

CHROM. 8620

HIGH-SPEED LIQUID CHROMATOGRAPHY OF POLYCHLORINATED BIPHENYLS AND RELATED COMPOUNDS

U. A. TH. BRINKMAN, J. W. F. L. SEETZ and H. G. M. REYMER

Department of Analytical Chemistry, Free Reformed University, Amsterdam (The Netherlands)

(Received July 21st, 1975)

SUMMARY

High-speed liquid chromatography in the system silica gel-dry *n*-hexane has been used to characterize the behaviour of a series of commercially available mixtures of chlorinated biphenyls, viz., Aroclor 1221-1268. An attempt has been made to assign a large number of peaks in the chromatograms of the Aroclors to the individual constituents. To this end, retention times and UV spectra were recorded for 47 polychlorinated biphenyls. The dependence of retention and spectral characteristics on the chlorine content of the substituted biphenyls is discussed.

INTRODUCTION

The separation, identification and quantitative determination of polychlorinated biphenyls (PCBs) and chlorinated pesticides are problems of much current interest. In the literature, gas chromatography and (reversed-phase) thin-layer chromatography are the methods generally recommended for analysis (for a review, see *e.g.*, ref. 1). Up to now, the use of high-speed liquid chromatography (HSLC) appears to have been considered by only a few workers²⁻⁴, no doubt largely due to the fact that the electron-capture detector, and thus gas chromatography, is well suited for the determination of polyhalogen compounds. This detector has a sensitivity for this type of compound that is several orders of magnitude greater than that of detectors commercially available for liquid chromatographs. On the other hand, HSLC often opens the possibility of creating very sharp and rapid separations. Moreover, more sophisticated UV detectors having a UV range down to 200-210 nm have recently become available, and the construction of an electron-capture detection system suitable for HSLC has been announced⁴. A study of the potentialities of HSLC for PCB and pesticide analysis, therefore, appears to be fully justified.

MATERIALS AND METHODS

A Siemens S100 liquid chromatograph equipped with an automatic injection system (10 μ l) and a Zeiss PM2 DLC UV detector was used. In order to avoid contamination of the samples and to obtain reproducible results, it was found necessary

to rinse the needle of the injection system 6–7 times between runs. Baseline noise was eliminated by inserting an RC filter (τ_{RC} , 0.9 sec) at the output of the recorder, as recommended by Brooker⁵. Long-term drift was decreased by using a home-made thermostatted detector cell (volume, 8 mm³; pathlength, 1 cm)* instead of the Siemens cell. The column was a stainless-steel tube, 25 cm \times 3 mm I.D., pre-packed with 5- μ m Lichrosorb SI 60 silica gel (Merck, Darmstadt, G.F.R.). The number of theoretical plates of the column was calculated to be *ca.* 24,000 per metre (for decachlorobiphenyl) at the optimum flow-rate of 0.3 ml/min. Unless otherwise stated, *n*-hexane (pesticide grade; Merck) dried over molecular sieve 5A was used as the mobile phase. The column was thermostatted at a temperature of $27 \pm 1^\circ$.

The PCB samples investigated included Aroclor 1221, 1232, 1242, 1248, 1254, 1260 and 1268, produced by Monsanto (St. Louis, Mo., U.S.A.). As is well known, the Aroclor products are characterized by their four-digit number: the first two digits represent the type of molecule (12, chlorinated biphenyl), while the latter two digits give the weight percent of chlorine. Biphenyl and decachlorobiphenyl were purchased from Aldrich Europe (Beers, Belgium), and small samples of 46 PCBs were received as free gifts from Dr. O. Hutzinger (University of Amsterdam, Amsterdam, The Netherlands). The pesticides used in the present study were *o,p'*-DDD, *o,p'*-DDE, *p,p'*-DDE, *o,p'*-DDT, *p,p'*-DDT (all from Aldrich), hexachlorobenzene (K and K, Plainview, N.Y., U.S.A.), heptachlor (Riedel de Haen, Seelze-Hannover, G.F.R.) and aldrin.

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

RESULTS AND DISCUSSION

Aroclors, biphenyl and pesticides

An illustrative example of the separation of eight common pesticides, decachlorobiphenyl and biphenyl is shown in Fig. 1. Apart from a minor exception (heptachlor precedes *p,p'*-DDE), the order of elution agrees with that observed by Leon⁶ using (classical) microcolumn chromatography on silica gel containing 5% (w/w) of water, using *n*-hexane and 60% benzene in *n*-hexane as the mobile phases. The efficient separations of *e.g.* *o,p'*- from *p,p'*-DDT, and of *p,p'*-DDT from its metabolite *p,p'*-DDE, merit attention. Under the prevailing conditions, the peaks of *o,p'*-DDT and *o,p'*-DDE are not resolved. However, on increasing the efficiency of the chromatographic system by decreasing the flow-rate to *ca.* 0.3 ml/min, two separate peaks are observed, *o,p'*-DDE preceding *o,p'*-DDT. The figure also demonstrates that for all of the pesticides investigated excepting the DDEs, detection at or around 205 nm is distinctly superior (10–80-fold increase) to that at 254 nm, the wavelength generally used in photometric detectors for liquid chromatographs.

The chromatographic behaviour of PCBs and pesticides is compared in Fig. 2, where chromatograms of a mixture of Aroclors (Aroclor 1232, 1248, 1254 and 1268) and the mixture already shown in Fig. 1 are superimposed. Evidently, no useful overall separation of the PCBs and the common pesticides results. However, from a more detailed study of the behaviour of the individual Aroclors (*cf.* the arrow in Fig. 2),

* Construction details available on application.

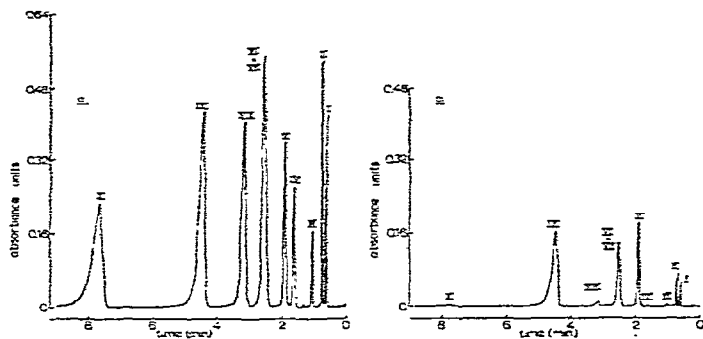


Fig. 1. HSLC of 8 common pesticides, decachlorobiphenyl and biphenyl in the system silica gel-dry *n*-hexane at (a) 205 and (b) 254 nm. I, Hexachlorobenzene; II, decachlorobiphenyl; III, aldrin; IV, heptachlor; V, *p,p'*-DDE; VI, *o,p'*-DDE; VII, *o,p'*-DDT; VIII, *p,p'*-DDT; IX, biphenyl; X, *p,p'*-DDD. Conditions: column, 25 cm \times 3 mm I.D. filled with 5- μ m Lichrosorb SI 60; flow-rate, 4 ml/min; temperature, $27 \pm 1^\circ$.

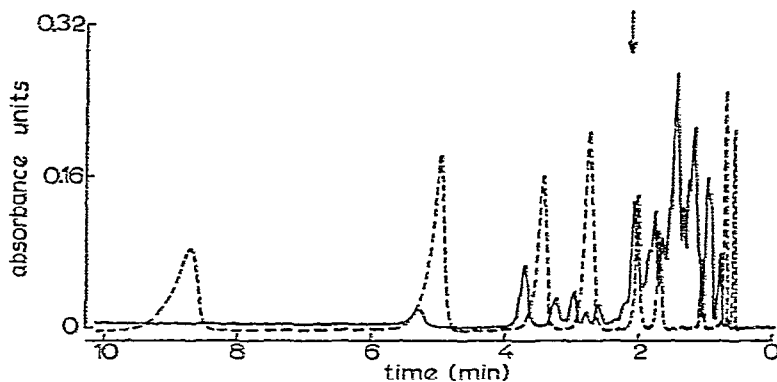


Fig. 2. HSLC of a mixture of pesticides (broken line; *cf.* Fig. 1) and a mixture of Aroclor 1252, 1246, 1254 and 1268 (solid line). The arrow marks the time at which the elution of the major peaks of Aroclor 1254 is complete. Conditions as in Fig. 1, except for flow-rate (3.5 ml/min); UV detection at 205 nm.

one may conclude that the highly chlorinated products, *i.e.*, Aroclor 1254, 1260 and 1268, are quantitatively separated from the DDTs, *o,p'*-DDE and *p,p'*-DDD; this is in agreement with recent findings of Aitzetmüller⁷. Besides, it should be borne in mind, that all Aroclors can be separated very effectively from pesticides such as BHC, endosulfan, endrin, dieldrin and methoxychlor, all of which display retention times in the present chromatographic system much longer than that of *p,p'*-DDD.

The data recorded in Figs. 3-5 show clearly that the chromatograms of each of the Aroclors have a characteristic pattern and range of retention times. As with the pesticides, detection at 205 nm instead of at 254 nm effects an increase in sensitivity, especially with the more highly chlorinated Aroclors (*cf.* Fig. 6b). To quote an example, at 200-210 nm, with the latter type of products, the patterns can be recognized down to 1-4 ppm. The introduction of a perchlorination step prior to analysis (see below) reduces this detection limit by about an order of magnitude. Also, it can be seen that the retention times of the Aroclors decrease with increasing chlorine

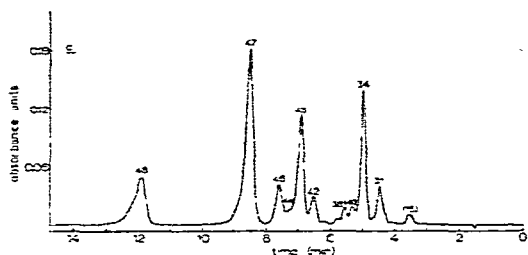


Fig. 3.

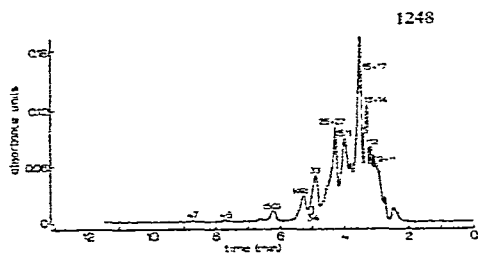
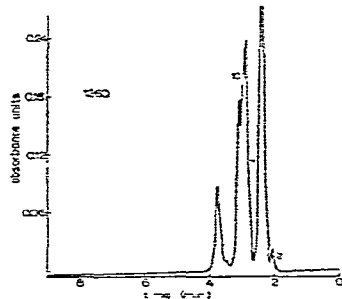
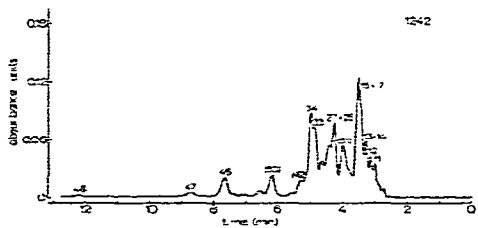
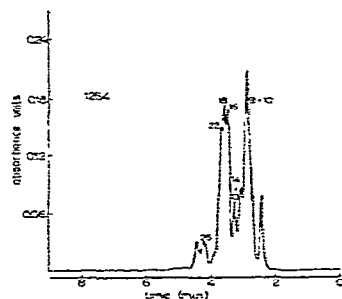
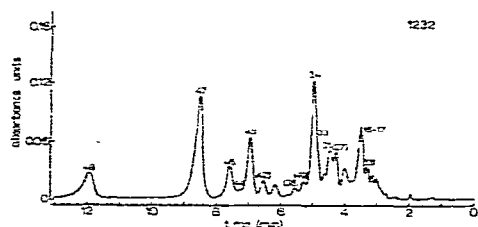
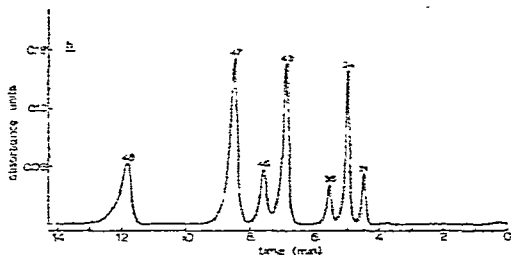


Fig. 4.

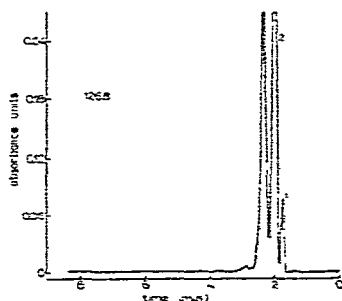


Fig. 5.

Fig. 3. HSLC of (a) Aroclor 1221 (220 ppm in *n*-hexane) and (b) an artificial mixture having a composition roughly equal to that calculated by Willis and Addison¹³ (cf. Table II). Conditions as in Fig. 1, except for flow-rate (1.4 ml/min); UV detection at 205 nm. In Fig. 3a, tentative assignments are indicated by brackets.

Fig. 4. HSLC of Aroclor 1232 (235 ppm), 1242 (205 ppm) and 1248 (245 ppm) dissolved in *n*-hexane. Conditions as in Fig. 3. Tentative assignments are indicated by brackets.

Fig. 5. HSLC of Aroclor 1254 (225 ppm), 1260 (235 ppm) and 1268 (220 ppm) dissolved in *n*-hexane. Conditions as in Fig. 3.

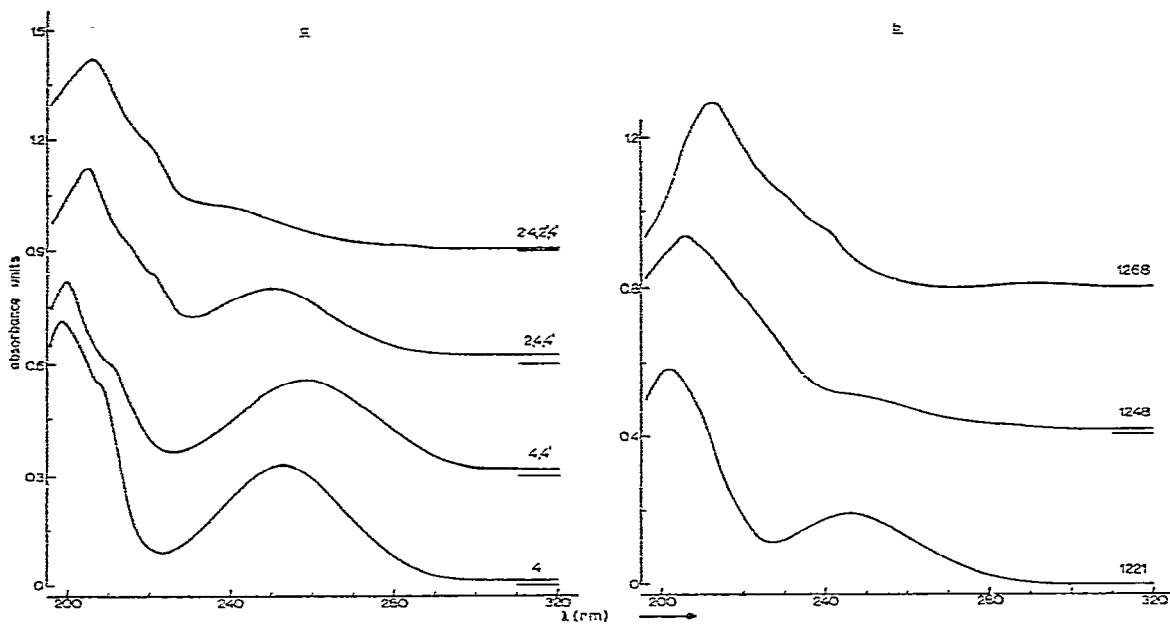


Fig. 6. UV absorption spectra of (a) 4-mono-, 4,4'-di-, 2,4,4'-tri- and 2,4,2',4'-tetrachlorobiphenyl; (b) Aroclor 1221, 1248 and 1268. Concentrations: 20 ppm PCB in *n*-hexane; 22 ppm Aroclor in *n*-hexane. Cell path, 2 mm.

content. As was first noticed by Aitzetmüller, such behaviour is opposite to that observed in gas-liquid and reversed-phase thin-layer chromatography, where the retention increases with increasing chlorine content. A detailed discussion of this aspect is given in the next section.

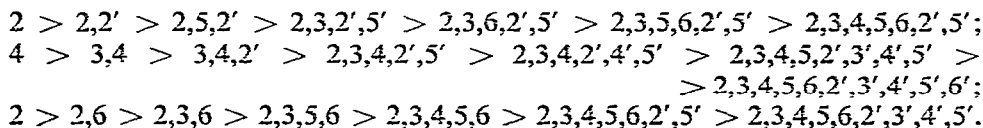
It is interesting that the water content of the *n*-hexane used as the mobile phase plays a decisive role in the separation, especially with the less highly chlorinated PCBs. A typical set of data is as follows: when the water content of the mobile phase decreases from 50 to 2 ppm, the retention time of biphenyl increases *ca.* 2-fold, whereas the retention times of decachlorobiphenyl and the major peaks of Aroclor 1254 show only a 1.1- and *ca.* 1.2-fold increase, respectively. The sharp separation of biphenyl from even the lower PCBs is important, because various recent procedures⁸⁻¹⁰ for the rapid qualitative and semi-quantitative analysis of PCB-containing mixtures are based on perchlorination with, *e.g.*, SbCl_5 , the fully chlorinated decachlorobiphenyl subsequently being determined by electron-capture gas chromatography. With this method of analysis, compounds such as chlorinated pesticides, polychlorinated naphthalenes and biphenyl may be thought to interfere. Fortunately, chlorinated pesticides are converted into products with retention times which are much longer than that of decachlorobiphenyl; under suitable conditions, the polychlorinated naphthalenes are also degraded into non-interfering products. Biphenyl, however, if it comes from sources other than the Aroclors, must be absent from the samples to be analyzed, as it is converted quantitatively into decachlorobiphenyl. Therefore, HSLC in the present chromatographic system is recommended as a purification step prior to analysis by perchlorination. Preliminary experiments have been carried out

successfully in our laboratory. The only loss stems from the biphenyl present in the Aroclors (1221 and 1232) themselves. This compares very favourably with, e.g., the results obtained by Müller *et al.*¹¹, who use a treatment with dichromate-sulphuric acid in order to eliminate interference by pesticides: losses of over 85% occur with some mono- and di- and even a trichlorobiphenyl; the recovery for tetra- and higher substituted biphenyls is 80% or more.

Individual PCBs

In order to study more closely the dependence of the retention times of the constituents of the Aroclors on their chlorine content, HSLC was carried out on 47 individual PCBs and biphenyl. Without exception, a single peak was observed in the chromatograms of these "99% pure PCB isomers". The relevant data are summarised in Table I; for clarity, the position numbering is kept separate for each ring.

The results demonstrate that, in general, retention times decrease with increasing chlorine content, as is to be expected on the basis of the chromatograms shown in Figs. 3-5. However, no strict relationship exists, the retention varying widely with both number and position of the chlorine atoms in the biphenyl nucleus. Illustrative examples are 2,4,6,2',4',6'-hexachlorobiphenyl and 2,6,2',6'-tetrachlorobiphenyl, which display unexpectedly small and large retention times, respectively. Closer inspection of the data reveals that the introduction of an increasing number of chlorine atoms into a particular PCB leads to a decrease in the retention time, as is manifest from such series as:



As the only major exception to this rule, the introduction of one, or even two, chlorine atoms into a PCB in such a way that 2,3-substitution results generally effects an increase in the retention time. To quote some examples: 2,3,2',3'-tetrachlorobiphenyl has a longer retention time than has 3,3'-dichlorobiphenyl, and the same applies to 2,3,4-trichlorobiphenyl compared with 2,4-dichlorobiphenyl; 2,3,4,2',3',4'-hexachlorobiphenyl is retarded much more than are several tri- (2,4,4') and tetra- (2,4,2',4' and 2,4,3',4') substituted biphenyls. In this context, one should also mention the 2,3,6,2',3',6'- and 2,3,4,2',3',4'-hexachlorobiphenyls, which have much longer retention times than has the isomeric 2,4,6,2',4',6'-hexachlorobiphenyl.

Table I also summarizes data on the UV absorption spectra of the PCBs; four spectra are shown in Fig. 6a. Agreement with results in the literature¹² is good; however, large discrepancies were noted in a few instances when comparing the molar extinction coefficients. The spectra of the individual PCBs were recorded chiefly with a view to selecting the proper wavelengths during detection in HSLC. However, in view of their diagnostic value, particularly with respect to the degree of chlorine substitution *ortho* to the phenyl-phenyl bond, further discussion appears to be justified. This discussion will follow the lines of a recent review by Hutzinger *et al.*¹², who based their conclusions on data for a more limited number of PCBs.

The UV spectrum of biphenyl features two important maxima, the so-called

TABLE I

HSLC RETENTION TIMES AND UV SPECTRAL DATA FOR 50 PCBs AND BIPHENYL

HSLC conditions: silica gel (5- μ m Lichrosorb SI 60)—dry *n*-hexane; flow-rate, 1.4 ml/min; UV detection at λ_{max} ; temperature, $27 \pm 1^\circ$. UV conditions: solutions of PCBs in *n*-hexane; Beckman Acta C III spectrometer.

No.	Substituted PCB	$t_{ret.}$ (min)	$\lambda_{max.}$ and $\lambda_{sk.}$ in nm ($\epsilon \cdot 10^{-3}$ in l-mole ⁻¹ ·cm ⁻¹)	
			Main band	κ band
1	2,3,4,5,6,2',3',4',5',6'	1.70	215 (114), 227, 240	
2	2,3,4,5,6,2',3',4',5'	2.00	213 (84), 229, 240	
3	2,4,6,2',4',6'	2.05	203 (70)*, 208, 221, 229	
4	2,3,4,5,2',3',4',5'	2.25	209 (63), 217, 229, 238	
5	2,3,5,6,2',3',5',6'	2.30	211 (97), 231, 239	
6	2,3,4,5,6,2',5'	2.50	215 (73)*, 228, 240	
7	2,3,4,5,2',5'	2.85	214 (58)	
8	2,3,5,6,2',5'	2.90	211 (65), 240	
9	2,4,5,2',5'	2.95	211 (51), 220, 229	
10	2,3,4,2',4',5'	3.00	212 (60), 228	240-245
11	2,4,2',4'	3.05	206 (57), 218, 230-240	
12	2,4,3',4'	3.15	210 (51), 219	251 (18)
13	2,5,3',4'	3.30	214 (46), 226	248 (13)
14	2,4,2',5'	3.35	205 (57), 225	240-245
15	2,4,4'	3.45	205 (49), 214, 220	250 (17)
16	2,4,5,2',3'	3.50	209 (58), 210, 229	240-245
17	2,5,4'	3.55	204 (43), 225 (29)	248 (14)
18	2,3,4,2',5'	3.60	211 (51), 207 (50), 220, 228	245
19	2,5,3'	3.65	213 (44)	240 (10)
20	2,3,4,2',3',4'	3.65	203 (56), 212 (55), 221, 228	240-245
21	2,3,6,2',3',6'	3.75	206 (82), 220-225, 234	
22	2,3,6,2',5'	3.80	203 (57), 220-225, 230-235	
23	2,3,4,5,6	3.95	214 (65), 225-235, 240	
24	3,5	4.15	206 (42), 210 (42), 221 (30)	251 (17)
25	3,3'	4.25	212 (52)	249 (15)*
26	2,3,2',5'	4.25	201 (63), 210, 219, 228	
27	3,4,2'	4.30	209 (51)	245 (13)
28	2,3,4,5	4.30	209 (49), 224	248 (12)
29	2,4,5	4.45	207 (42), 221	247 (12)
30	2,4,6	4.45	204 (53), 221, 229	240 (9)
31	4,4'	4.50	200 (46), 210	258 (21)
32	2,3,5,6	4.55	208 (54), 220, 230, 239	
33	2,5,2'	4.85	199 (47)*, 209, 230	
34	2,4'	5.00	202 (46), 208, 218	244 (15)
35	2,3,2',3'	5.20	204 (55), 215, 226	240
36	2,5	5.30	203 (41), 221 (29)	240 (11)
37	2,6,2',6'	5.50	200 (71)*, 218, 227	
38	2,4	5.55	203 (47), 211	244 (15)
39	3,4	5.80	205 (43), 210, 219	254 (20)
40	2,3,4	6.10	205 (47), 221, 228	247 (12)
41	2,3,6	6.15	205 (49), 224, 234	
42	3	6.50	204 (41), 207, 218	248 (16)
43	4	7.00	199 (47), 208	253 (21)
44	2,3'	7.25	204 (42), 218	242 (10)
45	2,6	7.45	203 (45), 219, 228	240 (6)
46	2,2'	7.55	199 (39), 205, 212	230-240
47	2	8.55	201 (45), 211	239 (11)
48	Biphenyl	11.90	201 (46), 205	246 (18)
49	2,4,2'	5.30		
50	2,3,2'	6.20		
51	2,5,2',5'	3.95		

* Some 20-40% higher values are quoted in ref. 12.

main band with λ_{\max} at 201 nm and a shoulder at *ca.* 205 nm, and a second maximum at 246 nm, generally referred to as the κ band, which is attributed to the conjugated biphenyl system with the contributions of both phenyl rings. With the mono- and dichlorobiphenyls, the main band changes its position only slightly compared with biphenyl, both hypso- and bathochromic shifts being observed. With the more highly chlorinated biphenyls, the main band almost invariably shifts to longer wavelengths over a distance generally not exceeding 10–12 nm. As a rule, one, two or even three shoulders occur in the main band in the region between 210 and 240 nm; occasionally, they form into an additional peak, notably with 2,5-di-, 3,5-di- and 2,5,4'-trichlorobiphenyl. As for the intensity of the main band, the molar extinction coefficient, ϵ_{main} , varies between *ca.* 40,000 and 60,000 for all but the highly *ortho*-substituted (2,6,2' and 2,6,2',6') biphenyls. With the latter type of PCBs, typical values of ϵ_{main} are between 60,000 and 110,000.

Changes in the κ band are more marked than they are for the main absorption band. PCBs with no or only one chlorine atom *ortho* to the phenyl-phenyl bond have relatively strong and well formed κ bands in the 240–260-nm range. Compared with biphenyl, *meta*, and even more so *para*, substitution induces a shift towards the visible region, while the introduction of a (single) *ortho* substituent causes a small hypsochromic shift. *Ortho* substitution, moreover, leads to a marked decrease in the intensity of the κ band; whereas the ratio $\epsilon_{\text{main}}/\epsilon_{\kappa}$ has a value of 2–2.5 for biphenyl and most non-*ortho*-substituted PCBs, this ratio increases to 3–4 upon the introduction of a chlorine atom in the 2-position. Even more drastic changes occur on introducing further chlorine atoms *ortho* to the phenyl-phenyl bond. Owing to the considerable hindrance to free rotation of the rings, and the resulting loss in coplanarity, a sharp decrease in ϵ_{κ} ensues, while the band itself shifts still further towards the ultraviolet region and shows up as a shoulder on or contiguous to the main absorption band*.

The above conclusions, which confirm and extend those previously reached by Hutzinger and his colleagues, are illustrated by the UV spectra of Aroclor 1221, 1248 and 1268 shown in Fig. 6b. The main band, which has λ_{\max} 202 nm with Aroclor 1221, shifts to longer wavelengths (206 and 213 nm) on increasing the chlorine content of the Aroclors. At the same time, the prominent κ band observed at 245 nm in Aroclor 1221 reduces to a broad, flat band with Aroclor 1248, while a mere shoulder at *ca.* 240 nm occurs with Aroclor 1268.

Composition of the Aroclors

A preliminary study has been made of the composition of the Aroclors. It should be borne in mind that in this paper we merely intend to demonstrate the potentialities of HSLC in the analysis and identification of PCBs and other such compounds. Peaks have been assigned by combining literature data on gas chromatographic analyses of the Aroclors^{13–17} and the HSLC results collected in Table I. No attempt has yet been made to confirm further our conclusions by, *e.g.*, recording UV, IR or mass spectra for individual peaks and comparing the results obtained with

* As for the occurrence of so-called β bands¹² in highly hindered substituted biphenyls (λ 265–305 nm; $\epsilon < 250$; *cf.* Fig. 6b, Aroclor 1268), these do not appear to be particularly important in the present study, and therefore are not taken into consideration.

those for authentic synthetic material. However, HSLC is a technique well suited to this type of work.

Aroclor 1221. Gas chromatography reveals¹³⁻¹⁵ the presence of 7-12 PCBs in Aroclor 1221; data on the qualitative and quantitative analysis are summarized in Table II. An HSLC chromatogram is shown in Fig. 3a: 17 peaks occur, 9 of which can be assigned to individual PCBs mentioned in the table; the peak identification numbers correspond to those in Table I. The peak at $t_{ret.}$ 5.30 min, which is also present in the chromatograms of Aroclor 1232 and 1242, may be due to 2,4,2'-trichlorobiphenyl, the only constituent identified in gas chromatography, which was not available in the present study. Our results are confirmed by comparing the chromatogram of Aroclor 1221 with that of an artificial mixture having a composition roughly equal to that calculated by Willis and Addison¹³ (Fig. 3b). The most notable differences are the absence of peaks at $t_{ret.}$ 6.50 and 3.45 min, which may be ascribed to 3-monochlorobiphenyl (*cf.* Table II) and, presumably, 2,4,4'-trichlorobiphenyl (*cf.* ref. 13), respectively.

For quantitative analysis, peak areas were measured for all peaks and weight percentages of the constituents were calculated on the assumption that ϵ_{205} is approximately equal ($\pm 5\%$) for all PCBs present. Inspection of the UV spectra shows that this assumption is valid for all known constituents except 2,2'- and 4,4'-dichlorobiphenyl, for which a correction was applied. The results in Table II show good agreement with the data published by Willis and Addison¹³ and Webb and McCall¹⁵. The weight percent of chlorine calculated from our data is 20.8%, which lies within the manufacturers specifications (20.5-21.5%).

TABLE II
COMPOSITION OF AROCLOR 1221

Substituted chlorobiphenyl	Qualitative analysis		Quantitative analysis (% w/w)		
	Ref. 14	This work	Ref. 13	Ref. 15	This work
Biphenyl	+	+	12.7	6.7	13
2-mono	+	+	28.4	31.8	33
3-mono	+	±			4
4-mono	+	+	18.7	19.3	17
2,2'-di	+	+	9.2	10.1	7.5
2,3'-di	+	±			<0.5
2,4-di		+	3.5	2.8	1.5
2,4'-di	+	+	13.6	20.8	15
4,4'-di	+	+	6.2	4.6	5
2,4,2'-tri	+	+?			1
Unidentified peaks	2	7	7.7	3.9	3

Aroclor 1232, 1242 and 1248. Chromatograms of Aroclor 1232, 1242 and 1248 are shown in Fig. 4. Interpretation was made with the help of gas-chromatographic analyses published by Webb and McCall¹⁴. Unequivocal assignment is possible for 10 or more peaks with all three products, while the peaks at $t_{ret.}$ 5.55 and 6.50 min are attributed to 2,4-di- and 3-monochlorobiphenyl, respectively, on the basis of the results obtained for Aroclor 1221. For the peaks at $t_{ret.}$ 3.3 and 4.3 min, Webb and McCall do not indicate the presence of any of the four PCBs concerned (*cf.* Fig. 4; Aroclor

1242 and 1248) in Aroclor 1232. However, as the proportions of 2,5,3',4'-tetra- and 3,4,2'-trichlorobiphenyl increase relative to those of 2,4,2',5'-tetra- and 2,3,2',5'-tetrachlorobiphenyl, respectively, when going from Aroclor 1248 to 1242, it can be concluded that in Aroclor 1232 these peaks are chiefly due to the former two PCBs.

Lastly, for 2,3,2'-tri- and 2,5,2',5'-tetrachlorobiphenyl, two relatively prominent constituents¹⁴ of the Aroclors under investigation, inspection of the chromatograms in Fig. 4 suggests that the peaks at $t_{\text{ret.}}$ 6.20 and 3.95 min may be attributed to them. The fairly large retention time assigned to the trichloro compound is in keeping with the picture outlined above for 2,3- and 2,2'-substituted biphenyls.

Aroclor 1254, 1260 and 1268. Chromatograms of Aroclor 1254, 1260 and 1268 are presented in Fig. 5. Comparison with Fig. 4 shows that a drastic change occurs on going from Aroclor 1248 to 1254: in the latter product, peaks having $t_{\text{ret.}}$ greater than *ca.* 4.5 min are virtually absent. This is in agreement with the results in refs. 14 and 16, neither of which report the presence of significant proportions of mono-, di- and/or trichlorobiphenyls in Aroclor 1254. For the rest, the lack of a sufficient number of PCBs and of literature data, and the relatively small differences in retention times observed with the more highly substituted chlorobiphenyls allow us to assign a few peaks only.

However, to quote an example, Aroclor 1260 consists of about equal proportions (40%) of hexa- and heptachlorobiphenyls¹⁵, predicted structures for two very prominent hexachloro compounds being 2,3,4,2',4',5'- and 2,3,4,5,2',5'-hexachlorobiphenyl¹⁷. According to Table I, the retention times of these PCBs lie within the range of retention times of one of the two major peaks shown in the chromatogram of Aroclor 1260. This strongly suggests that this peak is due chiefly to the hexa-substituted isomers, a suggestion supported by the fact that it is virtually absent from the chromatogram of Aroclor 1268, and that the peak at $t_{\text{ret.}}$ 2.3–2.6 min may be assigned primarily to hepta-substituted PCBs.

With Aroclor 1268, which has an average of 8.7 chlorine atoms per molecule, the peak at $t_{\text{ret.}}$ 2 min, still very small with Aroclor 1260, will be due at least partly¹⁶ to 2,3,4,5,6,2',3',4',5'-nonachlorobiphenyl. Also, it is worth noting that Aroclor 1268 is the only member of the Aroclor series that contains a significant amount of deca-chlorobiphenyl.

CONCLUSION

The application of HSLC in the qualitative and quantitative analysis of mixtures of PCBs and/or pesticides has a high potentiality. In order to achieve sufficient sensitivity, UV detection should be carried out at or around 205 nm.

The system silica gel-dry *n*-hexane is well suited for the separation of biphenyl from PCBs prior to quantitative analysis of PCB-containing mixtures by perchlorination procedures. Conversion of mixtures of PCBs into decachlorobiphenyl and subsequent analysis by HSLC permits the detection of PCBs down to *ca.* 100 ppb.

Use of the system silica gel-dry *n*-hexane is also recommended for the study of the composition of mixtures of PCBs such as the Aroclor 1221–1268 series. As, in general, retention times increase considerably with decreasing chlorine content, the results obtained with the lower Aroclors are superior to those obtained with the more highly chlorinated products. To quote an example, in the case of Aroclor 1221, 17

peaks have been observed in HSLC compared with 12, or less, in various gas chromatographic analyses.

REFERENCES

- 1 L. Fishbein, *Chromatography of Environmental Hazards*, Vol. II, Elsevier, Amsterdam, 1973, Ch. 24.
- 2 F. Eisenbeiss and H. Sieper, *J. Chromatogr.*, 83 (1973) 439.
- 3 S. H. Byrne, J. A. Schmit and P. E. Johnson, *J. Chromatogr. Sci.*, 9 (1971) 592.
- 4 F. H. Willmott and R. J. Dolphin, *J. Chromatogr. Sci.*, 12 (1974) 695.
- 5 G. Brooker, *Anal. Chem.*, 43 (1971) 1095.
- 6 V. Leoni, *J. Chromatogr.*, 62 (1971) 63.
- 7 K. Aitzetmüller, *J. Chromatogr.*, 107 (1975) 411.
- 8 O. W. Berg, P. L. Diosady and G. A. V. Rees, *Bull. Environ. Contam. Toxicol.*, 7 (1972) 338.
- 9 J. A. Armour, *J. Ass. Offic. Anal. Chem.*, 56 (1973) 987.
- 10 N. J. A. de Boer, U. A. Th. Brinkman and R. B. Geerdink, in preparation.
- 11 W. Müller, H. Rohleder, W. Klein and F. Korte, *GSF-Bericht Ö 104*, München, 1974.
- 12 O. Hutzinger, S. Safe and V. Zitko, *The Chemistry of PCBs*, CRC Press, Cleveland, 1974, Ch. 10.
- 13 D. E. Willis and R. F. Addison, *J. Fish. Res. Bd. Can.*, 29 (1972) 592.
- 14 R. G. Webb and A. C. McCall, *J. Ass. Offic. Anal. Chem.*, 55 (1972) 746.
- 15 R. G. Webb and A. C. McCall, *J. Chromatogr. Sci.*, 11 (1973) 366.
- 16 D. L. Stalling and J. N. Huckins, *J. Ass. Offic. Anal. Chem.*, 54 (1971) 801.
- 17 D. Sissons and D. Welti, *J. Chromatogr.*, 60 (1971) 15.
- 18 A. Thruston, *PCB Newsletter*, No. 3, July 1971.