Analyses of Polychlorinated Biphenyls in Bird Tissues and Aroclor Standards with Gas Chromatography and Mass Spectrometry

by

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Polychlorinated biphenyls (PCB's) consist of complex mixtures of isomeric biphenyls with various numbers of chlorine atoms substituted on the biphenyl rings. There are some 210 theoretically possible isomers of PCB's with 102 probable combinations (WIDMARK, 1968). Aroclor 1254 produced by Monsanto Chemical Company has been shown to have more than 50 components when analyzed by capillary gas chromatography columns (SESSONS and WELTI, 1971). In order to observe changes due to metabolism, it is desirable to relate peaks seen on gas-liquid chromatography (GLC) chromatograms to the PCB isomers composing the peaks. Also, HUTZINGER et al. (1972) has shown that in environmental samples, the pattern of PCB peaks change with trophic levels. WEBB and McCALL (1972) have related the relative retention times of the peaks seen on GLC chromatograms to the number of chlorines in the isomers which compose each peak for six different Aroclors. BAGLEY et al. (1970) demonstrated that most of the PCB peaks occuring in bald eagle carcasses were identical to components of Aroclor 1254 using combined GLC-mass spectrometry.

Many environmental samples are routinely monitored for insecticides and PCB's in this laboratory. Therefore a study was done to relate the peaks identified by GLC to the PCB isomers present in each peak as determined by mass spectrometry (MS).

Methods and Materials

Double-crested Cormorants (Phalacrocorax a. auritus) and eggs and White Pelicans (Pelecanus erythrorhynchos) were collected from rookeries at Dry Lake and South Waubay Lake, South Dakota. Eggs and carcasses did not necessarily come from the same birds. Four samples each of cormorant carcass and egg and three samples

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of pelican fat were extracted, saponified, oxidized and thin-layered as described by GREICHUS et al. (1972). These samples were employed in making comparisons between samples and PCB standards and in determining relative retention times (rrt). Each sample type was pooled before analysis to obtain the GLC/MS data given in the figures and table. Aroclor standards were obtained from Monsanto Company, St. Louis, Missouri.

The instrument used for GLC analysis was a Varian Aerograph HY-FI model 600D equipped with a model S-R 1 mv Sargent recorder and an electron capture detector cell with an 8 mc ^{63}Ni source. Column, injector and detector temperatures were 200C, 210C and 280C respectively. A 1/8 inch o.d. x 6 foot borosilicate glass column packed with a 1:1 mixture of 15% Qf-1 and 10% DC-200 Silicone on 80/100 mesh Chromosorb W (H/P A.W. DMCS) was operated isothermally with a flow rate of 40 ml per min of nitrogen carrier gas.

In the calculation of rrt, p,p'-DDE was assigned the value of 100. Under the conditions used for GLC analysis in this study both Aroclor 1254 and 1260 had peaks with the same rrt as p,p'-DDE.

Mass spectrometry analyses of Aroclors and samples were obtained with a Finnigan Model 3000 Peak Identifier GLC/MS equipped with a Gohlke separator. A 1/8 inch i.d. by 5 foot borosilicate glass column was packed with 3% OV-1 on 60/80 mesh Gas Chrom Q with a helium carrier gas flow rate of 40 ml per min. Temperatures of the column, injector and accessory were 200C, 210C and 210C respectively. The ionization potential was 70 ev.

Results and Discussion

Comparison of the PCB patterns of the four cormorant carcasses and four eggs revealed a number of changes (Fig. 1). Peaks with rrt of approximately 147, 195, 280 and 337 became relatively smaller while peak 128 increased in eggs in comparison to carcass. GRANT et al. (1971) found that the liver was the main site of Aroclor 1254 metabolism as rats with livers damaged by carbon tetrachloride could not metabolize PCB's as rapidly as rats with normal livers. The peak at 100 in egg contained p,p'-DDE as well as PCB's because p,p'-DDE apparently was not totally converted to 4,4'-dichlorobenzophenone during purification of the PCB's in the sample. No other interferring compounds were detected in the samples by MS.

The pattern for the peaks in pelican fat (Fig. 1) resembled those in cormorant carcass except that there was sometimes a peak

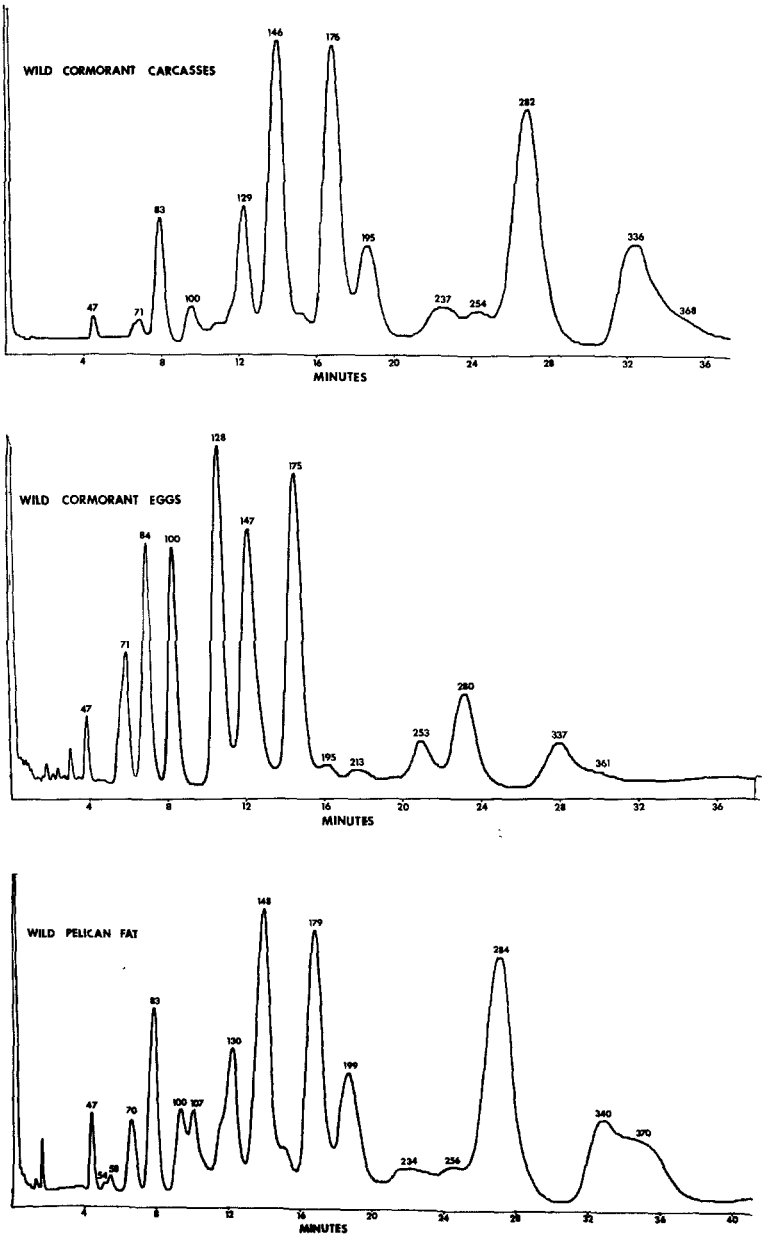

Fig. 1 - Relative retention times of GLC tracings of cormorant carcasses, eggs and pelican fat. Conditions: 1/8" o.d. x 6' glass column with 1:1 mixture of 15% QF-1 and 10% DC-200 on 80/100 mesh Chromosorb W operated at 200C with a nitrogen gas flow rate of 40 ml/min. p,p'-DDE = 100.

at rrt 107 in fat but not in carcass or egg. This peak is prominent in Aroclor 1254 (Fig. 2). The peak at rrt 70 was consistently smaller than the peak at 83 in the samples while they were about the same size in Aroclors 1254 and 1260 (Figs. 1 and 2). GRANT et al. (1971) observed that peaks with shorter rrt were metabolized to a greater degree than those with longer rrt

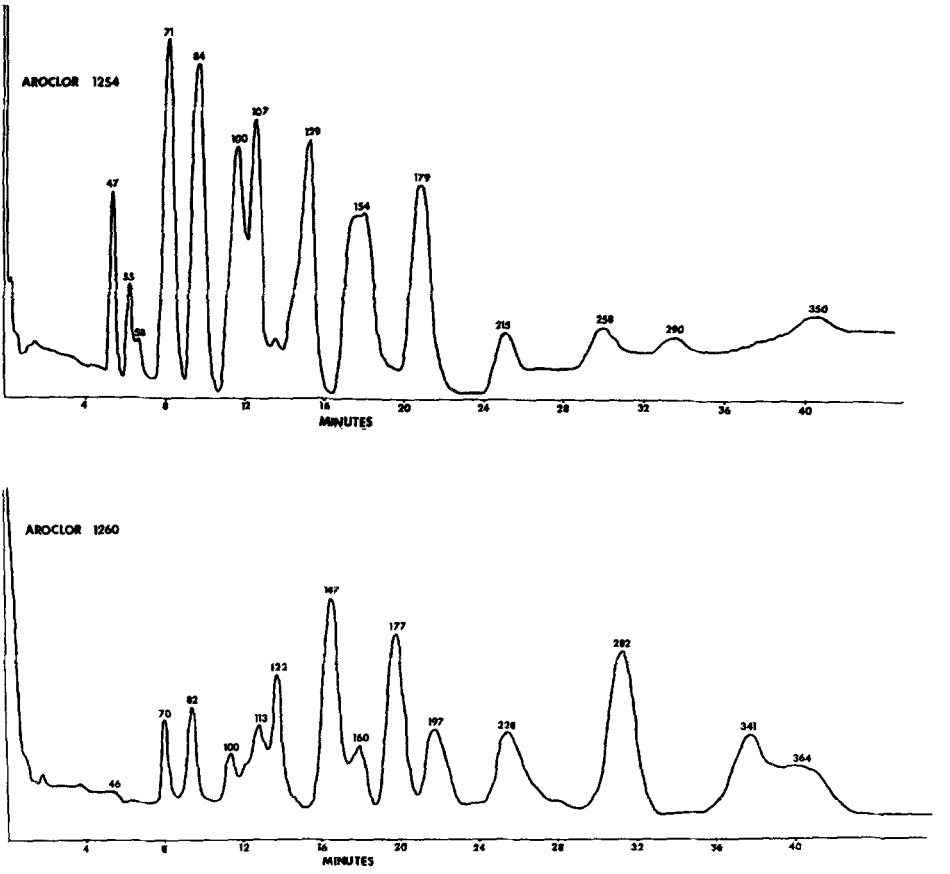

Fig. 2 - Relative retention times of GLC tracings of Aroclor 1254 and 1260. Conditions: 1/8" o.d. x 6' glass column with 1:1 mixture of 15% QF-1 and 10% DC-200 on 80/100 mesh Chromosorb W operated at 200 C with a nitrogen gas flow rate of 40 ml/min. p,p'-DDE = 100.

TABLE I

Relative retention times and mass spectra data for PCB residues in carcasses, eggs and fat of birds.

Cormorant Carcasses			Cormorant Eggs			Pelican Fat		
rrt[1]	m/e	No. Chlorines	rrt	m/e	No. Chlorines	rrt	m/e	No. Chlorines
47			47			47	290	4
53			53			53	290	4
57			57			58	290	4
71			71	290	4	70	290	4
				324	5		324	5
83	324	5	84	324	5	83	324	5
100			100	324	5	100	324	5
						107	324	5
129			128			130	324	5
							358	6
146	324	5	147	324	5	148	358	6
	358	6		358	6			
176	358	6	175	358	6	179	358	6
195	358	6	195			199	392	7
	392	7						
			213	358	6			
				392	7			
237						234	392	7
254	392	7	253	358	6	256		
				392	7			
282	392	7	280			284	392	7
336	392	7	337	392	7	340	392	7
368	426	8	361			370	426	8

[1]Retention times relative to p,p'-DDE = 100 as measured from first appearance of solvent peak.

TABLE II

Relative retention times and mass spectra data
for Aroclor Standards

Aroclor 1254			Aroclor 1260		
rrt[1]	m/e	No. Chlorines	rrt	m/e	No. Chlorines
47	290	4	46	290	4
55	290	4			
58	290	4			
71	324	5	70	324	5
84	324	5	82	324	5
100	324	5	100	324	5
				358	6
107	324	5			
	358	6			
			113	358	6
			122	358	6
129	358	6			
			147	358	6
154	358	6			
			160	392	7
179	358	6	177	358	6
			197	392	7
215	358	6			
	392	7			
			228	358	6
				392	7
258	392	7			
			282	392	7
				426	8
290	392	7			
			341	392	7
350	392	7			
			364	426	8

[1]Retention times relative to p,p'-DDE=100 as measured from first appearance of solvent peak.

in rats fed Aroclor 1254. Comparison of the pattern of PCB's in the cormorant egg to those in Aroclors 1254 and 1260 showed that peaks with rrt of 195 or greater became relatively smaller in the egg.

The peak at about 129 in the samples was found in Aroclor 1254 but not in 1260 while peaks at about 282 and 340 were prominent only in Aroclor 1260. BAGLEY et al. (1970) found most of the peaks occurring in eagle carcass had rrt values similar to peaks found in Aroclor 1254. However, a peak identified as No. 13 in eagle samples was not present in Aroclor 1254. This peak appeared to have a rrt similar to peak 195 in cormorant and pelican samples and was present in Aroclor 1260 (Figs. 1 and 2).

The PCB's in Aroclors 1254 and 1260 and in the biological samples were identified by GLC/MS. The numbers of chlorines contained in the PCB's were determined by observing the appropriate m/e values of the molecular ions and verified by the relative intensities of the peaks in the molecular ion clusters. The rrt of the major peaks in the gas chromatograms of the cormorant and pelican samples (Table I) could be correlated with the rrt of the major peaks in the standards (Table II) via their relative intensities and identical mass spectral fragmentation patterns. Multiple component peaks were verified by observation of the correct molecular weight and molecular ion clusters for each PCB.

It was concluded that all major PCB's occuring in cormorant carcass and eggs and in pelican fat also occurred in Aroclor 1254 and/or 1260. The pattern of PCB's found in the biological samples was not the same as those in Aroclor 1254 or 1260 and further changes in pattern were seen when comparing cormorant carcass and eggs.

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LITERATURE CITED

BAGLEY, G. E., W. L. REICHEL, and E. CROMARTIE. J. Assoc. Office. Anal. Chem. **53**, 252 (1970).

GREICHUS, Y. A., A. GREICHUS, and R. EMERICK. 164th Natl. Meeting A.C.S., Symposium on PCB's. New York, N. Y. August 28 - Sept. 1 (1972).

GRANT, D. L., W. E. J. PHILLIPS, and D. C. VILLENEUVE. Bull. Environ. Contam. Toxicol. 6, 102 (1971).

HUTZINGER, O., S. SAFE, and V. ZITKO. Analabs, Research Notes 12, 1 (1972).

SISSONS, D., and D. WELTI. J. Chromatogr. 60, 15 (1971).

WEBB, R. G., and A. C. MC CALL. 164th Natl. Meeting A.C.S., Symposium on PCB's, New York, N. Y. August 28 - Sept. 1 (1972).

WIDMARK, G. The OECD Study of Analysis of PCB. Report from the Institute of Anal. Chem., Stockholm, Sweden. (1968).