Ex. 4

A Method for the Quantitation of Polychlorinated Biphenyl (PCB) Isomers

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Polychlorinated biphenyls (PCB's) were first detected in 1966-67 in European wildlife (1-3). Since that time, workers in several European countries, the U.S., and Canada have reported the presence of PCB's in marine invertebrates, fish, birds, marine mammals, and humans (4-9). Attempts to quantitate the levels have been complicated by the fact that PCB's are not single compounds, but complex mixtures of isomers having varying degrees of chlorination.

Monsanto Chemical Company, the sole producer of PCB's in the U.S., markets them under the trade name 'Aroclor'. The products are designated by numbers; the first two digits represent the molecular type, and the last two digits give the weight percent of chlorine. Thus, Aroclor 1254 is a mixture of chlorinated biphenyls having an average chlorine content of 54% (5 Cl atoms).

Due to the complex nature of these mixtures, relative amounts of total PCB's have been estimated for tissue samples and whole organisms. Using electron-capture gas chromatography, the method described here allows for the quantitation of individual PCB isomers. The method is based on the fact that detector response is not the same for all PCB components, but depends on the degree of chlorination.

METHODS

Tissue samples were digested by a perchloric-acetic acid mixture (10) and the fat extracted in hexane. Cleanup methods employed by the Food and Drug Administration (11,12) were used which completely recovered PCB's along with the pesticide residues. After acetonitrile partitioning and Florisil column cleanup, PCB's were separated from the pesticides by column chromatography on silicic acid-Celite (13).

The determinative step was carried out under the following conditions:

Gas Chromatograph: Beckman Model GC-4, fitted with helium glow electron-capture detector; column: glass, 6 ft. x 4 mm I.D., packed with a 1:1 mixed bed of 5% DC-200 and 6% QF-1 on Chromosorb W (AW/DMCS).

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Operating Conditions: Column temperature 200°C., injector temperature 230°C., detector temperature 280°C; Carrier gas (He) flow rate, approximately 175 ml/min; Electrometer current 1.6 x 10^{-9} amps; Recorder: Beckman Model 1005 ten inch potentiometric, 1 mV, full scale deflection; chart speed 1 in/min; volume injected 4 µl.

Chromatograms of six PCB standards were obtained for Aroclor 1232, 1242, 1248, 1254, 1260, and 1262. These commercial mixtures correspond approximately to an average of two, three, four, five, six, and seven chlorine atoms per molecule, respectively. The Aroclors were dissolved in hexane to concentrations of 2 ng/µl. From preliminary injections it was found that a 4 µl injection placed peak heights on the linear portion of the standard curve for each Aroclor. Duplicate 4 µl injections were made, providing a total of 16 ng for each Aroclor chromatographed. Total peak area for each Aroclor was determined by the cut and weigh method.

The excellent resolution obtained with our operating conditions revealed a total of 32 different PCB isomers in the six Aroclor standards. The peaks were numbered 1 through 32 according to retention time. Individual peaks were identified as to chlorine content from mass spectrometry results of other workers (5,8,9). Thus, peaks 1 and 2 are monochlorobiphenyls; peaks 3 and 4, dichlorobiphenyls; peaks 5 through 8, trichlorobiphenyls; peaks 9 through 13, tetrachlorobiphenyls; peaks 14 through 16, pentachlorobiphenyls; peaks 17 through 22 and 24, hexachlorobiphenyls; peaks 23 and 25 through 28, heptachlorobiphenyls; peak 29, octachlorobiphenyl; peaks 30 and 31, nonachlorobiphenyls; and peak 32, decachlorobiphenyl. A standard injection of p,p'-DDE was made periodically to provide for a measure of relative retention time and to check detector sensitivity.

The amount of various chlorinated components in an Aroclor was determined by combining all peaks that corresponded to monochlorobiphenyls, all that were dichlorobiphenyls, etc. Where there was a problem of peak overlap, the 2 peaks were divided by a straight line drawn perpendicular from the minimum point between the peaks to the baseline. The cut and weigh method, using this technique, approximates automatic integration which operates on the same principle.

The semilogarithmic relationship of detector response (total peak area/ 16 ng) to average chlorine content was obtained for each Aroclor by the method of least squares. From this relationship, the theoretical response of the detector to each chlorinated biphenyl (mono through deca) was calculated (Fig. 1). These

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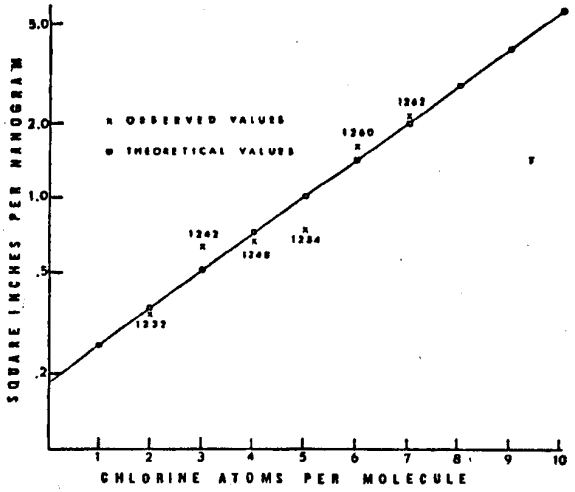

Figure 1. Detector response curve. Response to p,p'-DDE = 2.75 in^2/ng. (log y = 0.1512x - 0.7428)

values were then used to calculate the amount of chlorinated biphenyl represented by each peak in the sample chromatogram. The response curve was also used to determine the amount of each chlorinated component in the PCB standards.

RESULTS

When the individual peaks of Aroclor 1232 through 1262 were quantitated by means of the response curve, it was possible to determine the distribution of the 16 ng for each standard. Table I shows the peak area (ng) for each chlorinated species and the percentage of the 16 ng total injection accounted for in each standard.

The low percentage for Aroclor 1232 (62.5%) is apparently due to unchlorinated biphenyls in the mixture. Since this Aroclor has an average of only 2 chlorine atoms per molecule, a certain percentage of the molecules would have no chlorines. The distinct odor of these lower chlorinated Aroclors is in fact due

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to the presence of biphenyl in the mixture (14). These non-electron capturing particles do not contribute to total peak area and therefore the chromatogram does not account for the amount of material injected.

The low percentage for Aroclor 1254 (60.4%) may be due to the presence of unchlorinated biphenyls or other non-electron capturing impurities in the preparation. In the synthesis of Aroclors, the degree of chlorination is determined by either measuring the specific gravity or the viscosity of the mixtures. Therefore, it is possible that the average chlorine content of an Aroclor varies from batch to batch.

TABLE I

Distribution of chlorinated components in Aroclors.
Peak area (ng)

# Cl	1232	1242	1248	1254	1260	1262
1	1.466	.2607	-	-	-	-
2	2.472	1.950	.3681	-	-	-
3	2.732	5.681	3.223	.1896	-	-
4	1.931	4.321	4.559	1.108	-	-
5	1.111	2.541	3.868	3.092	1.139	.7123
6	.2927	.6039	1.225	4.609	5.590	4.316
7	-	-	-	.6696	5.678	7.034
8	-	-	-	-	.7770	2.199
9	-	-	-	-	.6700	1.338
10	-	-	-	-	.0910	.2850
Total	10.00	15.36	13.24	9.668	13.95	15.88
%	62.5	96.0	82.8	60.4	87.2	99.3

Fig. 2A is the chromatogram of a liver extract from the sea otter *Enhydra lutris*. The 50.6 g sample, which had about 2% fat content, contained 27 PCB isomers and had a total PCB level of 2.0 p.p.m.(total tissue). The chlorine content of each isomer is indicated below the peaks.

A chromatogram of Aroclor 1260 is provided (Fig.2B) for comparing the retention time of each isomer. An

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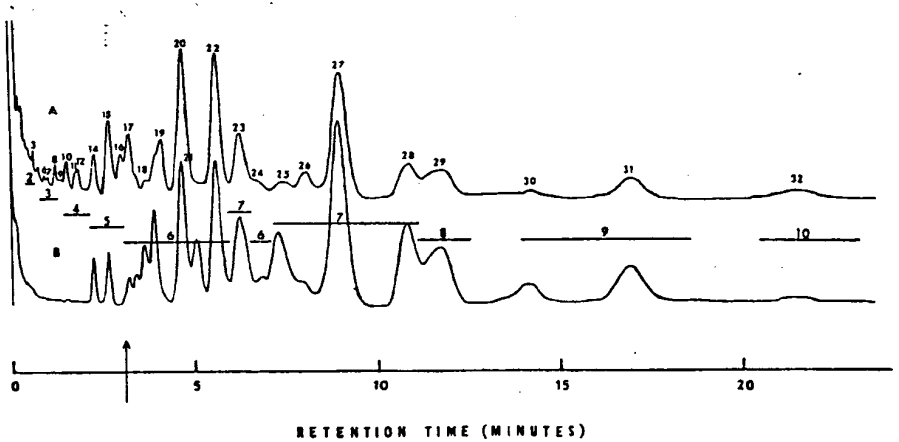

Figure 2. (A) Chromatogram of sea otter liver. (B) Chromatogram of Aroclor 1260. Arrow indicates retention time of p,p'-DDE. Peaks 3 and 6-12 identified from Aroclor 1232.

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arrow marks the retention time of p,p'-DDE relative to the PCB's.

Table II gives the amount of chlorinated biphenyl in ng and p.p.b. for each peak of Fig. 2A. As can be seen in Fig. 2A, there are no monochlorobiphenyls and only one dichlorobiphenyl isomer present in the sample. This may indicate that the lower chlorinated PCB's are less persistent in the environment. A previous study has shown that lower chlorinated components are metabolized in birds (5).

TABLE II

Quantitation of PCB components in sample.*
Peak area (ng)

Peak #	ng	p.p.b.	Peak #	ng	p.p.b.
3	.2686	41	20	1.423	210
6	.2036	30	21	.2285	35
7	.2598	39	22	2.013	300
8	.2212	33	23	.6775	100
9	.1116	17	24	.2964	45
10	.3099	47	25	.2738	41
11	.1537	23	26	.3679	56
12	.1537	23	27	1.419	220
14	.4358	67	28	.5632	85
15	.8995	140	29	.5195	79
16	.4463	68	30	.1821	27
17	.6397	97	31	.2642	39
18	.1964	30	32	.0822	12
19	.7990	120	Total	13.41	2024

*Sea Otter from Monterey, California.

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DISCUSSION

In quantitating levels in marine birds and fish, Risebrough (15) assumed that each PCB compound produced the same peak height with the electron-capture detector as the same amount by weight of p,p'-DDE. After summing the contributions of the individual peaks, the total was multiplied by a factor derived from measurements of standard solutions. Our results (Fig. 1), show that detector response is not the same for all isomers, but is dependent on the degree of chlorination. The principle of an electron-capture detector is based on the electron affinities of compounds. A polychlorinated compound can have 10^4 times the relative electron absorption coefficient as a monochloro compound and 10^6 times that of an unchlorinated compound (16).

Koeman et al. (5) semiquantitatively measured PCB residues by using one peak of a commercial mixture as a standard. Reynolds (17) used a similar method but based his quantitation on an average of two or more peaks. He reported his results as Aroclor 1254 or 1260 depending on the overall pattern of the chromatogram profile. Both of these methods assume that the peak(s) used as a standard give the same detector response as the rest of the peaks in the sample.

Several workers have compared the PCB components in field samples to Aroclor 1254 because the sample chromatograms were most similar to this mixture (8,18, 19). If Aroclor 1254 had been used as a standard for measuring levels in the sea otter liver, a value of 4.3 p.p.m. would have been obtained. This figure is over twice the 2.0 p.p.m. value that we calculated with the detector response curve. As stated before, there may be impurities in the Aroclor being used as a standard and any quantitation based on that one Aroclor would be erroneous. By using all six Aroclor standards, the errors due to impurities and unchlorinated biphenyls are minimized.

If the standard injection of p,p'-DDE shows that detector sensitivity has changed from when the response curve was calculated, a simple factor can be applied to the entire curve and all isomers quantitated as before.

We feel that this method should be used by all workers when reporting PCB levels found in field samples. A more accurate quantitation will be achieved when individual PCB components are synthesized for use as standards.

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