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Polychlorinated Biphenyls in Human Adipose Tissue

by FRANCIS J. BIROS, ANNITA C. WALKER and ANGELA MEDBERY

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Recent reports have demonstrated that chlorinated hydrocarbons are among the most abundant synthetic pollutants in the environment (1). Concern over the pollution hazard presented by this class of organic compounds has been deepened by the detection of polychlorinated biphenyls (PCB) in animal and environmental media. Since the first reported presence of PCB residues in wildlife tissues by Jensen (2), attention has been focused on elucidating the extent of distribution of these materials in wildlife species, especially in fish and birds (3). The occurrence of PCB in any sample, in addition to being toxicologically significant in living systems (4), presents a difficulty in the analysis of certain chlorinated pesticidal chemicals (5). Adequate discussions of the properties and use of PCB compounds, their distribution in the environment, and their interference with pesticide residue analysis have been presented previously (1-6).

The analytical evidence for the presence of PCB in environmental samples has been based on mass spectrometric analysis (7), retention times in gas chromatographic analysis (1, 5, 6, 8), chlorine content by microcoulometric analysis (6), relative inertness to nitration and dehydrochlorination procedures which are used to confirm common chlorinated pesticides (9), and indirect evidence from the relative distribution of these compounds in the environment (1). The most conclusive spectroscopic data have been the mass spectra of these compounds obtained with a combined gas chromatograph-mass spectrometer from extracts of fish, seabirds, conifer needles, and human depot fat (7).

The present work was initiated because of the lack of detailed information about the mass spectral properties of PCB. The relative chemical and thermal inertness of these materials renders them particularly suitable for analysis by gas chromatography-mass spectrometry. Furthermore, recent success utilizing combined gas chromatography-mass spectrometry for pesticide residue analysis (10) and the availability of human tissue suspected to contain PCB prompted us to examine the behavior of PCB during combined gas chromatography-mass spectrometry and, if possible, to confirm the presence of these compounds in human tissue samples. It must be emphasized that the origin of the PCB materials in the human adipose tissue described in this work is uncertain at this time. The data are presented as a preliminary description of the gas chromatographic-mass spectrometric behavior of PCB and of the analytical methodology applicable to the analysis of these compounds in human tissue by gas chromatography-mass spectrometry.

Experimental

The combined gas chromatograph-mass spectrometer system used in this study has been described previously (10). The gas chromatographic

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column was a stainless steel capillary, 100 feet x 0.020 inches i.d. and coated with OV-1 silicone oil, obtained from the Perkin-Elmer Corp., Norwalk, Conn. Programmed temperature analyses were made both for the six Aroclor 1200 series standard materials[1] and the tissue extracts. Figures 1 and 2 depict chromatograms obtained for Aroclors 1254 and 1260 of these series and detail the temperature programming conditions used. The molecular separator and gas inlet temperatures were maintained at 210°C and 215°C, respectively. All mass spectra were recorded at 80 ev electron energy with 2300 v accelerating voltage; the filament emission current was 100 μa. Chromatograms were recorded from the total ion current monitor. Helium carrier gas flow rate was 4 ml/min. The injector temperature was 175 °C. Mass spectra were scanned magnetically over the range m/e 5 to m/e 500 in 6 seconds.

Two samples of human adipose tissue were examined by gas chromatography-mass spectrometry. Previous analysis by microcoulometric and electron-capture gas chromatography had indicated the presence of PCB residues. The final analytical scheme used for preparation of the samples was the modified Mills procedure (11). The samples, designated A and B for discussion purposes, were estimated to contain 200 ppm and 600 ppm total PCB, respectively, as determined by

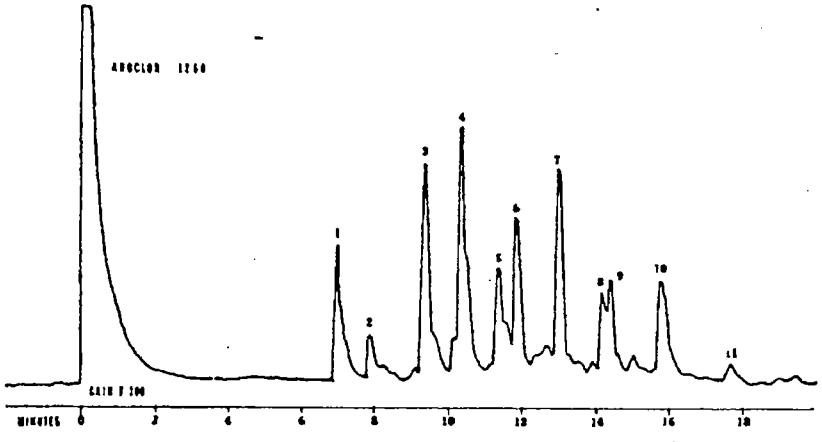


Figure 1. Total ion current monitor chromatogram of standard Aroclor 1254 mixture of polychlorinated biphenyls. Programmed temperature analysis: 2 min at 185°C, to 210°C at 5°C/min, isothermal at 210°C. (See text for remaining instrumental parameters and partial peak identification.)

[1]The Aroclor 1200 series PCB standard materials were provided by the Monsanto Chemical Co., St. Louis, Mo. as a gift to the Pesticides Repository of this laboratory.

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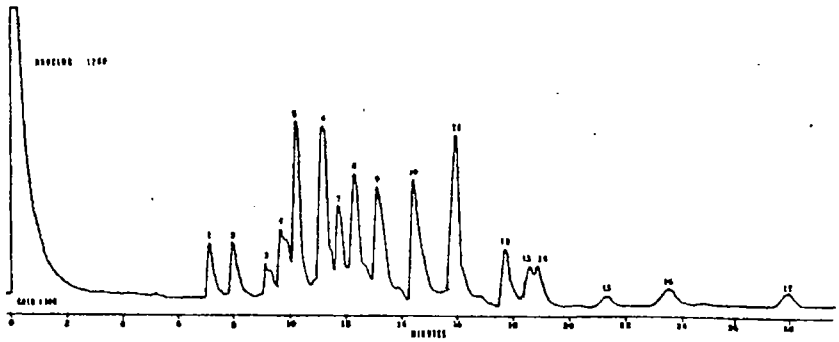


Figure 2. Total ion current monitor chromatogram of standard Aroclor 1260 mixture of polychlorinated biphenyls. Programmed temperature analysis: 2 min at 200°C, to 230°C at 5°C/min, isothermal at 230°C. (See text for remaining instrumental parameters and partial peak identification.)

electron-capture gas chromatography. Approximately 2 g portions of tissue were extracted with petroleum ether, partitioned with acetonitrile, and subjected to fractionation and cleanup by Florisil column chromatography. All of the PCB compounds were eluted in the first fraction (6% ethyl ether in petroleum ether) and this volume of eluate (200 ml) was carefully concentrated to a final volume of 10 µl. A 2 µl aliquot was then analyzed by gas chromatography-mass spectrometry. The results of the analyses for samples A and B are illustrated in Figures 3 and 4, respectively.

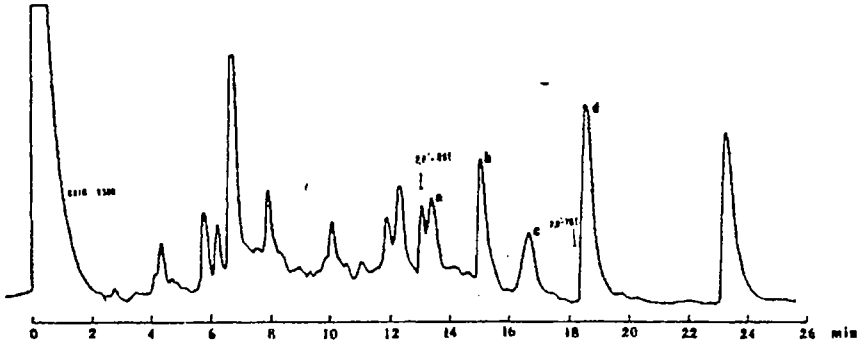


Figure 3. Total ion current monitor chromatogram of human adipose tissue extract A. Programmed temperature analysis: 5 min at 180°C, to 210°C at 5°C/min, isothermal at 210°C.

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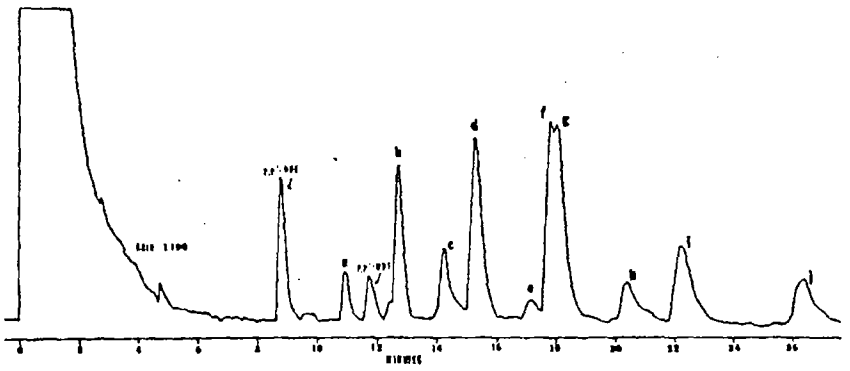


Figure 4. Total ion current monitor chromatogram of human adipose tissue extract B. Programmed temperature analysis: 2 min at 190°C, to 230°C at 5°C/min, isothermal at 230°C.

Results and Discussion

Separation efficiency for the individual PCB compounds was increased when the Aroclor standards were analyzed by capillary column gas chromatography-mass spectrometry. Resolution was better than that reported in the literature for standard analytical packed columns. No evidence of thermal degradation of any of the PCB compounds was found. It is apparent from the non-symmetrical nature of several of the peaks in both standard Aroclor chromatograms that all PCB isomers and/or chlorine homologs were not completely separated and resolved. However, satisfactory mass spectra were obtained for all of the major numbered peaks in Figures 1 and 2. All seriously overlapping peaks appeared to be isomers of the same PCB. No attempt was made to elucidate the structure of individual PCB components other than to establish the molecular weight and number of chlorine atoms. All components of the Aroclors gave molecular ion groups of high intensity, as would be expected from highly chlorinated biphenyl structures. In addition, the characteristic isotopic distribution pattern (12) corresponding to the number of chlorine atoms in the parent ion and chlorine-containing fragment ions, was observed. Two noteworthy features of the spectra were the relatively intense fragment ions produced by consecutive loss of chlorine atoms from the parent ion and the presence of intense doubly charged fragments within the mass spectra of most of the PCB compounds. Figures 5 and 6 illustrate mass spectra obtained for a trichlorobiphenyl isomer in Aroclor 1232 and a heptachlorobiphenyl isomer in Aroclor 1260, respectively. Thus, peaks 1 and 2 in the chromatogram of Aroclor 1254 were shown to be tetrachlorobiphenyls; peaks 3 through 6, pentachlorobiphenyls; and peaks 7 through 11, hexachlorobiphenyls. Similarly, peaks 1 and 2 in Aroclor 1260 were identified as pentachlorobiphenyls, peaks 3 through 6 as hexachlorobiphenyls, peaks 7 and 9 through 12 as heptachlorobiphenyls, peaks 13 and 14 as octachlorobiphenyls, peaks 15 and 16 as nonachlorobiphenyls, and peak 17 as decachlorobiphenyl.

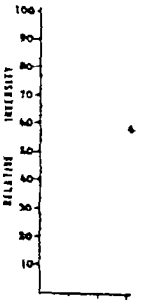


Figure 5.
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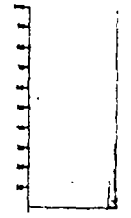


Figure 6.
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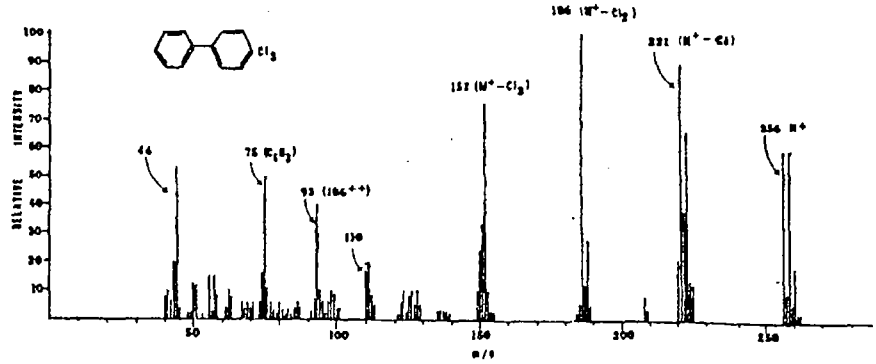


Figure 5. Mass spectrum of a trichlorobiphenyl contained in standard Aroclor 1232.

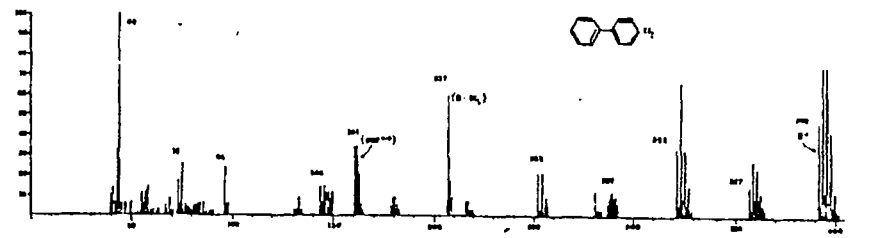


Figure 6. Mass spectrum of a heptachlorobiphenyl found in standard Aroclor 1260.

Examination of the mass spectra of the components of adipose tissue sample extract A (Fig. 3) revealed that peaks a, b, c, and d were polychlorinated biphenyls whose mass spectra were indistinguishable from those obtained respectively for peaks 6, 7, 9, and 10 of Aroclor 1254 (Fig. 1). The mass spectrum of peak d (an isomer of hexachlorobiphenyl, mol. wt. 358) is reproduced in Figure 7. 2,2-Bis(p-chlorophenyl)-1,1-dichloroethylene (p,p'-DDE) was also confirmed in this adipose tissue sample. 1,1-Bis(p-chlorophenyl)-2,2,2-trichloroethane (p,p'-DDT), a chlorinated pesticide frequently found in human adipose tissue, was not detected. Under the conditions of the analysis, however, p,p'-DDT overlaps considerably with PCB peak d. Additionally, p,p'-DDT is easily dechlorinated thermally to 2,2-bis (p-chlorophenyl)-1,1-dichloroethane (p,p'-DDD) on stainless steel gas chromatographic columns, particularly at the low concentration levels found in this sample. The remaining peaks observed in the total ion current monitor trace of adipose tissue extract A were non-chlorinated materials of lipid composition assumed to be natural constituents of adipose tissue carried through the analytical scheme.

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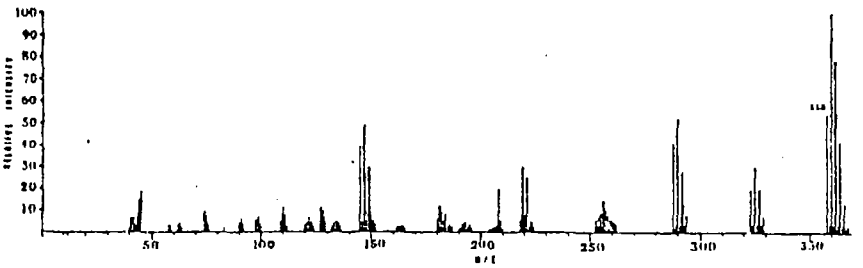


Figure 7. Mass spectrum of a hexachlorobiphenyl contained in human adipose tissue sample A (Peak d, Fig. 3).

Injection of adipose tissue extract B into the gas chromatograph-mass spectrometer resulted in the total ion current monitor trace illustrated in Fig. 4. Examination of the mass spectra obtained for peaks a through j showed that they were identical to those obtained for peaks 7 and 9 through 17 in the Aroclor 1260 standard materials (Fig. 2). In addition, p,p'-DDE and p,p'-DDT, which were present at higher levels than those encountered in sample A, were also confirmed in this adipose tissue sample.

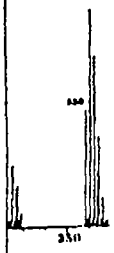
The results detailed here demonstrate the ease with which PCB compounds can be identified in human adipose tissue by combined gas chromatography-mass spectrometry using existing analytical methodology (12). Highly diagnostic mass spectra were obtained for each PCB component of Aroclor 1254 and 1260 standard materials and PCB isolated from two human adipose tissue samples. The presence of p,p'-DDE and p,p'-DDT residues within the same samples were also confirmed by mass spectrometry. High resolution capillary gas chromatographic columns may be used to separate pesticide residues such as p,p'-DDE and p,p'-DDT from PCB compounds. However, in those instances where insufficient separation of residue components occurs, mass spectrometric techniques are available for the identification of unresolved components in gas chromatographic effluents (13).

Summary

Among the chlorinated hydrocarbon synthetic pollutants in the environment, polychlorinated biphenyls represent a class of compounds possessing mammalian toxicity and recently found to be present as residues in the tissues of various wildlife species, although they are not in use as biocidal compounds. Two human adipose tissue samples examined by combined gas chromatography-mass spectrometry were shown to contain substantial quantities of polychlorinated biphenyls ranging from pentachlorobiphenyl to decachlorobiphenyl and including at least fourteen isomers and chlorine homologs. Although the origin of the PCB compounds found in the adipose tissue is ununknown, the data are presented as a description of the gas chromatographic-mass spectrophotometric behavior of PCB and of the analytical methodology applicable to the analysis of these compounds in human tissues by gas chromatography-mass spectrometry.

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