A Gas Chromatographic-mass Spectrometric Comparison of Polychlorinated Biphenyl Residues in the Japanese Quail Brain to an Aroclor Standard

by

D. J. CALL, Y. A. GREICHUS, and J. J. WORMAN

Experiment Station Biochemistry and Chemistry Departments
South Dakota State University, Brookings 57006

Polychlorinated biphenyl (PCB) residues have been discovered in biological tissues in recent years (BAGLEY et al., 1970; GREICHUS et al., 1973a; KOEMAN et al., 1969). PCB storage in liver, brain, and muscle has been reported (DAHLGREN et al., 1971). Analyses of biological residues by combined gas-liquid chromatography (GLC) and mass spectrometry (MS) revealed their patterns to most closely resemble those of two PCB standards, Aroclors 1254 and 1260 (BAGLEY et al., 1970; GREICHUS et al., 1973b). However, the biological residue chromatograms had certain changes in their overall patterns as compared to the standards. This study was designed to (1) compare the residue patterns in brains of quail fed Aroclor 1260 to the Aroclor 1260 standard, (2) identify the residue components by mass spectrometry, and (3) determine the specific changes in the biological samples.

MATERIALS AND METHODS

Seven Japanese quail (Coturnix coturnix japonica) 7 weeks of age were fed 1,000 parts per million (ppm) Aroclor 1260 for 21 days or until death. Aroclor 1260 was supplied by Monsanto Chemical Company, St. Louis, Missouri. It was dissolved in soybean salad oil and thoroughly mixed into the diet of Purina Game Bird Chow. Four of the 7 birds died from this treatment, with deaths occurring as early as day 12 of the treatment. The remaining birds were sacrificed on day 22. The heads of all birds were frozen for later brain residue analyses.

Brains were removed and wet weights taken on a Mettler balance. Whole brains were homogenized and dried in a Florisil-sodium sulfate mixture. Procedures for extraction, purification and quantitation of PCB's were essentially those of GREICHUS et al. (1973a). PCB's were extracted with 750 ml of 70:30 (v:v) petroleum ether and dichloromethane on a 20 by 600 mm Pyrex

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chromatographic column packed with 50 grams of Florisil, 60/100 mesh (Fisher Scientific Co.).

GLC analyses were performed on a Varian Aerograph HY-FI Model 600 D gas chromatograph with an 8 mc ^{63}Ni detector. A borosilicate glass column, 1/8 inch o.d. x 5 feet in length, was packed with a 1:1 mixture of 15% QF-1 Silicone (Fluoro) and 10% DC-200 Silicone on 80/100 mesh Chromosorb W (H/P A.W. DMCS). The nitrogen carrier gas was operated at a flow rate of 40 ml/min, and the operating temperatures for injector, column, and detector were 210 C, 190 C, and 280 C, respectively.

The PCB's in the standard and brain were identified by GC/MS. The appropriate *m/e* values of the molecular ions were observed to determine the number of chlorines contained in the PCB's. Chlorine numbers were verified by the relative intensities of the peaks in the molecular ion clusters. Brain extracts were pooled for MS analysis. The instrument used was a Finnigan Model 3000 Peak Identifier GLC/MS equipped with a Gohlke separator. A 1/8 inch i.d. x 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/min. Temperatures of the column, injector, and accessory were 200 C, 210 C, and 210 C, respectively; and the ionization potential was 70 ev.

Statistical treatment of the peak area percents for peaks of similar retention times in the standard and brain chromatograms consisted of the t-test for paired and unpaired data as appropriate (STEEL and TORRIE, 1960).

RESULTS AND DISCUSSION

Comparison of the quail brain chromatograms with the Aroclor 1260 standard chromatograms revealed some gross changes in the patterns. Representative chromatograms are shown in Figure 1. Quail brain peaks with relative retention times (rrt's) of about 72, 84, 122, and 160 were significantly smaller ($p<0.01$) than the corresponding standard peaks (Table 1). There were no peaks at rrt's of 46, 100, 113, or 228 in the biological samples. New peaks occurred in the brains at the rrt of 238, and occasionally at 108. Brain peaks were larger ($p<0.01$) at rrt's of about 147, 176, 107, 280, and 336 than the corresponding standard peaks (Table 1).

BAILEY and BUNYAN (1972) found that pigeons (Columbia livia) and Japanese quail metabolized PCB's at a rate generally dependent on the amount of chlorine in the molecule. They suggested that some lower chlorinated isomers are metabolized very rapidly. Many

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of the peaks representing lower chlorinated compounds disappeared from the tissue of Japanese quail fed PCB's (KOEMAN et al., 1969), and in the eggs of mallards (Anas platyrhynchos) fed Aroclor 1254 (HEATH et al., 1972). Considerable diminution of the first two major chromatogram peaks occurred in tissue samples, eggs and feces of ring doves (Streptopelia risoria) fed 10 ppm Aroclor 1254 (LINCER and PEAKALL, 1973). Rats administered Aroclor 1254 metabolized the shorter retention time components to a greater extent than the longer retention time components (GRANT et al., 1971).

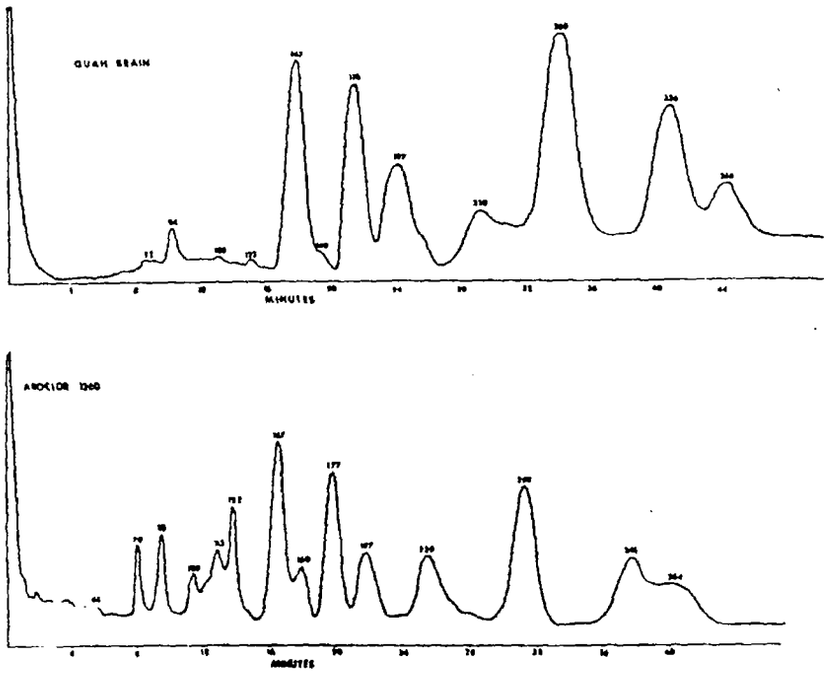

Figure 1. GLC tracings of the brain residues in Japanese quail fed 1000 ppm Aroclor 1260 and the Aroclor 1260 standard. Average retention times (p,p'-DDE = 100) are given above the component peaks. Conditions: 1/8" o.d. x 5' glass column with 1:1 mixture of 15% QF-1 and 10% DC-200 on 80/100 mesh Chromosorb W operated at 200 C with a nitrogen gas flow rate of 40 ml/min.

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TABLE I

Comparative GLC and mass spectral data for the components of Aroclor 1260 standard and Aroclor 1260 brain residues in Japanese quail.

Aroclor 1260 Standard						Quail Brain				
Peak rrt[1]	m/e	Cl no.	Area %	Nanogram %[2]	Response factor[3]	Peak rrt	m/e	Cl no.	Area %	Nanogram %
46	290	4	--	--	--	--	--	--	--	--
70	324	5	2.8	2.7	0.0009	72	--	--	0.1**	0.1
82	324	5	3.6	4.7	0.0012	84	324	5	1.6**	2.2
100	324	5	2.6	3.8	0.0015					
	358	6				108	--	--	0.1	--
113	358	6	7.8	3.3	0.0004					
122	358	6	6.6	12.3	0.0018	122	358	6	0.3**	0.7
147	358	6	11.6	14.1	0.0012	147	358	6	17.1**	24.4
160	392	7	3.9	4.9	0.0012	160	--	--	0.3**	0.4
177	359	6	10.2	12.4	0.0012	176	--	--	17.0**	24.1
197	392	7	6.0	9.3	0.0015	197	392	7	10.8**	19.2
228	358	6								
	392	7	8.7	9.8	0.0011					
						238	--	--	2.7	--
282	392	7	17.5	11.0	0.0006	280	392	7	27.8**	19.7
	426	8					426	8		
341	392	7	11.3	4.2	0.0003	336	392	7	16.4**	5.8
364	426	8	7.4	4.0	0.0005	366	426	8	5.9	3.5

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

[2] Percent of total quantity of standard injected into gas chromatograph as taker from WEBB and MC Call (1972).

[3] Detector responses (ng PCB/area) to the standard components.

**Significantly different from the standard at the p<0.01 level.

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Loss of a peak at the rrt of 228 was observed in wild cormorant (Phalacrocorax auritus) carcasses and eggs, and in wild pelican (Pelecanus erythrorhynchos) fat (GREICHUS et al., 1973b). A peak appeared in cormorant carcasses at rrt 237 and in pelican fat at 234, which does not correspond to any peak in either Aroclor 1254 or 1260. This may correlate with the new peak at rrt 238 in the quail brain of this experiment.

The more highly chlorinated compounds have longer retention times (Table 1). The relatively larger size of the peaks with longer retention times in brain tissue would indicate that these more highly chlorinated compounds were preferentially stored in the brain lipids over the less chlorinated compounds, and/or were less readily metabolized.

WEBB and MC CALL (1972) determined the nanogram percent of PCB in each GC peak by combined GC-MS analysis and analysis by GC with a Coulson electrolytic conductivity detector. This allowed the determination of the empirical formulae for the compounds and the absolute amount of chlorine represented by each peak. Mean response factors (ng PCB/area) for each peak were determined in the present experiment by analyzing known amounts of 7 standards and measuring the peak areas. Brain samples were chromatographed and peak areas were multiplied by the corresponding response factors, giving the PCB nanogram quantities for the peaks in the brain.

Comparison of the nanogram percentages of the standard peaks with the peaks in the brain revealed the same trends as comparison of the peak area percentages (Table 1). The nanogram percentages for peaks at rrt's of about 72, 84, 122, and 160 were smaller in the brains than in the standards, and the nanogram percentages for peaks at rrt's of 147, 176, 197, 280, and 336 were relatively larger in the brain. However, the differences in peak area percentages and nanogram percentages from standard to brain were not in close agreement for most peaks, indicating the importance of determining detector response to the various components. Others have stated the importance of this (ROTE and MURPHY, 1971; WEBB and MC CALL, 1972).

Nanogram quantities were not determined for the peaks in quail brain at rrt's 108 and 238, as there were no corresponding standard peaks for determination of the necessary response factors. As a result, the remaining brain nanogram percent values are slightly higher than if these peak values had been included.

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The results of this experiment showed that qualitative and quantitative changes in the Aroclor 1260 standard occurred within the bodies of the quail, and the less chlorinated compounds in comparison to the more highly chlorinated compounds were stored in relatively smaller quantities in the brain and/or were more readily metabolized.

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