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IDENTIFICATION OF THE INDIVIDUAL POLYCHLORINATED BIPHENYLS IN A MIXTURE BY GAS–LIQUID CHROMATOGRAPHY

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SUMMARY

Retention indices were computed for all of the 210 possible chlorinated biphenyls on 13 gas chromatographic liquid phases. All possible pairwise comparisons of retention indices were made for each liquid phase, each pair of liquid phases and each set of three liquid phases. On the basis of a closeness criterion of 10 ($\Delta RI = RI_a - RI_b = 10$), those combinations of three or fewer liquid phases which could distinguish between nearly all possible pairs of chlorinated biphenyls were selected. Further considerations such as column efficiencies, analysis time required, resolution achievable and availability led to the selection of several common liquid phases for the qualitative and, in some cases, quantitative analysis of the individual components of mixtures of polychlorinated biphenyls. A few specific applications are discussed.

INTRODUCTION

The persistence of the ubiquitous pollutants polychlorinated biphenyls (PCBs) in the environment and the synthesis of individual biphenyls for research into the mechanisms of toxicity mandate an analytical effort for the forseeable future. Mixtures of PCBs encountered in commercial preparations, tissue extracts and the like are usually of such complexity as to defy complete resolution into their individual components on any one packed chromatography column, although an approximation of complete resolution is possible on appropriate support-coated open capillary columns[1].

In some cases such as reaction mixtures from attempted syntheses of radioactively labeled PCBs[2], some tissue extracts[3,4] and fractions from reversed-phase chromatography of commercial PCB mixtures[5], resolution on packed columns is sufficient theoretically to permit qualitative identification of most if not all of the individual PCBs present. The first objective of the present study was to provide listings of retention indices[6,7] for all possible PCBs on several packed gas chromatography columns to aid investigators in qualitative identification.

Sissons and Welti[1] reported that retention indices (*RI*) for PCBs could be calculated by summing the 1/2 (*RI*) values for the two substituted rings. We subse-

quently[8] extended this observation to show that it applied equally well for five liquid phases in addition to the Apiezon L used by Sissons and Welti. In the present study we found no exceptions to rough additivity of 1/2 (*RI*) values on 13 liquid phases for PCBs having up to three chlorine atoms in each ring, or for any octa-, nona- or decachlorobiphenyl. PCBs having four or five chlorines in one ring and none in the other did deviate very significantly from additivity of 1/2 (*RI*) values, and had to be treated separately.

Gas–liquid chromatography (GLC) of PCBs has almost always been performed on relatively non-polar liquid phases. In what follows we discuss the advantages of using polar and non-polar liquid phases in combination for reliable identification of individual PCBs.

MATERIALS AND METHODS

GLC liquid phases and solid supports were from the suppliers mentioned previously[8]. Reference PCBs were either synthesized here or obtained commercially; the sources have been listed previously[9]. The individual PCB standards used in this study included biphenyl substituted with chlorine in the 2-; 3-; 4-; 2,6-; 2,3-; 2,4-; 2,4′-; 3,3′-; 4,4′-; 3,4-; 2,4,4′-; 2,4,5-; 3,4,3′-; 2,6,2′-; 2,5,2′-; 2,5,4′-; 2,3,5-; 2,6,2′ 6′-; 2,5,2′,5′-; 2,4,2′ 4′-; 2,3,2′,3′-; 2,3,4,6-; 2,3,4,5-; 2,3,5,6-; 3,5,3′,5′-; 3,4,3′,4′-; 2,4,5,2′,5′-; 2,3,4,2′,5′-; 2,3,4,5,6-; 2,3,6,2′,3′,6′-; 2,3,4,2′,3′,4′-; 2,4,6,2′,4′,6′-; 2,4,5, 2′,4′,5′-; 2,3,5,2′,3′,5′-; 3,4,5,3′,4′,5′-; 2,3,4,5,2′,3′,4′,5′-; 2,3,5,6,2′,3′,5′,6′-; and 2,3, 4,5,6,2′,3′,4′,5′,6′-positions (38 compounds), as well as biphenyl itself.

Retention indices were measured using C-16 through C-28 *n*-alkanes as described elsewhere[7]. Half-indices[1] were determined either by taking half of the retention index for a symmetrical PCB (*e.g.*, 3,4,5,3′,4′,5′-hexachlorobiphenyl), or by subtracting half of the retention index for biphenyl from the retention index of a chlorinated biphenyl having only one ring substituted.

The GLC columns used in this study and the column temperatures at which retention indices were determined are listed in Table I. In all cases, helium was used as the carrier gas at a flow-rate of 35 ml/min for 1/8-in. columns and 50 ml/min for the 1/4-in. diameter column. Injection port and hydrogen flame ionization temperatures were 250° and 280°, respectively. All retention times were corrected for column dead-space by subtracting the retention time of methylene chloride. Quantitative analysis involved measuring peak areas with a Spectra-Physics System IV computing integrator and correcting the peak areas for the relative molar response of the hydrogen flame detector to each component as described previously[8].

For each column, the number of theoretical plates relative to the peak shape of 2,3,5,2′,3′,5′-hexachlorobiphenyl, the analysis time through the elution of decachlorobiphenyl and the retention index difference required for a resolution of 0.5 (considered minimal for the distinction between one and two closely eluting components) were calculated by standard methods[10]. Availability of consistent preparations of the liquid phases was subjectively estimated from manufacturers' literature. Pairwise comparisons of retention indices were performed using a PDP-11/40 computer.

TABLE I

CHROMATOGRAPHY COLUMNS USED

Number	*Packing*	*Dimensions (cm)*	*Temperature (°C)*
1	10% OV-101, 80–100-mesh Chromosorb W HP	300 × 0.2	200
2	5% Versilube F-50, 80–100-mesh Chromosorb W HP	275 × 0.2	200
3	6% Apiezon L, 100–120-mesh Chromosorb W AW	200 × 0.2	205
4	10% OV-210, 80–100-mesh Supelcoport	330 × 0.2	200
5	10% OV-3, 100–120-mesh Gas-Chrom Z	300 × 0.2	200
6	10% OV-17, 80–100-mesh Supelcoport	300 × 0.2	200
7	10% OV-25, 100–120-mesh Gas-Chrom Z	300 × 0.2	200
8	10% AN-600, 100–120-mesh Chromosorb W HP	300 × 0.2	190
9	10% OV-225, 80–100-mesh Supelcoport	250 × 0.2	190
10	3% CHDMS*, 100–120-mesh Gas-Chrom Q	400 × 0.2	200
11	5% Halocarbon K-352, 80–100-mesh Supelcoport	200 × 0.5	195
12	5% Poly MPE, 80–100-mesh Gas-Chrom Z	200 × 0.2	200
13	3% Dexil 410, 90–100-mesh Anakrom AS	200 × 0.2	200

* Cyclohexanedimethanol succinate.

RESULTS

Table II lists the 1/2 (*RI*) values from which, by summing pairs, all 2730 retention indices (210 PCBs × 13 columns) can be calculated. The average deviation between calculated and observed retention indices for the 38 standard PCBs was 2.7, less than the reproducibility in measuring retention indices (± 0.03% of the *RI*).

Table III lists the number of theoretical plates seen for each packed column using 2,3,5,2′,3′,5′-hexachlorobiphenyl as an arbitrary determinant. Two situations must be considered: the need to identify a single PCB (or a mixture of completely resolved PCBs), and the need to ascertain that each PCB "peak" contains only a single PCB. Table III also lists the number of theoretical plates needed to ensure detection of two peaks whose retention indices differ by only 10 units. This would permit selection of an optimum column length for each column.

When all possible pairwise comparisons (21,945 per column) were made using a "closeness criterion" of 10 as a definition of distinguishability, it was found that no single column could resolve all of the PCBs. Column 4 (OV-210) was closest, with 465 indistinguishable pairs out of 21,945 possible. Column 7 (OV-25) was poorest, with 607 pairs not distinguished.

Considering combinations of two columns, OV-3 plus CHDMS was the best with only 41 indistinguishable pairs. Combinations 5+12 or 7+12 (OV-3 or OV-25 plus Poly MPE) were close, with 55 indistinguishable pairs. Five sets of three columns were nearly equivalent. Columns 5+8+12 (OV-3, AN-600 and Poly MPE) and columns 8+12+13 (AN-600, Poly MPE and Dexil 410) reduced the number of indistinguishable pairs to 10, while combinations 1+7+12 (OV-101, OV-25 and Poly MPE), 3+6+10 (Apiezon L, OV-17 and CHDMS) or 6+8+12 (OV-17, AN-600 and Poly MPE) gave only 9 indistinguishable pairs. Using a closeness criterion of 10, it appeared that five columns (*e.g.*, 1+4+7+12+13) would be required to reach zero indistinguishable pairs. Indistinguishable pairs are listed for some of the sets of three columns in Table IV.

TABLE II

1/2 (*RI*) VALUES FOR ALL RING SUBSTITUTION PATTERNS

Substitution pattern (1/2 PCB)	*Column No.*												
	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*
None	698	704	747	830	733	828	877	870	916	1000	733	977	772
2	811	814	831	950	852	964	1024	1003	1055	1127	856	1095	886
3	877	888	932	1067	921	1026	1083	1100	1146	1252	950	1221	966
4	887	896	945	1084	928	1047	1096	1120	1169	1273	962	1245	980
2,6	921	923	931	1072	967	1085	1164	1128	1188	1254	964	1220	987
2,5	960	970	997	1139	1003	1116	1175	1174	1220	1319	1025	1285	1037
2,4	970	974	1010	1135	1014	1115	1184	1173	1229	1322	1037	1303	1046
2,3	998	999	1026	1180	1046	1167	1253	1232	1299	1370	1069	1351	1088
3,5	1023	1026	1096	1223	1060	1154	1204	1249	1292	1424	1109	1404	1113
3,4	1072	1075	1138	1309	1126	1253	1310	1344	1401	1525	1169	1494	1182
2,4,6	1038	1037	1076	1179	1070	1176	1250	1205	1259	1363	1109	1351	1097
2,3,6	1088	1068	1085	1257	1112	1246	1327	1309	1360	1438	1140	1401	1149
2,3,5	1120	1113	1168	1270	1159	1272	1335	1334	1391	1490	1200	1486	1165
2,4,5	1132	1126	1178	1331	1165	1278	1340	1351	1396	1514	1216	1497	1214
2,3,4	1168	1166	1211	1405	1212	1354	1440	1433	1480	1605	1269	1568	1270
3,4,5	1233	1238	1313	1500	1275	1404	1470	1536	1584	1710	1360	1709	1360
2,3,5,6	1203	1197	1189	1383	1240	1355	1430	1391	1420	1500	1210	1530	1263
2,3,4,6	1200	1190	1182	1390	1212	1360	1435	1400	1419	1480	1208	1504	1265
2,3,4,5	1210	1200	1207	1430	1245	1375	1440	1571	1520	1577	1310	1621	1423
2,3,4,5,6	1345	1309	1349	1546	1352	1478	1559	1576	1736	1677	1421	1793	1439
2,3,5,6*	1257	1256	1273	1444	1314	1393	1481	1471	1536	1612	1276	1637	1307
2,3,4,6*	1255	1234	1268	1450	1286	1419	1509	1473	1535	1592	1362	1644	1309
2,3,4,5*	1341	1339	1380	1556	1377	1502	1593	1581	1654	1750	1467	1772	1422
2,3,4,5,6*	1407	1429	1433	1650	1472	1631	1723	1687	1748	1782	1578	1913	1521

* These values apply when only one ring is substituted.

TABLE III

COLUMN EFFICIENCIES

Column No.	*Number of theoretical plates*		*Elution time (min) decachlorobiphenyl*
	*Observed***	*Required****	
1	2652	3306	41.4
2	3166	2763	118
3	3032	2530	132
4	3516	4356	55.2
5	2856	2763	301
6	3833	3179	250
7	3264	3286	292
8	1414	3687	63.5
9	3203	3324	390
10	1960	3711	369
11	491	4287	18.9
12	1854	3486	71.8
13	1372	3253	332

* For identifications see Table I.

** From $N = 16\,(d/w)^2$ for 2,3,5,2′,3′,5′-Hexachlorobiphenyl.

*** For $R = 0.5$, $RI_2 - RI_1 = 10$; from $\Delta RI = \ln \alpha / B$, $N = [2R\,(\alpha+1)/(\alpha-1)]^2$, ln (rel. retention time) $= B(RI) - A$.

TABLE IV

PAIRS OF PCBs NOT DISTINGUISHED AT A CLOSENESS CRITERION OF 10

*1 + 7 + 12**	*5 + 8 + 12*	*6 + 8 + 12*
2,2′-; 2,6-	2,2′-; 2,6-	3,4′-; 3,4-
3,4′-; 3,4-	3,4′-; 3,4-	2,5,2′-; 2,3,6-
2,5,4′-; 2,4,3′-	2,6,3′-; 2,3,2′-	2,5,2′,6′-; 2,3,6,2′-
2,4,2′,5′-; 2,3,5,2′-	2,5,2′-; 2,3,6-	2,4,2′,5′-; 2,3,5,2′-
3,4,2′,4′-; 3,4,5,2′-	2,5,2′,6′-; 2,3,6,2′-	2,4,2′,4′-; 2,3,5,6-
2,4,6,2′,3′-; 2,3,5,2′,6′-	2,4,4′-; 2,3,4-	2,3,4′-; 3,4,2′-
2,3,6,3′,5′-; 2,4,5,2′,4′-	2,4,2′,5′-; 2,3,5,2′-	2,4,6,3′,5′-; 2,3,4,5-
2,3,5,2′,4′-; 2,4,5,2′,5′-	2,4,2′,5′-; 2,4,5,2′-	2,3,6,3′,5′-; 2,3,4,4′-
2,3,5,3′,4′-; 2,3,4,3′,5′-	2,3,4′-; 3,4,2′-	2,3,5,6,2′,5′-; 2,3,4,6,2′,4′-
	2,3,5,2′,5′-; 2,3,5,6,4′-	

* Combination (set) of columns used. For identification see Table I.

Table V shows an analysis of a very crude reaction mixture from the attempted synthesis of 2,5,4′-trichlorobiphenyl. The sample was run on OV-3, AN-600 and Poly MPE columns. Only four peaks matched the same PCB on all three columns, revealing that peaks comprising 62% of the total GLC peak area were due to compounds other than PCBs, *i.e.*, impurities, by-products and starting materials. Most of the peaks could easily have been misidentified as various PCBs had only one column been used.

TABLE V

ANALYSIS OF CRUDE 2,5,4′-TRICHLOROBIPHENYL

The values in the squares do not necessarily correspond horizontally to the same component.

Retention indices			*Identification*	*Area (%)*
OV-3	*Poly MPE*	*AN-600*		
1771	2325	2102	2,3-Dichlorobiphenyl	0.25
1853	2467	2222	3,4′-Dichlorobiphenyl	5.54
1933	2532	2285	2,5,4′-Trichlorobiphenyl	31.14
2009	2575	2349	2,5,2′,5′-Tetrachlorobiphenyl	1.14
2073	2744	2433	Other products, not PCBs	61.93
2103	2942	2539		
2161	3074	2579		
2228	>3074	2616		
2267		>2616		
2361				
2478				
2531				

Table VI shows a complete quantitative analysis of Aroclor 1221, a commercial mixture of PCBs containing approximately 21% (w/w) of chlorine. Sufficient resolution for accurate quantitation of this sample required the use of six different columns. Peak areas were corrected for relative molar response as indicated previously[8]. Summing the chlorine content as a function of each component gave a theoretical content of 21.16% of chlorine for this sample.

TABLE VI

ANALYSIS OF AROCLOR 1221, BATCH NO. AM-25

Chlorine substitution pattern	*Retention indices (observed)* *						*Corrected mole-%* **
	1	*3*	*5*	*8*	*10*	*12*	
0 (Biphenyl)	1396	1495	1467	1740	2001	1955	15.85
2-	1508	1573	1583	1870	2125	2072	32.14
3-	1575	1677	1655	1963	2250	2199	2.73
4-	1586	1692	1661	1986	2275	2220	19.07
2,6-	1620	1677	1702	1993	2255	2199	0.33
2,2'-	1620	1662	1702	2011	2255	2188	4.81
2,4-	1667	1758	1748	2040	2320	2280	2.72
2,5-	1656	1740	1736	2040	2320	2261	0.18
2,3'-	1689	1758	1774	2102	2380	2316	3.07
2,4'-	1700	1776	1774	2119	2401	2338	10.17
3,3'-	1756	1858	1841	2210	2500	2440	0.72
3,4'-	1764	1878	1853	2222	2526	2468	1.22
3,4-	1764	1888	1853	2210	2526	2468	1.20
4,4'-	1775	1888	1853	2248	2547	2491	3.65
2,4,2'-	1780	1842	1865	2175	2450	2400	0.32
2,5,2'-	1780	1827	1853	2175	2450	2380	0.64
2,5,3' + 3.5,2'-	1836	1928	1920	2260	2562	2520	0.17
2,5,4'-	1846	1948	1932	2292	2600	2520	0.23
2,3,3'-	1876	1955	1966	2330	2624	2571	0.07
2,4,4'-	1857	1955	1944	2292	2600	2550	0.19
3,4,2'-	—	—	—	2351	2648	—	0.07
2,5,2',5'-	1920	1990	2001	2351	2624	2571	0.14
2,4,5,2',5' + 2,4,5,2',4'-	—	2171	2169	—	—	—	0.12
Others	Not identified, seen on only one column						0.01

* Columns identified by numbers in Table I.

** Average of 9 determinations.

DISCUSSION

A complete table of retention indices for 210 PCBs on 13 columns is not provided here as it would require an excessive amount of space and can easily be generated from the data in Table II. The absolute validity of the assumption that retention indices for all PCBs can be estimated by summing appropriate 1/2 (*RI*) values cannot be tested until all 210 PCBs have been synthesized in pure form. Our experience suggests that the retention indices derived from Table II will be within 0.03% of those observed for all PCBs having up to and including three chlorine atoms in each ring, those tetra- and pentachlorobiphenyls having one ring unsubstituted, all octachloro- and nonachlorobiphenyls, decachlorobiphenyl and all PCBs differing by not more than one in the numbers of chlorine atoms in the two rings. Caution should be exercised in applying the predicted retention indices to PCBs having more than three chlorine atoms in one ring and less than three in the other. However, the last-mentioned group is seldom, if ever, seen in environmental samples.

Table III, which indicates the number of theoretical plates required to achieve adequate separation for a closeness criterion of 10 retention index units to apply, may be misleading as it refers to the column temperatures listed in Table I. In many

cases *ca.* 200° may not be optimum for a particular liquid phase, and better results might be achieved by a systematic variation of temperatures. In practice, we found that two columns, Apiezon L and OV-225, are capable of resolving a complex mixture of PCBs such as the Aroclors into the greatest number of visible peaks, especially if a slow temperature program from 150° to 250° is used. However, we have never attempted to select a "best" single liquid phase.

As the most discriminating pair of liquid phases, we could recommend OV-3 or OV-25 and Poly MPE. Analysis times (Table III) are not completely unreasonable for these pairs, while the other computer-recommended pair, OV-3 and cyclohexanedimethanol succinate, would require an 8-m column of the latter and hence an analysis time of over 12 h through decachlorobiphenyl.

The poor column efficiency of Halocarbon K-352, and doubts about its availability in reproducible batches, discourage our inclusion of this packing in a recommended triplet, unless octachloro- through decachlorobiphenyls are of major interest. Similarly, Dexil 410 is no longer being manufactured. Thus, we recommend either OV-101 (375 cm) + OV-25 (300 cm) + Poly MPE (400 cm), or OV-3 (or OV-17) (either one 300 cm) + AN-600 (780 cm) + Poly MPE (400 cm) as the best combination of three. If, in addition to these three, Apiezon L and/or OV-225 are used for the sake of their excellent resolution properties, very high confidence in PCB identifications is realistic.

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