

Occurrence of Polychlorinated Biphenyls in Humans*

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In 1967 the Human Monitoring Survey (1) was established by the Pesticides Program of the U.S. Department of Health, Education and Welfare (now, Division of Pesticide Community Studies, E.P.A.) to determine on a national scale the levels and trends of certain more commonly-used pesticide chemicals in the general population. Initially, this program was limited to the identification and measurement of chlorinated hydrocarbon residues in human adipose tissue and blood serum. The Michigan Department of Public Health has been one of several state health laboratories participating in the analysis of samples, collected by the State Services Branch of the Division of Pesticide Community Studies, from hospitals located in approximately 30 states. For the purpose of obtaining poolable data, the state laboratories use methodologies recommended by the Perrine Primate Laboratory, E.P.A. (Florida), which is responsible for methodology development, the operation of the quality control program, and serves as a repository of reference standards.

Since 1966 our laboratory has been conducting routine analysis of human adipose samples for organochlorine pesticide residues by gas chromatography, utilizing electron capture detection. In many instances, we observed numerous un-

identified peaks recurring in a repeated pattern on chromatograms, some eluting much later than p,p' DDT. Our attention became focused on polychlorinated biphenyls (PCBs), after the Perrine Primate Laboratory alerted laboratories involved with the human monitoring program and community studies on pesticides of the possible interference of pesticide residue analysis by these compounds. This was prompted by early reports of research workers in Europe and the United States finding PCB residues in animal and environmental media (2, 3, 4). Early confirmation of some of these compounds had been obtained with a combined gas chromatograph-mass spectrometer from extracts of fish, sea birds, conifer needles, and some samples of human depot fat (5).

In March, 1969, one of Michigan's state laboratories found 19 ppm DDT in canned coho salmon during routine residue analysis. Our residue analysis on a similar sample seemed to corroborate this finding. However, considerable interference by "artifact" peaks was evident on the chromatogram, causing difficulty in both qualitative and quantitative analysis (2, 6) of pesticide residues. To a lesser extent, similar peaks had been observed with some human adipose samples. These unknown peaks were ascertained later as polychlorinated biphenyls when compared with several Monsanto Aroclors by electron capture gas chromatography and microcoulometry.

Prior to receipt of Monsanto Aroclors, we found a readily available source of a PCB indicator, e.g., residue analysis report forms. One of us had known by previous experience that carbonless copy paper has on one side a coating of microcapsules containing an Aroclor solution of a colorless dye (16). An extract of this paper pro-

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duced a chromatogram which matched a considerable portion of the interference observed with residues in coho salmon and human adipose tissue.

During the year following the coho salmon incident, our laboratory found substantial levels of polychlorinated biphenyls in several samples of human adipose tissue collected through the Human Monitoring Survey program. Three of these examples are given here to illustrate our findings.

Materials and Equipment

Materials and glassware used for extraction of samples and preparation cleanup of extracts are described fully elsewhere (7, 8).

A Micro-Tek Model 220 gas chromatograph (Tracor, Inc.) was used. It was equipped with two parallel-plate, tritium-source electron capture detectors, Vycor glass inserts installed at inlet ports to accommodate off-column injection, and U-shaped Pyrex glass columns (6 ft. \times $\frac{1}{4}$ in. o.d.). Column packings (9) supplied by the Perrine Primate Laboratory consist of 1.5%OV-17/1.95%QF-1 (Column 1) and 4%SE-30/6%QF-1 (Column 2) on 80/100 mesh Chromosorb W HP. Operating temperatures of columns, detectors, and inlet were 200°C, 210°C, and 225°C, respectively. Carrier gas was nitrogen at flow rates of 65 ml/min through column 1 and 80 ml/min through column 2.

Reference standards of 1200 series Aroclors and organochlorine pesticides were obtained from the Pesticides Repository, Perrine Primate Laboratory, E.P.A., Perrine, Florida. Aroclors, products of Monsanto Company, are designated by numbers, such as 1260 which represents a mixture of chlorinated biphenyls having an average chlorine content of 60%.

For thin-layer chromatography, glass plates (Analtech, Inc.) precoated with aluminum oxide G, 250 microns thick, impregnated with 3.3% silver nitrate were used.

Method

The analytical scheme used for the preparation of these samples was the modified Mills procedure (8). One to three grams of tissue were extracted with hexane, 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 reduced in a Kuderna-Danish concentrator to 10 ml. A 5- μ l aliquot was injected into the gas chromatograph. The extracts were diluted as required to obtain peaks within the linear range of the detector.

For certain selected samples, the polychlorinated biphenyls were separated from the organochlorine pesticide residues by application of the method of Armour and Burke (10). The 6% Florisil eluate was chromatographed on the silicic acid-Celite column with 250 ml of petroleum ether, which eluted the PCBs. The organochlorine pesticides were recovered next after elution with 200 ml of acetonitrile-hexane-methylene chloride (1:19:80).

Results and Discussion

In Fig. 1, electron capture chromatograms of human adipose tissue extract M69305 (6% Florisil eluate) and a standard solution of Aroclor 1260 are presented to illustrate that some of the "artifact" peaks in this tissue sample match those in the PCB standard. On the 4%SE-30/6%QF-1

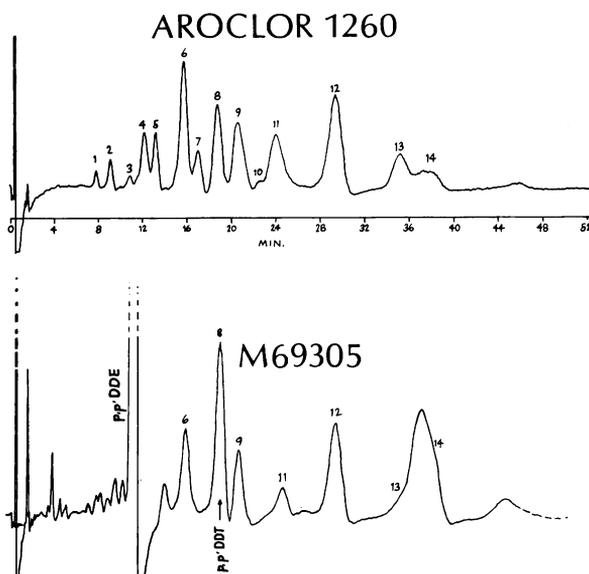


FIGURE 1. GLC-EC chromatogram of Aroclor 1260 (1.5 ng) and human adipose sample M69305. GLC conditions: 6' \times $\frac{1}{4}$ " 4% SE-30/6% QF-1 column, oven temp. 200°C, and nitrogen flow 75 ml/min.

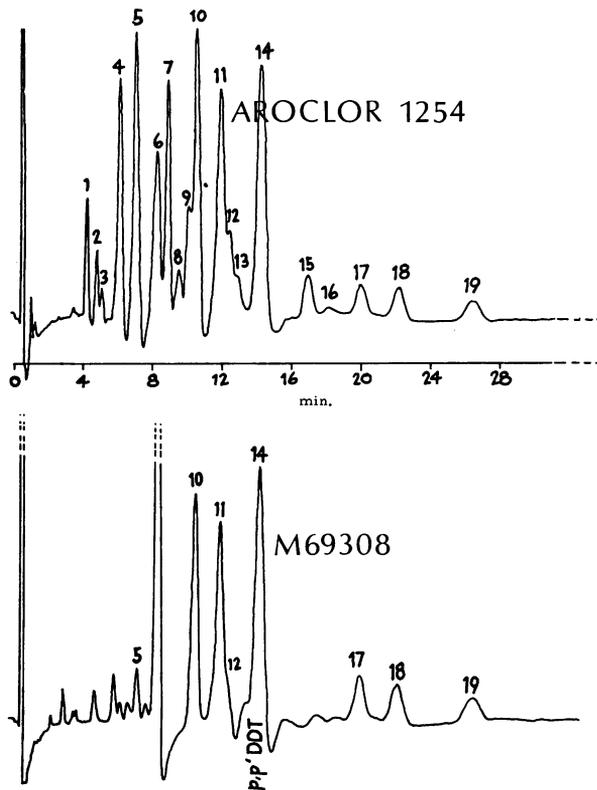


FIGURE 2. GLC-EC chromatogram of Aroclor 1254 (5.1 ng) and human adipose sample M69308. Peak 1' is 2,2',5,5'-tetrachlorobiphenyl, and peak 5 is 2,2',-4,5,5'-pentachlorobiphenyl. GLC conditions: 6' x 1/4'' 4% SE-30/6% QF-1 column, oven temp. 200°C, and nitrogen flow 75 ml/min.

column, p,p'DDT is completely masked by Peak 8 of Aroclor 1260; however, no interference is encountered with the quantitation of p,p'DDE.

On the basis of a major peak following p,p'DDT, the level of PCBs in this sample was estimated to be seven parts per million as Aroclor 1260. For estimating PCB levels by gas chromatography, we use Peak 12, preferably on 1.5%OV-17/1.95%QF-1 column, as a reference in Aroclor 1260 which has the following retention time relative to aldrin ($R_t = 1.00$):

Column	Aroclor		
	Peak	p,p'DDE	p,p'DDT
OV-17/QF-1	6.4	2.23	4.18
SE-30/QF-1	4.83	1.82	3.12

In this sample, PCBs ranging from hexachlo-

robiphenyls to decachlorobiphenyl were confirmed by combined gas chromatography-mass spectrometry (11).

Fig. 2 shows that tissue extract M69308 contains some components which are present in Aroclor 1254. Approximately 20 ppm PCBs as Aroclor 1254 was determined by gas chromatography. Biros (11) confirmed the presence of pentachlorobiphenyls and hexachlorobiphenyls in addition to p,p'DDE. The largest unlabeled peak following Peak 5 in sample M69308 is p,p'DDE. The level of p,p'DDT in this sample was too low for confirmation by mass spectrometry, and its peak is masked by Peak 14 of Aroclor 1254 on the SE-30/QF-1 column used in this illustration.

In the spring of 1970, we found approximately 100 ppm PCBs in a sample of subcutaneous adipose tissue (M70227) which was obtained from a hospital in southeastern Michigan. The subject was a white male, age 77, whose demise followed a pulmonary thrombo embolism.

An electron capture chromatogram of the 6% Florisil column eluate of this sample is illustrated in Fig. 3 and is compared with a chromatogram of Aroclor 1260. This extract contains late eluting isomers that are also components of Aroclor

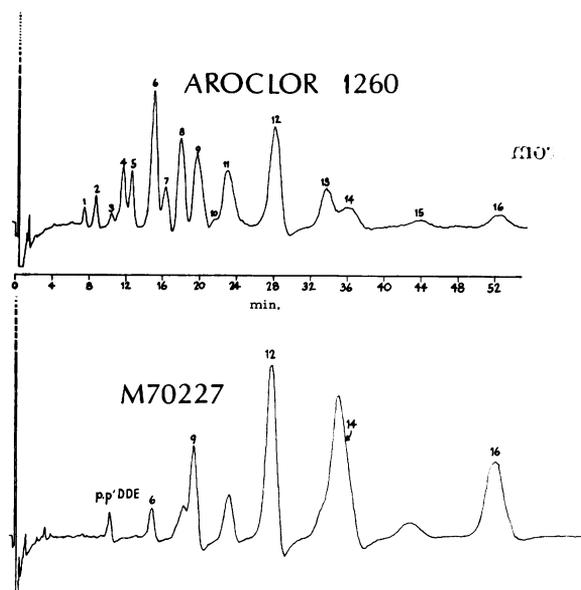


FIGURE 3. GLC-EC chromatogram of Aroclor 1260 (2.1 ng) and human adipose sample M70227 (30.5 µg; 6% Florisil fraction). GLC conditions: 6' x 1/4'' 6% SE-30/6% QF-1 column, oven temp. 200°C, and nitrogen flow 80 ml/min.

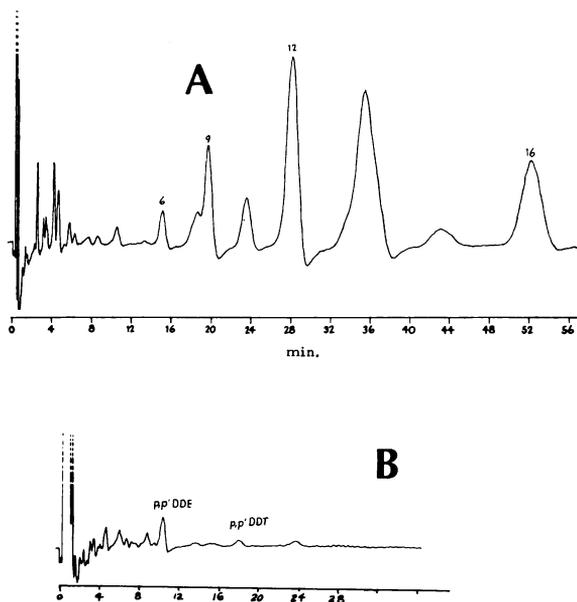


FIGURE 4. GLC-EC chromatogram showing separation of PCB (A) and organochlorine pesticides (B) by method of Armour and Burke. GLC conditions same as Fig. 3. Weight of sample injected 30.5 μ g.

1268, which makes matching to any particular Aroclor difficult. Fig. 4 shows the separation of PCBs from organochlorine pesticides by the method of Armour and Burke (10), although some of the p,p'DDE eluted in Fraction A in this case. On the basis of a chromatogram of the second eluate from the silicic acid-Celite column resulting from a subsequent effort, residues of 0.37 ppm p,p'DDT and 0.81 ppm p,p'DDE were found in the fat of this sample. Using the combined gas chromatograph-mass spectrometer, Biros (12) confirmed the presence of p,p'DDE in M70227 as well as ten polychlorinated biphenyls ranging, from hexachloro- to nonachloro-compounds. These compounds are found in Aroclors 1260, 1262, and 1268.

Further examination of other tissues of the same autopsy case revealed a good correlation of PCB levels when compared on the basis of extractable lipids as demonstrated in Table 1. These data are good estimates calculated from the height of a peak corresponding to Peak 12 of Aroclor 1260 on the OV-17/QF-1 column. PCB peaks in M70227 appear to be a complex mixture of several Aroclors not usually observed in human adipose tissue.

Efforts to determine the source of PCBs in sample M70227 have not been successful. Indications are that this individual was exposed to PCBs during his lifetime. As PCBs enter a biological system, the less chlorinated isomers are either metabolized or excreted, which changes the composition of original Aroclors, so that chromatograms of these mixtures from biological samples do not match exactly those of reference standards. This has also been observed with PCBs in M70227. In fact, more than 4,000 human adipose samples examined in our laboratory have produced chromatograms that do not exactly match those of standard Aroclor solutions. We have found the distribution of PCBs in samples, such as M70227, to be uniform throughout the sample as received, indicating that these samples have not been contaminated by PCBs during handling. As for all samples, M70227 was collected from an autopsy examination through the cooperation of pathologists, according to directions specified by the State Services Branch of the Division of Pesticide Community Studies, E.P.A.

As many investigators of PCBs have found, one of the principal sources of polychlorinated biphenyls in the biological system is the food chain (5, 13, 14). Coho salmon, for example, had been a source of PCBs in food until it exceeded the action level of 5 ppm.

Another possible avenue of human exposure to polychlorinated biphenyls may be indicated by their presence in some house dust (vacuum sweepings). Our examination of house dust from residences of occupationally-exposed population of southwestern Michigan revealed that several samples contained up to 180 ppm PCBs, mainly Aroclor 1254.

Table 1. Levels of PCB's in Various Tissues Related to Sample M70227*

Tissue	% Lipid ext'd	PCB level, ppm	
		Wet weight basis	Fat basis
S. Adipose*	41.1	75	180
S. Adipose	63.9	100	155
S. Adipose	32.8	50	150
P. Adipose	44.1	50	115
Liver	0.08	2	250
Kidney	0.06	0.75	125

Table 2. Distribution of PCB Levels in Adipose Tissue of the General Population—Analysis by Gas Chromatography

Level, ppm	Samples	Percent of total
Negative	4	2.0
Trace to <1.0	109	55.6
1.0-2.0	72	36.7
>2.0	11	5.6

As mentioned previously, levels of polychlorinated biphenyls in human tissue extracts illustrated in Figs. 1, 2, and 3 were determined by electron capture gas chromatography using a major peak after p,p'DDT (Peak 12) as a reference peak for Aroclor 1260. Since April, 1971, our laboratory has run semiquantitative analysis of polychlorinated biphenyls in samples of human adipose tissue by both electron capture gas chromatography and thin-layer chromatography. The latter based on total chlorine is an adaptation of a procedure reported by Mulhern et al., (7, 15). After gas chromatographic analysis is completed, the remaining extract is evaporated, and pesticide residues are dehydrochlorinated with ethanolic potassium hydroxide and oxidized with chromic oxide. The concentrated extract of PCBs remaining is spotted on a thin-layer plate, and the developed spots after exposure to ultraviolet light are compared with several spots of standard Aroclor 1260 at different concentrations. The limit of detection is 250 ng of Aroclor 1260. Results in Tables 2 and 3 indicate that both methods provide similar information, but thin-layer chromatography is preferred. On the basis of samples received and analyzed by our laboratory, these data show the distribution of PCB levels in adipose tissue of the general population.

Table 3. Distribution of PCB Levels in Adipose Tissue of the General Population—Analysis by Thin-Layer Chromatography

Level, ppm	Samples	Percent of total
Negative	20	10.3
Trace to <1.0	87	44.8
1.0-2.0	70	36.1
>2.0	17	8.8

Summary

According to samples of human adipose tissue received by our laboratory, 41-45% of the general population have levels of 1.0 ppm or more (wet weight) polychlorinated biphenyls with isomers from Aroclors 1254, 1260, 1262, and 1268. The presence of PCBs ranging from pentachlorobiphenyls to decachlorobiphenyl has been confirmed in selected samples, M69305, M69308, and M70227, by the use of combined gas chromatography-mass spectrometry.

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