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STRUCTURAL IDENTIFICATION OF POLYCHLORINATED BIPHENYLS IN COMMERCIAL MIXTURES BY GAS–LIQUID CHROMATOGRAPHY, NUCLEAR MAGNETIC RESONANCE AND MASS SPECTROMETRY

D. SISSONS AND D. WELTI
Unilever Research Laboratory, Colworth House, Sharnbrook, Bedford (Great Britain)

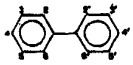
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SUMMARY

The major constituent polychlorinated biphenyls in Aroclor 1254 have been characterised by high resolution nuclear magnetic resonance and mass spectrometry following separation by liquid–solid and gas–liquid chromatography. The retention indices of these compounds together with those of forty synthesised polychlorinated biphenyls have been used to predict the structures of the remaining Aroclor 1254 constituents, together with all those of Aroclor 1242 and 1260.

INTRODUCTION

Polychlorinated biphenyls (PCBs) exhibit a high degree of biological and chemical stability. As they are lipid soluble they tend to accumulate in food chains and, since 1966, have been detected in a wide range of marine organisms, sea birds and even human adipose tissue[1–6]. Particularly high levels have been found in marine samples taken from the Baltic[6] and the Pacific coastal waters of the U.S.A.[7]. Quantitative analytical data are only approximate because of the large number (210) of PCBs that are theoretically possible—up to ten chlorine atoms may be substituted in the molecule as indicated below:



Quantitative results are estimated by relating the peak areas obtained from extracts of PCBs in an electron-capture (EC) gas–liquid chromatography (GLC) chromatogram to the corresponding peak area(s) produced by a known weight of a commercial PCB mixture or an insecticide such as DDE. Certain authors[8–11] have shown that the patterns of GLC peaks produced by extracts of marine organisms are very similar to those produced by commercial PCB products containing 54% or 60% chlorine. While this is true for species indigenous to rivers and well defined sea-areas, *e.g.*

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estuaries, the situation may be quite different in large sea-areas such as the North Sea. The PCBs that are present in some species of sea fish have been shown in this laboratory to vary widely in type and quantity and bear little resemblance to any commercial product[19]. In spite of a growing interest in the toxicology of PCBs, relatively few data have been published on this aspect to date. All available information relates to trials involving commercial products[12] although individual PCBs may well have differing toxicities.

Both from the analytical and toxicological viewpoints, therefore, there is a need to think in terms of individual PCBs rather than complex mixtures, in order to progress to a greater understanding of the problems and significance of PCB contamination. As a first step in this direction we have used a combination of analytical techniques to characterise the component PCBs in Aroclors 1242, 1254, and 1260 (commercial products manufactured by Monsanto Chemicals Ltd. containing 42%, 54% and 60% chlorine, respectively). Aroclor 1254 was fractionated on a column of alumina and the major PCBs in the fractions were separated and collected from a gas–liquid chromatograph. The separated PCBs were characterised by mass spectrometry (MS) and high resolution nuclear magnetic resonance spectroscopy (NMR) to determine the number and positions of the chlorine atoms associated with each compound. NMR was chosen in preference to IR spectroscopy as it is a more definitive technique although the latter has the advantage of requiring a smaller sample size.

The NMR spectra of chlorinated biphenyls which have been reported previously[14–17] were unhelpful in this instance. MAYO AND GOLDSTEIN[18] have analysed biphenyl itself on 60 and 100 MHz spectrometers.

The PCBs characterised by NMR, together with forty individually synthesised PCBs were subsequently analysed by high resolution GLC to determine their absolute retention values using the KOVÁTS[10] retention index (R.I.) system. Previous GLC data on PCBs have been restricted to relative retention times[20]. Attempts to derive accurate retention data based on internal standards were unsuccessful due to variations in conditions during the lengthy GLC runs of up to 16 h duration, and because of the severe limitations placed on the choice of standards due to the complex Aroclor chromatograms. The structures of the minor PCB constituents of Aroclor 1254 and all PCBs in Aroclors 1242 and 1260 have been predicted by a variation of the interpolation method suggested by EVANS AND SMITH[21].

EXPERIMENTAL

Fractionation of Aroclor 1254 on an alumina column

60 mg of Aroclor 1254 was washed onto a 25 × 1 cm column of 20 g activity grade I alkaline alumina (M. Woelm, Eschwege, G.F.R.) with five 1 ml volumes of hexane. All PCBs were eluted in 900 ml of hexane (flow rate of 1 ml/min), collected in 25 ml fractions. This fractionation was repeated with a further 60 mg of Aroclor and the corresponding fractions were added together. After suitable dilution, usually 100 or 1000 fold, each fraction was analysed by EC GLC to determine whether further separation of PCBs was required. Analyses were performed on a Pye Model 74 instrument fitted with a nickel-63 EC-detector and a 7 ft. × ¼ in. "pyrex" column of 2% Apiezon L + 0.02% Epikote resin on 80–100 mesh Varaport 30 packing material. Of a wide variety of stationary phases that were tested, Apiezon L gave the highest

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resolution of PCBs in Aroclor 1254. Argon was used as carrier gas at an inlet pressure of 25 p.s.i. and a flow of 80 ml/min. The column and injector were maintained at a temperature of 230° and the detector at a temperature of 325°.

Collection of GLC fractions for NMR

Alumina column fractions were separated into their component PCBs on a 7 ft. × ¼ in. GLC column similar to that described above but fitted into a Pye 104 chromatograph adapted for fraction collecting. The effluent gas was split in the ratio of twenty-five parts to the collection outlet and one part to the flame-ionisation detector. The temperature was programmed from 220–230° at 1°/min and the carrier gas flow was 40 ml/min of argon measured at 220°. The outlet to the collection trap was maintained at 250°. The traps were interchangeable straight metal tubes loosely packed with glass wool. They were screwed onto a threaded outlet tube and maintained at a temperature of approximately 0° above a tray of solid carbon dioxide. Trapping efficiencies of nearly 90% were obtained with 2,4,6-2′,4′,6′-hexachlorobiphenyl, but for other PCBs recoveries were often much lower. Trapped PCBs were eluted with light petroleum ether which was evaporated off and replaced with deuterochloroform or tetrachloroethylene before NMR analyses.

NMR

Spectra were obtained on a Varian HR 220 spectrometer. The temperature of the probe varied between ~ 13° at the beginning of a day to ~ 18° over the greater part of the day. This temperature difference was shown to be insignificant in the interpretation of spectra as selected spectra obtained at 55° showed only slight shifts in some resonance lines.

For most gas chromatographic fractions the sample size was small and several of the spectra were very weak. In these cases, the spectrometric scanning conditions resulted in the spectra being extremely noisy.

Combined GLC–MS

Aroclor mixtures were separated using a Perkin-Elmer 50 ft. × 0.02 in. I.D. support coated open tubular (SCOT) Apieson L column under the conditions described in the next section. The column effluent was split between the flame-ionisation detector and an AEI MS12 mass spectrometer. Approximately 10% of the column effluent was taken into the mass spectrometer ion source with no removal of the carrier gas. Peak homogeneity was monitored by repetitively scanning over the parent molecular ion region, observing an oscilloscope screen and recording several spectra during the elution of a peak.

The higher chlorinated PCBs in Aroclor 1260, which had very long retention times on the SCOT column, were analysed on the 7 ft. × ¼ in. O.D. column described previously. The larger sample sizes and shorter retention times enabled a higher PCB concentration to be obtained in the carrier-gas stream. The column was connected to the MS12 through a Watson-Biemann type fritted glass separator. The carrier gas flow rate was 40 ml/min of helium and the column temperature was maintained at 205°. PCBs fractionated from Aroclor 1254 and all the synthesised PCBs were examined either as effluents from the 7 ft. column or in the mass spectrometer direct-inlet system.

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High resolution GLC and retention indices

The Aroclors and synthesised PCBs were analysed on the SCOT Apiezon L column fitted in a Pye Model 104. A flame-ionisation detector was necessary as the *n*-alkanes which were added to samples for the determination of retention indices produced very small and inverted peaks with an electron capture detector. Helium was used as the carrier gas at a flow of 2.5 ml/min. The column was operated at 205°, which was near the maximum temperature permitted (225°) for this phase. The operating temperature was dictated by the need to examine all the PCBs under conditions that allowed both the higher chlorinated PCBs in Aroclor 1260 to be eluted quickly and the lower chlorinated species in Aroclor 1242 to be resolved adequately. Some column deterioration was noted at 205° and significant variations in the R.I.s of PCBs were evident after three weeks. The changes were almost entirely due to reduced retention volumes of the *n*-alkanes relative to those of the PCBs. To overcome this effect the R.I.s of well characterised PCBs were used as sub-standards, as the retention volumes of PCBs were constant relative to each other. Column efficiencies were constant at between 24 000 and 27 000 theoretical plates, depending on the PCB.

Several pairs of peaks, *e.g.* 2,4,5-2',4',5'- and 3,4-2',3',4'-, changed relative positions as the column temperature was increased between 190–210°. For this reason isothermal was preferred to temperature programmed gas chromatography.

R.I.s of the later PCBs in Aroclor 1260 were determined on the 7 ft. × ¼ in. Apiezon L column and were correlated graphically with the SCOT column R.I.s.

RESULTS

Fractionation on an alumina column

Only twenty-three distinct peaks were apparent in the gas chromatogram of

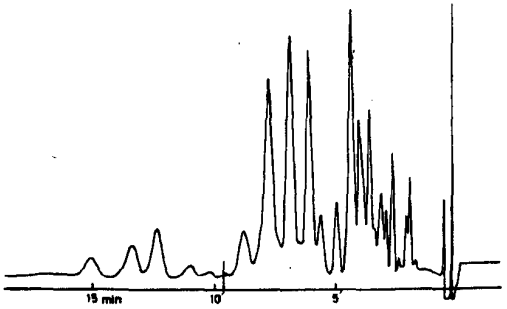


Fig. 1. 2 ng of Aroclor 1254 chromatographed on a 7 ft. Apiezon L packed column at 230°. Attenuation setting maintained at 2 × 10^2; electron capture detector.

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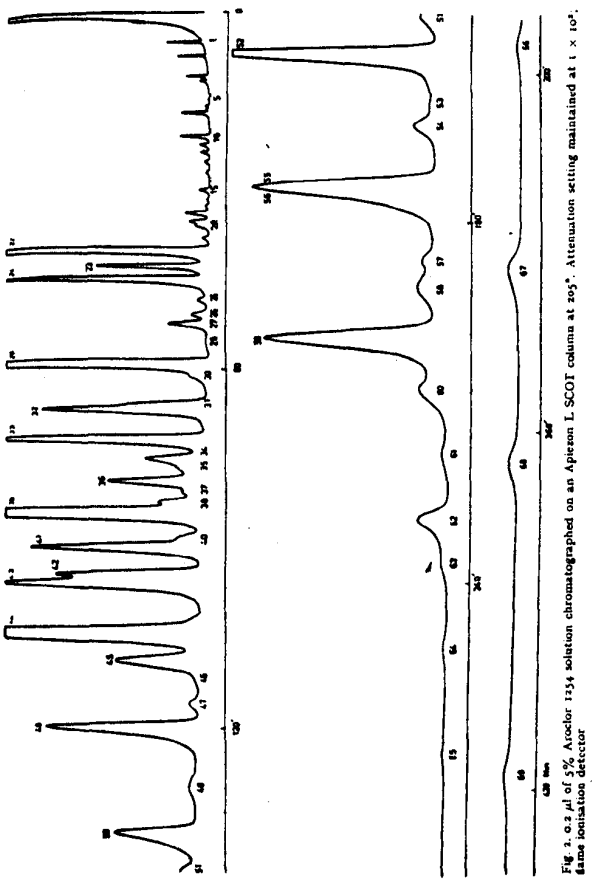


Fig. 2. 0.2 μl of 5% Aroclor 1254 solution chromatographed on an Apiezon L SCOT column at 205°. Attenuation setting maintained at 1×10^2. flame ionisation detector

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Aroclor 1254 (Fig. 1) analysed on the 7 ft. packed Apiezon L column at 230°. Similar GLC analyses of the hexane fractions obtained from the alumina column produced chromatograms containing a total of thirty-five peaks. This increase in the number of resolved peaks was due to the combination of the different elution patterns of the two systems. After characterisation of the PCBs by NMR, the order of elution of the substituted phenyl rings, assuming a constant second ring, was shown to be 2,4,5-, 2,5-, 2,3,4,5-, 2,4-, 2,3,6-, 3,4-, 2,3-, 2,3,4- from the alumina column compared to 2,5-, 2,4-, 2,3-, 2,3,6-, 3,4-, 2,4,5-, 2,3,4-, 2,3,4,5- from the GLC column.

Three PCBs, corresponding to peaks 39, 44 and 56 in Fig. 2, were sufficiently well resolved by alumina column fractionation to be analysed directly by MS and NMR. A further nineteen PCBs, including all the remaining major peaks in the Aroclor 1254 chromatogram, required further separation by GLC before MS and NMR analyses.

TABLE I

THE RETENTION INDICES, CHLORINE NUMBERS AND STRUCTURES OF THE MAJOR PCB CONSTITUENTS IN AROCLOR 1254

Peak No.	*R.I.*	*Chlorine No.*[a]	*NMR determined structure*	*Alternative predictions*	
				Structure	*R.I.*[b]
22	1994	4	2,5-2',5'	2-2',3',5'	1996
23	2010	4		2-2',4',5'	2012
				2,4-2',5'	2010
24	2022	4	2,3-2',5'	3-2',4',6'	2021
				2,4-2',4'	2027
		(5)		2,6-2',3',6'	2017
29	2089	5	2,5-2',3',6'	2,6-2',3',5'	2092
32	2119	5	2,3-2',3',6'		
33	2136	4	2,5-3',4'		
36	2159	5		2,5-2',3',5'	2159
39	2175	5	2,5-2',4',5'	2,4-2',3',5'	2175
				3,5-2',4',6'	2175
		(6)		2,6-2',3',4',6'	(2175)
				2,3,6-2',3',6'	2172
41	2191	5	2,4-2',4',5'	2,3-2',3',5'	2189
42	2203	5	2,3-2',4',5'		
43	2207	5	2,5-2',3',4'		
44	2228	5	3,4-2',3',6'	2,4-2',3',4'	2226
45	2238	—		2,3-2',3',4'	2240
				2,6-3',4',5'	2240
				3,4-3',5'	2238
				2,3,5-2',4',6'	2240
48	2264	6	2,3,6-2',4',5'	2,4-2',3',4',6'	(2263)
50	2299	6	2,3,4-2',3',6'		
52	2321	5	3,4-2',4',5'		
55	2356	5	3,4-2',3',4'		
56	2356	6	2,4,5-2',4',5'	2,5-2',3',4',5'	(2360)
				3,5-2',3',4',6'	(2357)
59	2390	6	2,3,4-2',4',5'	2,3-2',3',4',5'	(2391)
				2,4,6-3',4',5'	2388
		(7)		2,3,5-2',3',5',6'	(2390)

[a] Cl No. between brackets; Chlorine number of minor constituent not associated with accurate R.I.

[b] R.I. between brackets: Predicted R.I. using Table VIII.

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TABLE II

THE RETENTION INDICES, CHLORINE NUMBERS AND PREDICTED STRUCTURES OF THE MINOR PEAKS IN AROCLOR 1254

Peak No.	*R.I.*	*Chlorine No.*	*Structure*	*Predicted R.I.*[a]
1	1490	0	Biphenyl	1491[b]
2	1579	1	2-	1577[b]
3	1672	2	2-2′	1669[b]
4	1688	1	4-	1687[b]
5	1750	2	2-5′	1754
6	1758	—	2,4-	1756[b]
7	1763	2	2-3′	1770
8	1774	3	2-2′,6′	1765
9	1782	2	2-4′	1782[b]
10	1833	3	2-2′,5′	1831
11	1849	3	2-2′,4′	1848
12	1863	3	2-2′,3′	1863
			3-2′,6′	1866
13	1879	3	4-2′,6′	1878
14	1896	—	4-4′	1894[b]
15	1922	4	2-2′,4′,6′	1924[b]
16	1935	—	2-3′,5′	1932
			2-2′,3′,6′	1932
			3-2′,5′	1932
17	1943	—	4-2′,5′	1944
18	1952	3	3-2′,4′	1949
19	1963	3	3-2′,3′	1963
			4-2′,4′	1961[b]
20	1966	3	3-2′,3′	1963
			4-2′,4′	1961[b]
21	1980	3	2-3′,4′	1975
22	2040	4	4-2′,3′,6′	2041
			2,3-2′,4′	2041
23	2051	4	2-2′,3′,4′	2047
			2,3-2′,3′	2055
24	2058	4	2,3-4′,3′	2055
25	2072	4	2,5-3′,4′	2071
26	2097	—	3-2′,3′,5′	2097
			2,4-2′,4′,6′	2097
			2,5-3′,5′	2094
			2,6-2′,3′,5′	2092
27	2115	5	2,3-2′,3′,6′	2120
			2,3-2′,4′,6′	2112
28	2146	4	2-3′,4′,5′	2144
			3-2′,3′,4′	2148
29	2152	— (not 6)	3-2′,3′,4′	2148
			2,4-3′,4′	2154
30	2164	—	2,3-3′,4′	2168
			2,3,6-2′,4′,6′	2160
31	2169	—	2,3-3′,4′	2168
			2,3,6-2′,3′,6′	2173
32	2186	—	2,3-2′,3′,5′	2183
			3,5-2′,3′,6′	2185
33	2246	—	3-2′,4′,5′	2245
			2,4-2′,3′,5′,6′	(2241)
			2,5-2′,3′,4′,6′	(2247)
			2,3,5-2′,3′,6′	2248
34	2254	—	4-3′,4′,5′	2257
			2,3-2′,3′,5′,6′	(2256)
			3,5-2′,3′,5′	2259
			2,4,5-2′,4′,6′	2256

(continued on p. 22)

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TABLE II (*continued*)

Peak No.	*R.I.*	*Chloride No.*	*Structure*	*Predicted R.I.*[c]
50	2283		2,4,2',3',4',6'	(2279)
			2,6-2',3',4',5'	(2281)
			3,4-3',4'	2282
51	2310	5	2,5-2',4',5'	2305
			3,5-2',3',4'	2310
		(7)[a]	2,3,6-2',3',5',6'	2305
53	2335	5	2,3-3',4',5'	2338
		(7)[a]	2,3,6-2',3',4',6'	(2329)
54	2340	6	3,5-4',5',6'	(2345)
			2,3,5-2',4',5'	2330[b]
57	2371	—	2,4-2',3',4',5'	(2370)
			2,3,4-2',3',5'	2371
58	2379	6	2,3-2',3',4',5'	(2370)
			3,4-2',3',5',6'	(2379)
			2,3,4-2',3',5'	2371
60	2400	6	3,4-2',3',4',6'	(2401)
			3,4,5-2',3',6'	2396
61	2413	7	2,3,5-2',3',4',6'	(2414)
62	2425	6	2,3,4-2',3',4'	NMR
63	2433	7	2,3,6-2',3',4',5'	(2439)
			2,4,5-2',3',4',6'	(2428)
			2,4,6-2',3',4',5'	(2439)
64	2451	7	2,3,4-2',3',5',6'	(2450)
65	2466	7	2,3,4-2',3',4',6'	(2464)
66	2489	6	2,4,5-3',4',5'	2485[b]
67	2519	6	3,4-2',3',4',5'	NMR
68	2543	7	2,4,5-2',3',4',5'	(2541)
			3,4,5-2',3',5',6'	(2548)
69	2577	7	2,3,4-2',3',4',5'	NMR

[a] Smaller peaks eluted earlier than major parts of peaks 51 and 53, respectively.
[b] R.I. of synthesised compound.
[c] R.I. between brackets: Predicted R.I. using Table VIII.

NMR and MS

The structures of the major *Aroclor 1254* PCBs that were identified by NMR and MS after their separation and collection from a gas chromatograph are given in Table I. Three further minor peaks are included in Table II.

The NMR chemical shifts for both these PCBs and the synthesised compounds give a rational gradation of frequencies depending on the substitution patterns of both rings. Full details of the chemical shifts, the line splittings and structural assignments will be published separately[22].

The spectra of all the compounds examined from *Aroclor 1254* gave near first order spectra. The 2,3,4- and 2,3,6-substituted phenyls gave AB → AX systems; the 2,4-, 2,5- and 3,4-substituted phenyls gave ABX → AMX systems; and the 2,4,5- and 2,3,4,5-substituted phenyls gave two and one singlet(s), respectively. Under the spectrometer conditions used for running these weak spectra, and considering the 220 MHz spectrometer linewidth, the splitting due to *para*-coupling could not be observed.

The majority of the structural assignments in Table I were obtained from NMR data alone; the MS and R.I. evidence confirmed the results. However, in one or two cases an interplay of all three techniques allowed firm assignments to be made.

The fraction trapped as peak 24 was shown to be a mixture by GLC. The NMR

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TABLE III

THE RETENTION INDICES, CHLORINE NUMBERS AND PREDICTED STRUCTURES OF AROCLOR 1242 PEAKS

Peak No.	R.I.	Chlorine No.[a]	Structure	Predicted R.I.[a]
3	1672	2	4-2'	1669[b]
6	1765	2	2-3'	1770
8	1781	2	2-4'	1782[b]
9	1833	3	2-2',5'	1831
10	1849	3	2-2',4'	1848
11	1863	3	2-2',3'	1864
			3-2',6'	1866
12	1879	3	4-2',6'	1878
13	1896	2	4-4'	1894[b]
15	1935	3	4-3',5'	1932
			3-2',5'	1932
17	1951	3	3-2',4'	1949
18	1961	3	3-2',3'	1963
			4-2',4'	1962[b]
19	1966	3	3-2',3'	1963
			4-2',4'	1962[b]
20	1980	3	2-3',4'	1975
21	1996	4	2-2',3',5'	1996
			2,5-2',5'[b]	1994[b]
22	2011	4	2-2',4',5'	2012
			2,4-2',5'	2010
24	2024	4	3-2',3',6'	2029
			3-2',4',6'	2021
31	2092	3	4-3',4'	2088
		4	2,5-3',5'	2094
		(5)	2,4-2',4',6'	2097
			2,5-2',3',6'[b]	2089
34	2138	4	2,5-3',4'[b]	2138
35	2148	4	2-3',4',5'	2144
			3-2',3',4'	2148
1	1490	0	Biphenyl	1491[b]
2	1580	—	2-	1577[b]
4	1750	2	2,5-	1744
5	1758	—	2,4-	1736[b]
7	1774	3	2-2',6'	1765
14	1923	4	2-2',4',6'	1924[b]
			2,5-2',6'	1927
16	1944	3	4-2',5'	1944
23	2015	4	2-2',4',5'	2012
25	2028	4	3-2',4',6'	2029
			2,3-2',5'	2025
			2,4-2',4'	2027[b]
			2,6-3',5'	2028
			4-2',4',6'	2033[b]
26	2037	4	4-2',3',6'	2041
27	2041	4	2,3-2',4'	2041
28	2052	4	2-2',3',4'	2047
			2,3-2',3'	2055
29	2061	—	?	
30	2080	3	3-3',4'	2076
32	2121	4	2,3-3',5'	2125
			4-2',4',5'	2125
33	2129	4	2,3-3',5'	2125
			4-2',4',5'	2125
36	2167	4	4-2',3',4'	2160

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TABLE III *(continued)*

Peak No.	*R.I.*	*Chlorine No.*[d]	*Structure*	*Predicted R.I.*[c]
37	2174	4	2,3-3',4'	2168
38	2178	—	2,4-2',3',5'	2175
			2,5-2',4',5'[b]	2175[b]
			2,6-2',3',4',6'	(2175)
			3,5-2',3',6'	2183
			3,5-2',4',6'	2175
39	2195	5	2,4-2',4',5'[b]	2191
40	2207	5	2,3-2',4',5'	2206[b]
			2,5-2',3',4'[b]	2210
41	2212	5	2,5-2',3',4'[b]	2210
42	2232	5	2,3-2',3',4'	2226
			3,4-2',3',6'[b]	2228
43	2242	6	2,4-2',3',5',6'	(2241)
			2,5-2',3',4',6'	(2247)
			2,3,5-2',4',6'	2240
44	2268		2,4-2',3',4',6'	(2263)
			2,3,6-2',4',5'[b]	2264[b]
45	2287	–	2,6-2',3',4',5'	(2283)
			3,4-3',4'	2282[b]
			2,3,4-2',4',6'	2291

[a] Structures found in Aroclor 1254 by NMR determinations.
[b] R.I. of synthesized compound or NMR standard.
[c] *R.I. between brackets: Predicted R.I. using Table VIII.*
[d] Cl No. between brackets: Chlorine number of minor constituent not associated with accurate R.I.

spectrum of the mixture contained resonance lines compatible with the 2,3-, 2,4- and 2,5-dichlorophenyl groups. A chromatogram of Aroclor 1254 containing added 2,4-2',4'-tetrachlorobiphenyl showed that the retention time of the latter was not coincident with peak 24, although the trapped fraction could have included some 2,4-2',4'-compound from the tail of the peak. The spectrum also suggested that the other alternative R.I. structure 3-2',4',6'-tetrachlorobiphenyl was not present. Using the total NMR, MS and R.I. evidence, the most likely structure is 2,3-2',5'-tetrachlorobiphenyl.

The fraction trapped as peak 32 gave a very weak spectrum in tetrachloroethylene which contained lines typical of a 2,3,6-trichlorophenyl group. Other lines were too weak for certain correlation. However, the limited evidence is in agreement with the predicted GLC/R.I. structure of 2,3-2',3',6'-pentachlorobiphenyl.

Peaks 42 and 43 were always eluted closely to one another on every chromatogram. Two different fractionations of these peaks gave spectra which included lines typical of a 2,4,5-trichlorophenyl group in addition to those of 2,5-2',3',4'-pentachlorobiphenyl. This agrees with a GLC/R.I. predicted structure of 2,3-2',4',5'-pentachlorobiphenyl for peak 42.

The fraction trapped as peak 68 was shown by a subsequent GLC separation to contain a large number of impurities and, in addition, the spectrum was very weak. However, after the GLC/R.I. values suggested that the peak might be 2,4,5-2',3',4',5'-heptachlorobiphenyl it was possible to predict the frequencies of the three singlets in the spectrum from the total PCB NMR data. The three lines were, in fact, present at the predicted frequencies.

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TABLE IV

THE RETENTION INDICES, CHLORINE NUMBERS AND PREDICTED STRUCTURES OF AROCLOR 1260 PEAKS

Peak No.	*R.I.*	*Chlorine No.*[a]	*Structure*	*Predicted R.I.*[b]
14	2086	5	2,5-2′,3′,6′[a]	2086
			2,5-2′,4′,6′	2081
17	2168	6	2,3,6-2′,3′,6′	2171
18	2174	5	2,4-2′,3′,5′	2175
			2,5-2′,4′,5′[a]	2175[c]
			3,5-2′,4′,6′	2175
19	2230	6	2,4-2′,3′,5′,6′	(2241)
			2,3,5-2′,4′,6′	2240
45	2264	6	2,4-2′,3′,4′,6′	(2263)
			2,3,6-2′,4′,5′[a]	2264[c]
47	2296	6	2,3,4-2′,3′,6′[a]	2290
48	2300	7	2,3,6-2′,3′,5′,6′	(2303)
			2,4,6-2′,3′,5′,6′	(2295)
52	2358	6	2,5-2′,3′,4′,5′	(2360)
			3,5-2′,3′,4′,6′	(2357)
			2,4,5-2′,4′,5′[a]	2356[c]
55	2390	6	2,3-2′,3′,4′,5′	(2391)
			2,3,4-2′,4′,5′[a]	2391[c]
			3,4,5-2′,4′,6′	2388
		(7)	2,3,5-2′,3′,5′,6′	(2390)
57	2411	7	2,3,5-2′,3′,4′,6′	(2413)
			2,4,5-2′,3′,5′,6′	(2406)
58	2428	6	2,3,4-2′,3′,4′[a]	2425[c]
		7	2,4,5-2′,3′,4′,6′	(2428)
59	2432	7	2,4,5-2′,3′,4′,6′	(2428)
			2,4,6-2′,3′,4′,5′	(2430)
		8	2,3,5,6-2′,3′,5′,6′	2438[c]
60	2445	7	2,3,4-2′,3′,5′,6′	(2440)
			2,3,6-2′,3′,4′,5′	(2439)
61	2542	7	2,4,5-2′,3′,4′,5′	(2541)
68	2575	7	2,3,4-2′,3′,4′,5′[a]	(2575)
			3,4,5-2′,3′,4′,6′	(2570)
70	2724	8	2,3,4,5-2′,3′,4′,5′	(2726)

Peaks 4–21 in Aroclor 1254 chromatogram were also present in Aroclor 1260.

22	1994	4	2-2′,3′,5′	1996
			2,5-2′,5′[a]	1994[c]
23	2009	4	2-2′,4′,5′	2012
			2,4-2′,5′	2010
24	2013	—	2-2′,4′,5′	2012
			2,4-2′,5′	2010
25	2022	4	3-2′,4′,6′	2021
26	2024		2,3-2′,5′	2025
			2,4-2′,4′	2027[c]
27	2030	—	3-3′,5′	2033
			3-2′,3′,6′	2029
			4-2′,4′,6′	2033[c]
			2,4-2′,4′	2027[c]
			2,6-3′,5′	2028
28	2040	4	4-2′,3′,6′	2041
			2,3-2′,4′	2041
29	2050	4	2-2′,3′,4′	2047
			2,3-2′,3′	2055
30	2058	4	2,3-2′,3′	2055
32	2127	—	3-2′,4′,5′	2114
			2,3-2′,3′,6′	2114

(continued on p. 29)

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TABLE IV *(continued)*

Peak No.	R.I.	Chlorine No.[a]	Structure	Predicted R.I.[b]
33	2125	—	4-2',4',5'	2125
			2,3-3',5'	2125
34	2135	4	2,5-3',4'[a]	2138
35	2140	4	2-3',4',5'	2141
			3-2',3',4'	2148
36	2158	5	2,5-2',3',5'	2159
39	2192	—	2,3-2',3',5'	2189
			3,4-2',4',5'[a]	2191
40	2205	5	2,3-2',4',5'	2206[c]
41	2209		2,5-2',3',4'[a]	2210
42	2229	5	2,4-2',3',4'	2226
			3,4-2',3',6'[a]	2228
44	2254	6	2,3-2',3',5',6'	(2256)
			2,4,5-2',4',6'	2256
46	2283	—	2,3-2',3',4',6'	(2279)
			2,6-2',3',4',5'	(2283)
			3,4-3',4'	2282[c]
49	2321	5	2,4-3',4',5'	2323
			3,4-2',4',5'[a]	2319
50	2326	7	2,3,6-2',3',4',6'	(2329)
			2,4,6-2',3',4',6'	(2321)
51	2340	6	3,5-2',3',5',6'	(2335)
			2,3,5-2',4',5'	2340[c]
53	2372	—	2,4-2',3',4',5'	(2376)
			2,3,4-2',3',5'	2374
54	2379	—	2,4-2',3',4',5'	(2376)
			3,4-2',3',5',6'	(2379)
			2,3,4-2',3',5'	2374
55	2402	6	3,4-2',3',4',6'	(2401)
61	2460	7	2,3,4-2',3',4',6'	(2462)
62	2464	8	2,3,4,6-2',3',5',6'	(2464)
63	2486	8	2,3,6-2',3',4',5',6'	(2489)
			2,4,6-2',3',4',5',6'	(2481)
			2,3,4,6-2',3',4',6'	2490[c]
		6	2,4,5-3',4',5'	2488[c]
64	2518	6	3,4-2',3',4',5'[a]	(2516)
65	2522	7	2,3,5-2',3',4',5'	(2523)
67	2550	—	3,4,5-2',3',5',6'	(2548)
69	2578	8	2,3,5-2',3',4',5',6'	(2577)
			2,3,4,5-2',3',3',5'	(2581)
70	2587	8	2,3,4,5-2',3',5',6'	(2581)
71	2597	8	2,4,5-2',3',4',5',6'	(2593)
72	2603	8	2,3,4,5-2',3',4',6'	(2603)
73	2618	9	2,3,5,6-2',3',4',5',6'	(2624)
74	2640	8	2,3,4-2',3',4',5',6'	(2627)
e	—	9	2,3,4,6-2',3',4',5',6'	(2650)
75	2682	—	3,4,5-2',3',4',5'	(2685)
77	2738	—	3,4,5-2',3',4',5',6'	(2738)
78	2770	9	2,3,4,5-2',3',4',5',6'	(2768)
d	—	10	2,3,4,5,6-2',3',4',5',6'	2810[c]

[a] Structures found in Aroclor 1254 by NMR determinations.
[b] R.I. between brackets: Predicted R.I.'s using Table VIII.
[c] Cl No. between brackets: Chlorine number of minor constituent not associated with accurate R.I.
[d] Peaks observed on mass spectrometer oscilloscope, but not on GLC.
[e] R.I. of synthesised compound.

The MS results obtained from the GC/MS runs are quoted in Tables I, II, III and IV as the number of chlorine atoms associated with each PCB peak.

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Prediction of PCB structures using R.I.s

Retention indices are a more reproducible form of presenting logarithmic retention volumes of GLC peak maxima and are obtained with reference to the well defined retention values of a series of *n*-alkanes separated in the same chromatogram. The R.I. of a compound is directly proportional to its free energy of solution in a

TABLE V

½(R.I.) VALUES OF PHENYL RINGS CONTAINING UP TO THREE CHLORINE ATOMS

Ring structure	*½(R.I.) value*	*Derivation*	*R.I.*
Phenyl	747	biphenyl	1494
2	834	2-2′	1669
	829	2-	1377
3	935	3-3′	1871
	930	3-	1678
4	947	4-4′	1894
	940	4-	1687
2,3	1028	2,3-2′,4′,5′	2206
	1025	2,3-	1772
2,4	1013	2,4-2′,4′	2026
	1009	2,4-	1756
2,5	997	2,5-2′,5′	1994
2,6	930	2,6-2′,6′	1860
	932	2,6-	1679
3,4	1141	3,4-3′,4′	2282
	1134	3,4-	1881
3,5	1097	3,5-3′,5′	2194
	1095	3,5-	1842
2,3,4	1213	2,3,4-2′,4′,5′	2391
2,3,5	1162	2,3,5-2′,4′,5′	2340
2,3,6	1086	2,3,6-2′,4′,5′	2264
2,4,5	1178	2,4,5-2′,4′,5′	2356
2,4,6	1076	2,4,6-2′,4′,6′	2151
	1086	2,4,6-4′	2033
	1090	2,4,6-2′	1924
	1084	2,4,6-	1831
3,4,5	1310	3,4,5-2′,4′,5′	2488

stationary phase, which in turn is an approximately additive function of the groups constituting the molecule. Consequently any PCB molecule can be thought of as consisting of two chloro-substituted phenyl groups each with its own ½(R.I.) value. All PCBs are composed of twenty such basic groups and the R.I. of any PCB can therefore be estimated by adding together the ½(R.I.) values of the two component phenyl groups.

The ½(R.I.) values of all substituted phenyl groups containing up to three chlorine atoms and the R.I.'s of the PCBs from which they were derived are shown in Table V. Certain discrepancies are apparent between the ½(R.I.)s for the same ring structures calculated from different PCBs. These discrepancies are due to electronic and steric effects which vary according to the substitution patterns in the two rings under consideration. Electronic effects are induced by the introduction of chlorine atoms into the phenyl ring and vary according to the exact position of each substitution. The inclusion of chlorine atoms results in a change in the electronic

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TABLE VI

COMPARISON OF EXPERIMENTAL AND PREDICTED R.I.S OF PCBS

Structure	*R.I.*	
	Experimental	*Predicted*
2-4'	1784	1781
2,3,5-	1922	1925
2,4,4'	1960	1956
2,5-2',3',6'	2080	2083
2,5-3',4'	2136	2138
2,5-2',4',5'	2173	2174
2,4-2',4',5'	2191	2191
2,5-2',3',4'	2207	2210
3,4-2',3',6'	2228	2227
2,3,4-2',3',6'	2299	2299
3,4-2',4',5'	2321	2319
2,3,5-4',4',5'	2339	2340
3,4-4',3',4'	2356	2354
2,3,4-2',3',4'	2425	2424

distribution in each ring and varies the character of the phenyl–phenyl bond. The net effect is illustrated in Table V by the increased R.I. (2 to 7 units) for molecules substituted *in both rings compared to those substituted in only one ring.*

The steric effect, previously noted in methyl biphenyls[28,29], is caused by substitution of chlorine atoms in the *ortho* (2,2', and 6,6') positions in the rings. Increasing substitution in these positions progressively restricts free rotation of the phenyl groups and is accompanied by a reduction in R.I. This effect is illustrated in Table V by the smaller R.I. for 2,6- and 2,4,6-rings (-2 and -8 units, respectively) derived from molecules containing two *o*-chlorine compared to no *o*-chlorine in the second *ring.*

Any electronic and steric effects must be allowed for when predicting the R.I.s of PCBs, by using appropriate ½(R.I.) values derived from a similar type of PCB molecule. Using the data in Table V, the experimental and predicted R.I.s for a further range of PCBs of known structure are compared in Table VI. With only one exception, for which no obvious explanation can be given, agreement is within four R.I. units, which is consistent with our experimental error of ± 2 units in determining

TABLE VII

EXPERIMENTAL ½(R.I.) VALUES OF PHENYL RINGS CONTAINING 4 AND 5 CHLORINE ATOMS

Ring structure	*½(R.I.) value*	*Derivation*	*R.I.*
2,3,4,5	1364	2,3,4,5-2',3',4'	2577
	1378	2,3,4,5-3',4'	2519
	1380	2,3,4,5-	2127
2,3,4,6	1245	2,3,4,6-2',3',4',6'	2490
2,3,5,6	1219	2,3,5,6-2',3',5',6'	2438
	1273	2,3,5,6-	2020
2,3,4,5,6	1405	2,3,4,5,6-2',3',4',5',6'	2810

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R.I.s. Therefore structural predictions of PCBs in Aroclors 1242 and 1254 could be made with confidence.

Very few standard PCBs containing more than three chlorine atoms per ring were available for analysis but, from the limited data obtained (Table VII), it was evident that electronic and steric effects had a much greater influence on their R.I.s than on those of the lower chlorinated PCBs. This resulted in increased difficulty in predicting the R.I.s of some of the hexa-, and the hepta-, octa- and nona-substituted

TABLE VIII

INFERRED $\frac{1}{2}$(R.I.) VALUES FOR FOUR AND FIVE SUBSTITUTED PHENYL RINGS AS AFFECTED BY *ortho*-SUBSTITUTION IN THE SECOND RING

Substituted phenyl ring	$\frac{1}{2}$(*R.I.*) *values*		
	Two ortho	*One ortho*	*No ortho*
2,3,4,5	1353	1363	1375
2,3,4,6	1245	1250	1260
2,3,5,6	1219	1228	1238
2,3,4,5,6	1405	1415	1428

molecules. However, the relation between structure and R.I. was apparent when most of the later peaks in the chromatogram of Aroclor 1260 were studied. After allowance was made for electronic and steric effects, particularly in the case of the 2,3,4,6- and 2,3,5,6- rings joined to rings containing two, one or no *o*-chlorine atoms, the more obvious correlations for the octa- and nona-substituted compounds could be made, and then the remaining peak assignments fitted into place. In this way, using the inferred $\frac{1}{2}$(R.I.) values given in Table VIII, it was possible to allocate structures to all appropriate peaks in the Aroclor 1260 chromatogram. The values in Table VIII are conditional on the presence of at least two substituted chlorine atoms in the second phenyl ring. This condition was imposed because it was not clear what effect a greater imbalance in the number of chlorine atoms between the rings would have on R.I.s. In practice, the MS data giving the number of chlorine atoms present in each PCB enabled potential errors due to the above effects to be greatly reduced.

Typical chromatograms of Aroclors 1242, 1254 and 1260 obtained from the SCOT column are shown in Figs. 2–4. Tables I, II, III and IV list the R.I.s and chlorine contents of all PCB peaks present in the Aroclors. The tables also include the alternative PCB structures that have R.I.s predicted to be within an arbitrary ± 5 of each peak. The R.I.s of synthesised PCBs are used where applicable, and those that were derived from the inferred $\frac{1}{2}$(R.I.) values given in Table VIII are given in brackets in Tables I–IV. PCB molecules having a difference of more than two chlorine atoms between rings are not included as these would be unlikely to occur in Aroclor mixtures. For the sake of emphasis the major peaks in the chromatograms are presented first, in Tables III and IV, in order of increasing R.I.

The changes in $\frac{1}{2}$(R.I.) values found in this investigation are in line with changes in the NMR chemical shift data. When considering the protons in one ring of the molecule, the chemical shifts are affected by the overall presence of chlorine atoms in either ring, the substitution position of chlorine atom(s) in the ring being

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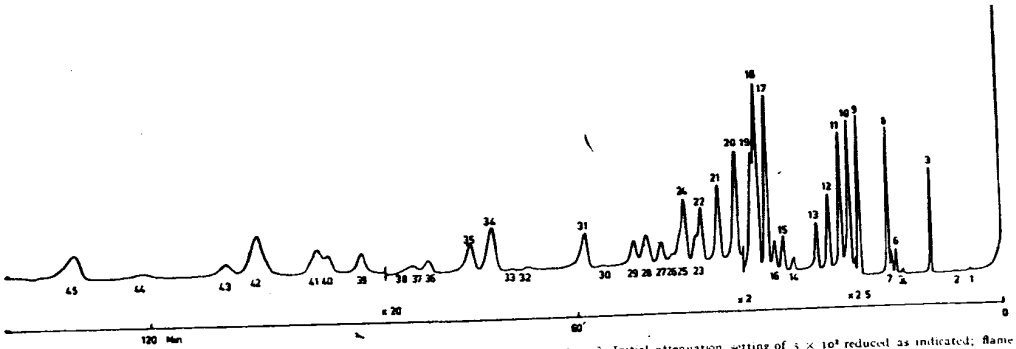


Fig. 3. 0.2 μl of 1% Aroclor 1242 solution chromatographed on an Apiezon L SCOT column at 205°. Initial attenuation setting of 5×10^2 reduced as indicated; flame ionisation detector.

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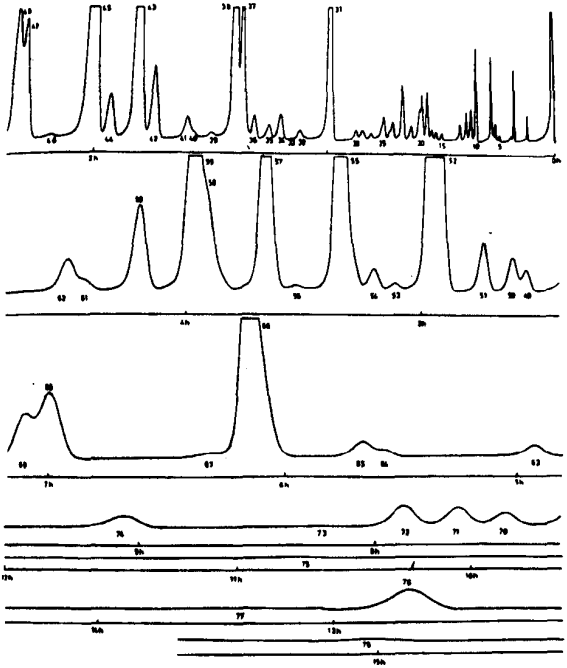


Fig. 4. 0.25 μl 10% Aroclor 1260 solution chromatographed on an Apiezon L SCOT column at 205°. Attenuation setting maintained at 1 × 10³; flame ionisation detector.

examined, and the number and relative location of the *o*-chlorine atoms causing steric hindrance in the molecule.

Allowing for variations in detector response, the SCOT column chromatograms indicate that the predominant species in Aroclor 1254 are pentachlorobiphenyls with smaller amounts of tetra- and hexachlorobiphenyls. This agrees well with the chlorine content of this Aroclor which corresponds to a mean value of five atoms per molecule. The most abundant chlorine substitutions are those in the 2,5-, 3,4-, 2,3,4-, 2,3,6-,

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2,4,5- and 2,3,4,5- positions. When alternative predicted structures are available, therefore, preference should probably be given to those containing these substitution patterns. It is noticeable that *certain substitutions, such as 2,4,6-, rarely occur* and others, such as 3-, 3,5- and 2,3-, occur only infrequently.

Aroclor 1242 contains predominantly trichlorobiphenyls with a lesser amount of dichloro- and tetrachloro-compounds. The mean chlorine value for this Aroclor is 3.1 atoms/molecule. The most frequent substitutions occur in the 2-, 2,4 and 2,5 positions. The predominant compounds in Aroclor 1260 were hexachlorobiphenyls with a significant level of heptachlorobiphenyl. The mean chlorine value of this Aroclor is 6.1 atoms/molecule. The most frequent substitution patterns were similar to those found in Aroclor 1254, although 2,3,5-, 2,3,4,6- and 2,3,5,6- structures were in greater evidence.

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