pyralenes 2000 and 3010; Pyralene 3010 showed a retention-time pattern (Fig. 1c) which was almost identical with that of Aroclor 1242. A peak due to 3-chlorobiphenyl, a very minor constituent of low-chlorinated PCB mixtures according to most workers (*cf.* ref. 2), was observed in all Pyralenes and also in the lower chlorinated Phenoclors and Clophens. This is in keeping with our previous data on the composition of Aroclors 1232, 1242 and, particularly, 1221. In our opinion, the discrepancy between the gas–liquid chromatographic (GLC) and HSLC results can be explained at least partly by the fact that the molar response of the electron-capture detector to 3-chlorobiphenyl is 5–6 times lower than that to both 2- and 4-chlorobiphenyl[3], whereas the response of the UV detector is similar for all three compounds[1].

Retention times in the system silica gel/dry *n*-hexane and UV spectral data for 11 PCBs are recorded in Table II. For ease of comprehension, some data from ref. 1 are also included in the Table. The following conclusions can be drawn.

(1) The previous[1] assignment of a peak with retention time (t_R) 3.95 min to 2,5,2′,5′-tetrachlorobiphenyl is now shown to be incorrect. This compound actually has a retention time of 3.50 min, which implies that it coincides with 2,4,5,2′,3′-pentachlorobiphenyl and forms part of the large band with t_R 3.50–3.80 min in Aroclor 1254. This is in keeping with GLC results[4], which indicate that the 2,5,2′,5′-isomer is one of the principal constituents of Aroclor 1254. The peak with t_R 3.95 min cannot

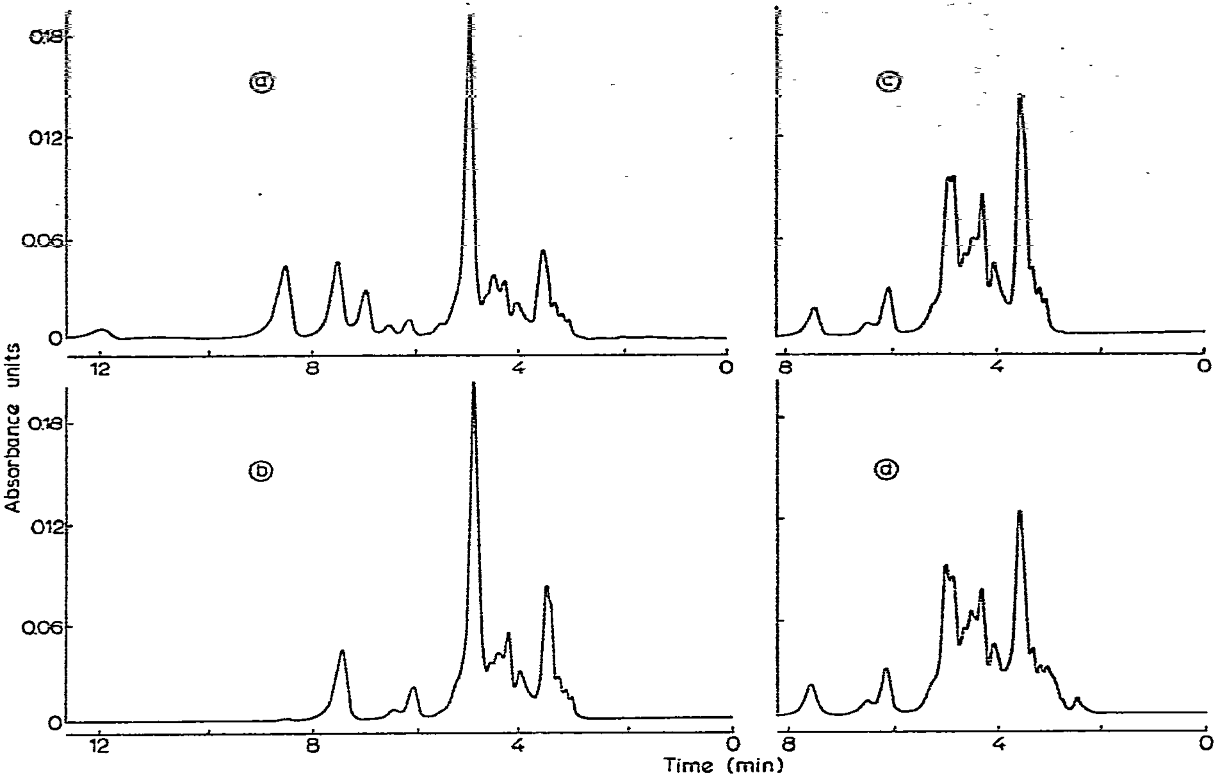

Fig. 1. HSLC of (a) Pyralene 2000, (b) Pyralene 1500, (c) Pyralene 3010 and (d) Clophen A30 (*ca.* 200 ppm in *n*-hexane). Conditions: column (25 cm × 3 mm I.D.) filled with 5-μm Lichrosorb SI 60; mobile phase, dry *n*-hexane; flow-rate, 1.4 ml/min; UV detection at 205 nm; temperature, 27 ± 1°. Peak assignments can be made with the help of the data published[1] for Aroclors 1232 and 1242.

be attributed to 2,3,2′,4′-tetrachlorobiphenyl (*cf.* Table II), since it is one of the PCBs shown to be absent[4] from Aroclors 1221–1254. The peaks observed in Aroclor 1254 with t_R 2.50 and 3.10 min can be assigned to 2,4,5,2′,4′,5′- and 2,3,6,2′,4′,5′-hexachlorobiphenyl, respectively.

(2) The availability of a large number of hexa- and heptasubstituted PCBs enables us to assign 10 peaks and shoulders in the HSLC chromatogram of Aroclor 1260 to individual PCBs. The interpretation was made with the help of GLC data of Sissons and Welti[5] for Aroclor 1260, and of Tas and co-workers[6,7] for Phenoclor DP6, which has a composition similar to that of Aroclor 1260 (see above). Since the resolution of PCBs in the system silica gel/dry *n*-hexane decreases with increasing chlorine content, the HSLC chromatogram of Aroclor 1260 (Fig. 2) was run at a flow-rate of *ca.* 0.3 ml/min (pressure, 16 bar), in order to optimize the separation efficiency of the HSLC system. A chromatogram of Aroclor 1268, run under the same conditions, is included in the figure. Comparison with the chromatogram for Aroclor 1260 allows the tentative assignment of two corresponding peaks to 2,3,4,5,2′,4′,5′-hepta- and 2,3,4,5,2′,3′,4′,5′-octachlorobiphenyl.

(3) The UV spectral data confirm and extend our earlier observations. Among

TABLE II

HSLC RETENTION TIMES AND UV SPECTRAL DATA FOR INDIVIDUAL PCBs

For HSLC and UV conditions, see text. Retention time data in the lower half of the table have been taken from ref. 1.

No.	*Substituted PCB*	t_R *(min)*	$\lambda_{max.}$ *and* $\lambda_{sh.}$ *in nm* ($\varepsilon \cdot 10^{-3}$ *in l/mole·cm)*	
			Main band	κ *band*
51	2,5,2′,5′	3.50*	201 (49), 213, 225	
52	2,3,2′,4′	3.90	205 (52), 228	235–240
53	2,3,6,2′,4′,5′	3.10	207 (54), 220–235	
54	2,4,5,2′,4′,5′	2.50	212 (57)	245–250
55	2,4,5,2′,4′,6′	2.25		
56	3,4,5,3′,4′,5′	2.90	222 (55), 218, 230	265 (24)
57	2,3,4,5,2′,3′,4′	2.90		
58	2,3,4,5,2′,3′,6′	3.05	208 (65), 218, 237	
59	2,3,4,5,2′,4′,5′	2.30		
60	2,3,4,5,2′,4′,6′	1.95		
61	2,3,4,5,3′,4′,5′	2.30		
22	2,3,6,2′,5′	3.80		
7	2,3,4,5,2′,5′	2.85		
10	2,3,4,2′,4′,5′	3.00		
21	2,3,6,2′,3′,6′	3.75		
4	2,3,4,5,2′,3′,4′,5′	2.25		
2	2,3,4,5,6,2′,3′,4′,5′	2.00		
1	2,3,4,5,6,2′,3′,4′,5′,6′	1.70		

* Value replaces the incorrect retention time quoted in ref. 1.

the new entries, 3,4,5,3′,4′,5′-hexachlorobiphenyl only exhibited a strong and well formed κ band at 265 nm, while such a band is absent in highly *ortho*-substituted PCBs such as 2,3,6,2′,4′,5′-hexa- and 2,3,4,5,2′,3′,6′-heptachlorobiphenyl. Further, the extinction coefficients, ε_{main}, are of the expected order of magnitude, 50,000–70,000, and $\lambda_{max., main}$ generally falls between 200 and 215 nm. Here, however, one should note the exceptionally large bathochromic shift of the main band of 3,4,5,3′,4′,5′-hexachlorobiphenyl, which has also been observed by Hutzinger *et al.*[8]. This shift appears to be in keeping with the behaviour of other non-*ortho*-substituted PCBs. For instance, 3,4,3′,4′-tetra-

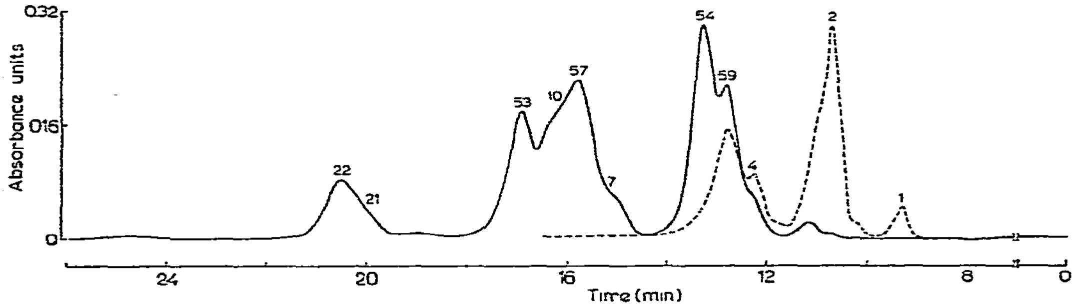

Fig. 2. Assignment of peaks to individual PCBs (*cf.* Table II) in HSLC chromatograms of Aroclors 1260 (——) and 1268 (– – –) (*ca.* 170 ppm in *n*-hexane). Conditions as in Fig. 1, except for the flow-rate (0.3 ml/min).

TABLE III

COMPARISON OF BEHAVIOUR OF TRI-, HEXA- AND HEPTA-SUBSTITUTED PCBs IN HSLC

Retention times are taken from Table II and ref. 1.

Trisubstituted PCB	t_R *(min)*	*Hexa-substituted PCB*	t_R *(min)*	*Hepta-substituted PCB*	t_R *(min)*
2,4,6	4.45	2,4,6,2′,4′,6′	2.05	2,3,4,5,2′,4′,6′	1.95
		2,4,5,2′,4′,6′	2.25		
2,4,5	4.45	2,4,5,2′,4′,5′	2.50	2,3,4,5,2′,4′,5′	2.30
		3,4,5,3′,4′,5′	2.90	2,3,4,5,3′,4′,5′	2.30
		2,3,4,2′,4′,5′	3.00		
		2,3,6,2′,4′,5′	3.10		
2,3,4	6.10	2,3,4,2′,3′,4′	3.65	2,3,4,5,2′,3′,4′	2.90
2,3,6	6.15	2,3,6,2′,3′,6′	3.75	2,3,4,5,2′,3′,6′	3.05

chlorobiphenyl has $\lambda_{max.}$ at *ca.* 211 nm (ref. 8), whereas the 2,3,2′,3′-, 2,5,2′,5′- and 2,6,2′,6′- isomers display maximum absorption at 204, 201 and 200 nm, respectively (Table II and ref. 1). A similar trend is observed with the *x,x*′-dichlorobiphenyls.

(4) Two general rules have been formulated[1] concerning the dependence of the retention time of PCBs on the number and position of chlorine atoms in the biphenyl nucleus: (a) retention generally decreases with increasing chlorine content; (b) 2,3-substitution distinctly promotes retention. The present data confirm the validity of both rules. Moreover, they help to demonstrate that the retention time of, *e.g.*, *a,b,d*′,*e*′-tetrachlorobiphenyls and *a,b,c,d*′,*e*′,*f*′-hexachlorobiphenyls is invariably intermediate between those of *a,b,a*′,*b*′- and *d,e,d*′,*e*′-tetrachlorobiphenyls, and *a,b,c, a*′,*b*′,*c*′- and *d,e,f,d*′,*e*′,*f*′-hexachlorobiphenyls, respectively. An example can be found in Table III.

In ref. 1, no conclusions were drawn regarding the effect of 2,6-substitution, although 2,6-substituted PCBs generally have rather long retention times; for example, 2,6,2′,6′-tetrachlorobiphenyl is even more retarded than the 2,3,2′,3′-isomer. This was due to the fact that 2,4,6,2′,4′,6′-hexachlorobiphenyl displayed an unexpectedly short retention time. In Table III, the behaviour of *x,y,z*-tri-, *x,y,z,x*′,*y*′,*z*′-hexa- and 2,3,4,5,*x*′,*y*′,*z*′-hepta-substituted PCBs is compared. The extraordinary behaviour of 2,4,6,2′,4′,6′-hexachlorobiphenyl is now seen to be the rule rather than the exception. With all three series of isomers, retention decreases in the order: 2,3,6 > 2,3,4 > 3,4,5 > 2,4,5 > 2,4,6. That is, whereas 2,6-substitution promotes retention, symmetrical 2,4,6-substitution strongly diminishes the interaction between the substituted biphenyl and the silica gel.

Thin-layer chromatography

In the literature, several workers have studied TLC of commercial PCB mixtures (*e.g.*, refs. 9–14). From their results, one may tentatively conclude that reversed-phase TLC is generally superior to TLC in its normal mode. In the present study, the relative merits of three systems have been assessed: (a) silica gel/*n*-hexane; (b) Kieselguhr impregnated with paraffin oil/acetonitrile–methanol–water (8:9:3)[13]; (c) Kieselguhr impregnated with paraffin oil/acetonitrile–methanol–acetone–water (20:

20:9:1)[13]. With the latter two systems, the developing solvent is saturated with paraffin oil before use.

Preliminary experiments on TLC in its normal mode showed that separations obtained with the system silica gel/*n*-hexane are superior to separations involving the use of, *e.g.*, aluminium oxide as stationary phase or isooctane as eluent. Moreover, thorough drying of both the silica gel plates and the hexane considerably increased the efficiency of the separation. Therefore, the following procedure[15] was used in all further experiments. A pre-coated silica gel plate was dried overnight at 150–160°. Subsequently, while the activated plate was still in the oven, the sandwich chamber was assembled with the help of a second glass plate and PTFE strips so as to leave a 1.5-cm wide margin of the silica gel plate for spotting. After leaving the plates in a desiccator for 30 min, small spots or *ca.* 1-cm wide zones of a 1–2% solution of a PCB mixture in decane were applied with the help of a pointed paperwick or an injection syringe. After evaporation of the decane (*ca.* 30 min), the plate was developed (*ca.* 30–60 min) with *n*-hexane (dried over molecular sieve 5A) for a run of length 10–20 cm. The spots were made visible under UV light (254 nm).

As for reversed-phase TLC, optimal results were obtained with home-made Kieselguhr plates. A mixture of 25 g of Kieselguhr and 60 ml of water was used for preparation[12] of the plates, employing a Camag spreading apparatus. The plates were dried overnight, dipped for 2 min in a 8% solution of paraffin oil in *n*-hexane or light petroleum (b.p. 40–60°) and subsequently dried overnight. Slightly inferior results were obtained when using pre-coated Kieselguhr-on-glass plates. Pre-coated Kieselguhr-on-aluminium foils yielded much less efficient separations. Moreover, with both types of ready-for-use layers, the presence of the fluorescent indicator detracted from the detection with tolidine reagent. After spotting and evaporation of the solvent (see above), development was carried out in a saturated rather than a sandwich chamber, using the solvent mixtures mentioned above. For a migration distance of 18 cm, the run time was *ca.* 1 h. After drying the chromatoplates for 30 min, the spots were detected by use of a 0.5% solution of tolidine in 80% ethanol containing 0.5% of glacial acetic acid, and subsequent irradiation with UV light (254 nm) for between 15 min and 16 h. Blue or blue-green, and occasionally brown, spots or zones were observed, and can be preserved for several weeks if the chromatoplates are stored in a refrigerator.

Chromatograms obtained with each of the three TLC systems are shown in Fig. 3. It should be noted that in all cases the number and/or intensity of the zones may vary slightly with the experimental conditions. However, this does not seriously detract from the use of TLC as a tool for discriminating between PCB mixtures. The following conclusions can be drawn.

The separations achieved in the present normal-mode TLC system are distinctly superior to those recorded in the literature, where PCB mixtures either migrate as a single spot[10,11] or are resolved into two or three spots[9] in a rather non-characteristic way. In addition, it should be noted that resolution of the slow-migrating PCB mixtures can be improved still further by overdevelopment; in this way, Aroclors 1221 and 1242 have been resolved into six well separated zones. Compared with reversed-phase TLC, normal TLC is more convenient and less time-consuming. Moreover, detection under UV light is far more rapid than is the tolidine–UV technique. On the other hand, with the latter method of identification, a complete range of spot

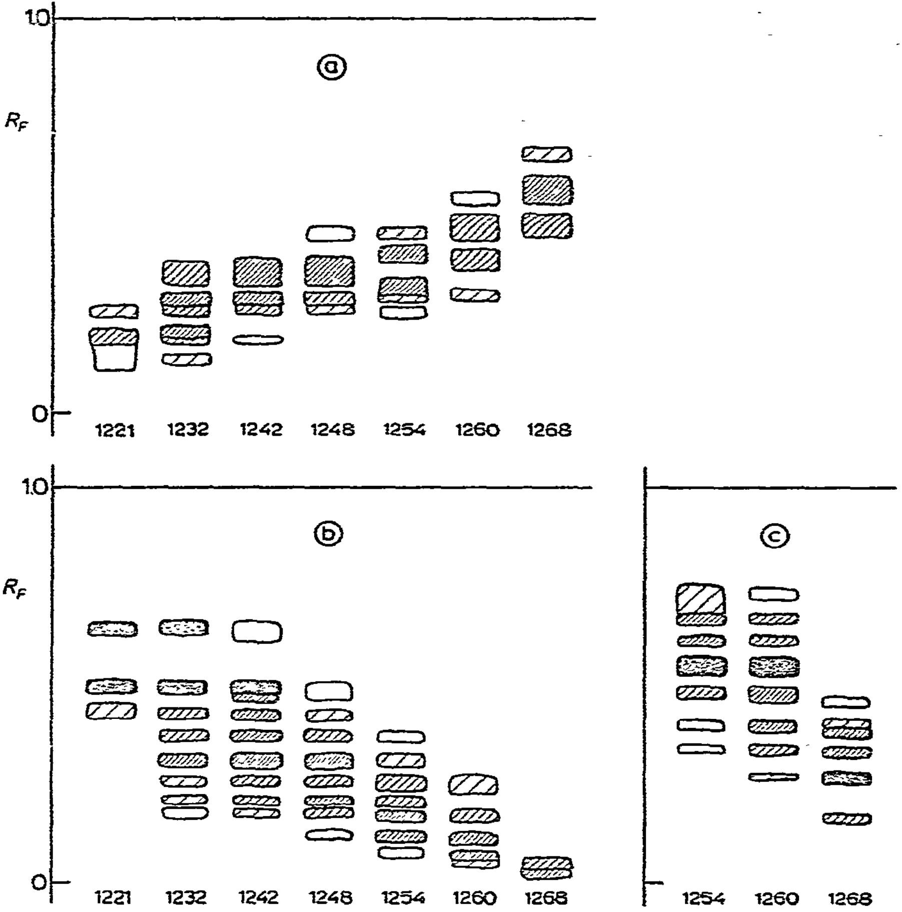

Fig. 3. TLC of Aroclors 1221–1268 in three systems: (a) silica gel/*n*-hexane; (b) Kieselguhr impregnated with paraffin oil/acetonitrile–methanol–water (8:9:3); (c) Kieselguhr impregnated with paraffin oil/acetonitrile–methanol–acetone–water (20:20:9:1). For details, see text.

intensities can be discerned, while with direct viewing under UV light the majority of the spots are of similar intensity. A further, and more crucial, drawback of normal-mode TLC is that the resolution is inferior to that in reversed-phase TLC. Excellent separations were obtained with both reversed-phase systems; multiple development, proposed by several workers[12–14], seems to be superfluous. For Aroclors 1221–1248, the solvent system acetonitrile–methanol–water (8:9:3) is recommended, while the more highly chlorinated Aroclors are best separated with acetonitrile–methanol–acetone–water (20:20:9:1). Reversed-phase TLC has been successfully used (*cf.* ref. 16) to demonstrate the similar compositions of Phenoclors DP5 and DP6, and Aroclors 1254 and 1260, respectively.

Finally, according to expectations based on our HSLC data, the R_F values of the Aroclors in normal-mode TLC increase, *i.e.*, retention decreases, with increasing chlorine content. In order to further confirm this conclusion, for several Aroclors the various zones on the thin-layer chromatograms were scraped off and extracted with

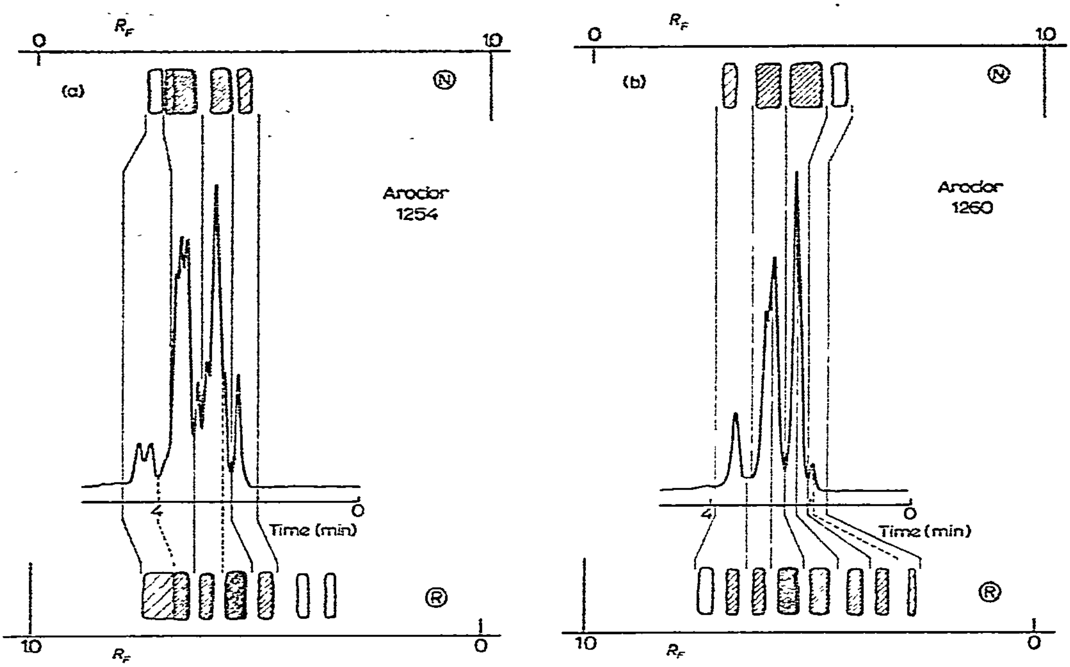

Fig. 4. Comparison of the behaviour of (a) Aroclor 1254 and (b) Aroclor 1260 in HSLC, and in normal (N) and reversed-phase (R) TLC. For conditions, see Fig. 3c (TLC) and ref. 1 (HSLC).

n-hexane, and the extracts were dried over molecular sieve 5A and analyzed by HSLC. Typical results are shown in Fig. 4. A similar procedure was followed for reversed-phase TLC, where according to expectations sorption should decrease with decreasing chlorine content. Despite some overlap of R_F/t_R ranges in the lower half of the figure, there is again a fairly strict relation between R_F sequence and order of elution time. It may be concluded that reversed-phase HSLC of PCB mixtures will yield a similar elution sequence of the individual constituents (although in reversed order) as does HSLC in the system silica gel/dry *n*-hexane. However, in view of the superiority of reversed-phase over normal TLC noted above, the resolution may well be superior.

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