Bull. Environm. Contam. Toxicol. 28, 97–104 (1982)

Polychlorinated Biphenyl and Other Chlorinated Hydrocarbon Residues in Adipose Tissue of Canadians

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The presence of organochlorine (OC) pesticides in adipose tissue of Canadians have earlier been reported by RITCEY et al. (1973) in the first nation-wide survey. At approximately the same time different chlorinated environmental pollutants were reported in human adipose tissue, such as hexachlorobenzene (HCB) (BRADY 1972), oxychlordane (BIROS 1973) and polychlorinated biphenyls (PCBs) (PESENDORFER 1973). This led to a national survey by MES et al. (1977) on chlorinated hydrocarbon residues in adipose tissue of Canadians, which incorporated these newly reported residues.

The data reported in this paper are part of a continuing monitoring program of chlorinated hydrocarbons in adipose tissue of Canadians in order to determine a possible trend in both the disappearance of restricted OC pesticides, such as p,p,-DDT, as well as the appearance of new environmental contaminants, such as hexachloro-1,3-butadiene (HCBD) (FISHBEIN 1979).

MATERIALS AND METHODS

Sampling

Human adipose tissues were obtained during autopsies on accident victims. The fat samples were collected in glass jars, previously washed, heated at 350°C and rinsed with residue free acetone and hexane and supplied with teflon lined screw caps. Samples were immediately frozen and kept frozen until analysed. A total of 99 samples was collected from the following provinces: Prince Edward Island (4), Nova Scotia (6), Quebec (39), Ontario (6), Manitoba (8), Saskatchewan (7), Alberta (8), British Columbia (19) and Yukon (2).

Analytical Methods

All solvents were glass distilled and free of interfering residues. Standards were gifts from the Environmental Protection Agency (U.S.A.) and 98-100% pure. Florisil, silicic acid, Celite, anhydrous Na_2SO_4 and glass-wool were treated as previously reported (MES and DAVIES 1978).

Extraction and Cleanup. Two gram samples were extracted with 100 ml of benzene:acetone (1:19 v/v) for 3 min. with a Silverson Homogenizer (Canlab Ltd). Extracts were filtered through glass wool and evaporated on an all-glass rotatory evaporator (< 30°C). Traces of benzene were removed by repeated evaporation with a few milliliters of hexane. The solvent free oily residue was redissolved in hexane and filtered through anhydrous Na_2SO_4 and the hexane evaporated to dryness. The residue was

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then transferred to a 15 mL graduated centrifuge tube, using acetone and the volume adjusted to 10 mL. A 0.5 ml aliquot was evaporated in a preweighed aluminum dish to determine benzene:acetone soluble lipids. Fat was removed from the remaining extract by low temperature precipitation (McLEOD and WALES 1972).

Separation. The fat free extract in ~ 1 mL hexane was chromatographed on a combined Florisil-silicic acid column as previously described (MES and DAVIES 1978) and the following fractions collected:

Fraction	Eluting solvent	Eluted compounds
I	35 mL hexane	HCBD, penta chlorobenzene (PCBz), HCB, p,p'-DDE, PCB in Arochlor 1260. Some PCBs in Aroclor 1242, Mirex.
II	40 mL of 20% CH_2Cl_2 in hexane	some PCBs in Aroclor 1242, α, β and γHCH, oxy-chlordane, trans-nonachlor, p,p'-DDE, o,p'-DDT, p,p'-DDT
III	40 mL of 60% CH_2Cl_2 in hexane	βHCH, heptachlor epoxide, dieldrin.

The fractions were concentrated to ~ 1 mL on a rotatory evaporator as above and transferred to 15 mL stoppered graduated centrifuge tubes, using hexane. The final volume was adjusted to 4 mL in most cases.

Identification and quantification. The fractions were chromatographed on a Varian 2100 series gas chromatograph with electron capture detector (Tritium foil).

Identification of individual residues was carried out on a 0.6 x 183 cm glass column, packed with 6% OV-210 + 4% SE-30 on chromosorb W (AW) 100/120 and a flow rate of 40-50 mL/min. Injection, column, and detector temperatures for the PCB determinations were 212, 199 and 250°C respectively, while for OC pesticides these temperatures were held at 220, 195 and 258°C. The β and γ HCH isomers were quantitated from chromatograms obtained on a column similar to the one above and under exactly the same GC conditions, but with 5% OV-210 as stationary phase.

In order to separate Mirex from PCBs fraction I was also chromatographed on a 0.5 mm x 36 m SCOT column, coated with SP 2100, using a Varian 1400 gas chromatograph with electron capture detector (Scandium Tritide). Injection, column and detector temperatures were 249, 225, and 241°C respectively. A linear flow rate of nitrogen of 21 cm/sec was maintained.

Aliquots (5 µL) of standard solutions of Aroclor 1260 (50×10^{-5} µg/µL), Aroclor 1242 (100×10^{-5} µg/µL) and other halogenated hydrocarbons ($0.25 - 10 \times 10^{-5}$ µg/µL, depending on individual response) were injected before and after every two sample injections.

Individual compounds were quantitated by measuring peak heights. PCBs were estimated by summation of peak heights of peaks 8 and 10-15 in Aroclor 1260. In Aroclor 1242, however, different peaks were used to estimate PCBs in fractions I and II. Peaks C to J of Aroclor 1242 in fraction I and peaks E and G-J in fraction II were used. (Fig. 1)

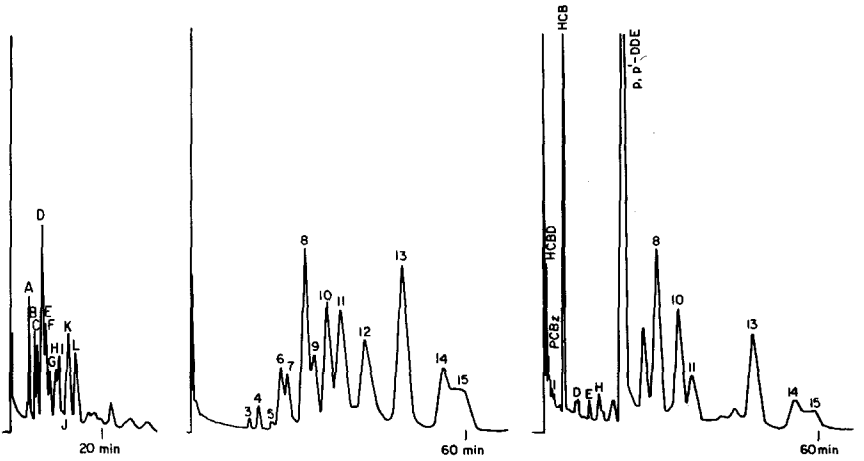

FIG. 1. Typical GC elution patterns of Aroclor 1242, 1260 and PCBs in human adipose tissue (from left to right respectively) on the mixed stationary phase GC column.

Confirmation of compounds in fraction I. The presence of HCB, HCBD, PCBz, p,p'-DDE and PCBs in fraction I were confirmed by further gas liquid chromatographic (GLC) in 5% OV-210.

Thin layer chromatography (TLC) separation of fraction I was carried out as described by MES et al., (1977), using 60% CH_2Cl_2 in hexane to elute the compounds from the adsorbent.

Appropriate fractions were re-chromatographed on a GLC column of mixed stationary phase as above and single ion monitored by mass-spectrometry (MS) for HCB (m/z = 284), HCBD (m/z = 260), PCBz (m/z = 250) and Mirex (m/z = 546), using a Varian Mat 311A coupled to a Varian 1400 series gas chromatograph by a two-stage Watson-Bieman separator at a resolution of 1000. A full scan probe analysis was performed on the same instrument for pentachlorobiphenyl (m/z cluster 224, 326, 328, 330), hexachlorobiphenyl (m/z cluster 358, 360, 362, 364) and heptachlorobiphenyl (m/z cluster 392, 394, 396, 398).

Confirmation of compounds in fraction II and III. Pesticides in fractions II and III were confirmed on 5% OV-210 as above.

TLC confirmation was carried out as above, except β and γ HCH, heptachlorepoxide, dieldrin and o,p'-DDT, which were re-chromatographed on 5% OV-210.

Single ion monitoring MS was carried out on appropriate fractions for β HCH (m/z = 290), γ HCH (m/z = 219), o,p'-DDT (m/z = 354 and 235), dieldrin (m/z = 380 and 345) and heptachlorepoxide (m/z = 390 and 353) as above. A full scan probe MS analysis was also carried out for oxychlordane [m/z clusters (420, 422), (424, 426), (385, 387, 389)], transnonachlor (m/z cluster 405, 407, 409), p,p'-DDE [m/z clusters (316, 318, 320), (246, 248), 210] and p,p'-DDT [m/z clusters (352, 354, 356), (317, 319), (235, 237)].

Controls. Samples were fortified with all compounds analysed for, except α HCH, Aroclor 1242, o,p'-DDT and Mirex. Fortification levels were 1.25 ppm for Aroclor 1260 and p,p'-DDE, 0.313 ppm for p,p'-DDT, 0.015 ppm for γ HCH and 0.05 ppm for all others. These fortifications were carried out in different combinations of pesticides in order to avoid overlapping of gas chromatographic retention times. At regular intervals during the survey solvent blanks were run through the entire analytical procedure.

RESULTS AND DISCUSSION

The results in Table 1 show the occurrence and very high incidence of environmental chemicals in adipose tissue of Canadians, as collected in 1976. All residues in Table 1 were confirmed by gas chromatography

TABLE 1

Chlorinated hydrocarbon residues in lipid extracts of adipose tissues of Canadians

Compound	μg/g wet weight		% of samples Containing
	Average[a] ± S.D.[b]	Range	residues
PCB, as Aroclor 1260	0.944 ± 0.902	0.040 - 6.801	100
PCB, as Aroclor 1242	0.307 ± 0.272	0.054 - 2.300	100
HCBD	0.004 ± 0.000	0.001 - 0.008	93
PCBz	0.002 ± 0.000	0.001 - 0.020	93
HCB	0.095 ± 0.103	0.010 - 0.667	100
α HCH	0.004 ± 0.000	0.001 - 0.043	97
β HCH	0.151 ± 0.459	0.016 - 4.413	100
γ HCH	0.003 ± 0.000	0.001 - 0.030	90
Oxychlordane	0.055 ± 0.026	0.005 - 0.186	100
t-Nonachlor	0.056 ± 0.032	0.015 - 0.244	100
Heptachlor epoxide	0.037 ± 0.045	0.004 - 0.404	100
Dieldrin	0.049 ± 0.030	0.003 - 0.211	100
p,p'-DDE	1.721 ± 1.390	0.034 - 7.819	100
o,p'-DDT	0.032 ± 0.058	0.005 - 0.578	96
p,p'-DDT	0.311 ± 0.493	0.016 - 3.998	100

[a] Average of 99 samples [b] Standard deviation.

(GC), TLC and MS in individual or pooled samples. PCBs in fractions I and II were confirmed by the presence of penta-, hexa-, and heptachlorobiphenyl. HCBD and PCBz residue determinations cannot be considered quantitative with eratic recoveries of 0-56 and 3-84 % respectively, causing the average recovery to be < 30%. Recoveries of other chlorinated hydrocarbon residues ranged from 70-100%. Figure 1 shows a typical GC elution pattern of HCBD, PCBz, HCB, PCBs and p,p'-DDE in fraction I of human adipose tissue extract.

Table 2 compares the present data with those obtained by RITCEY et al. (1973) in 1969 and MES et al. (1977) in 1972. In general it can be observed, that the average levels of PCBs, γ HCH and oxychlordane

TABLE 2

Trends in chlorinated hydrocarbon residues in adipose tissues of Canadians

Compound	µg/g wet weight		
	RITCEY et al.(1973)	MES et al.(1977)	THIS SURVEY
PCB, as Aroclor 1260		0.907	0.944
HCB		0.062	0.095
α HCH		0.004	0.004
β HCH		0.054	0.151
γ HCH	0.015	0.007	0.003
Oxychlordane		0.055	0.055
t-Nonachlor		0.065	0.056
Heptachlor epoxide	0.040	0.043	0.037
Dieldrin	0.122	0.069	0.049
p,p'-DDE	3.430	2.095	1.721
o,p'-DDT		0.031	0.032
p,p'-DDT	1.017	0.439	0.311
Total DDTs	4.543	2.571	2.064

appear to have remained constant; the average levels of γ HCH, t-nonachlor, heptachlor epoxide, dieldrin and total DDT appear to have decreased, and the average levels of HCB and β HCH appear to have increased.

The increase in HCB residue level probably reflects more the improved recovery in this study compared to the 1972 survey (MES et al. 1977), than an actual increase in the HCB body burden. The decrease in p,p'-DDT level between 1972 and 1976 was less than between 1969 and 1972. This could possibly be attributed to the fact that PCBs were not determined in 1969 and if present at that time could have contributed to the DDT level.

The geographic distribution of environmental contaminants is shown in Table 3 (MES et al., 1977). The results indicate higher residue levels of Aroclor 1242, HCB, γ HCH, oxychlordane, t-nonachlor, heptachlor epoxide, dieldrin and p,p'-DDE in the central region than in the Eastern and Western regions and Quebec. This is in contrast with reported values in the 1972 survey, where the residue levels of the Central region were consistently lower than in other regions, except for heptachlor epoxide. Although the levels of these residues were also higher than those found in Ontario and the Yukon, the latter two regions only represented a limited number of samples, especially in the case of populated Ontario. Only HCB, oxychlordane and heptachlor epoxide showed a statistically significant difference ($p < 0.05$) between the regions (Yukon not included).

There appears to be little difference in residue levels of males and females (Table 4), except for HCB, which is 2 x higher in females than males and β HCH which is higher in males. The difference in HCB was statistically significant.

Table 5 shows the residues in artificially grouped ages. Although the results indicate an increase in residue levels with age, except for β HCH and p,p'-DDT, the effect was only statistically significant ($p < 0.05$) for

TABLE 3

Regional distribution of chlorinated hydrocarbon residues in adipose tissue of Canadians.

Compound	Average µg/g wet weight ± S.D.					
	Region					
	Eastern[a]	Quebec	Ontario	Central[b]	Western[c]	Yukon
PCB, as Aroclor 1260	0.803 ± 1.094	0.890 ± 0.409	1.791 ± 1.468	0.779 ± 0.660	0.947 ± 1.233	1.398 ± 0.819
PCB, as Aroclor 1242	0.219 ± 0.304	0.293 ± 0.156	0.253 ± 0.073	0.416 ± 0.553	0.306 ± 0.181	0.367 ± 0.256
HCBD	0.002 ± 0.000	0.004 ± 0.000	0.004 ± 0.000	0.003 ± 0.000	0.003 ± 0.000	0.004 ± 0.000
PCBz	0.001 ± 0.000	0.002 ± 0.000	0.003 ± 0.000	0.002 ± 0.000	0.003 ± 0.000	0.001 ± 0.000
HCB	0.041 ± 0.020	0.072 ± 0.033	0.082 ± 0.048	0.149 ± 0.189	0.119 ± 0.121	0.126 ± 0.046
α HCH	0.002 ± 0.000	0.003 ± 0.000	0.004 ± 0.000	0.004 ± 0.000	0.006 ± 0.000	0.005 ± 0.000
β HCH	0.078 ± 0.087	0.074 ± 0.037	0.179 ± 0.318	0.126 ± 0.254	0.308 ± 0.848	0.089 ± 0.033
γ HCH	0.002 ± 0.000	0.002 ± 0.000	0.001 ± 0.000	0.004 ± 0.000	0.003 ± 0.000	0.003 ± 0.000
Oxychlordane	0.043 ± 0.017	0.047 ± 0.014	0.054 ± 0.017	0.074 ± 0.046	0.059 ± 0.028	0.076 ± 0.022
t-Nonachlor	0.047 ± 0.022	0.051 ± 0.020	0.048 ± 0.022	0.071 ± 0.057	0.059 ± 0.030	0.081 ± 0.025
Heptachlor epoxide	0.014 ± 0.000	0.029 ± 0.010	0.052 ± 0.069	0.076 ± 0.097	0.030 ± 0.014	0.042 ± 0.026
Dieldrin	0.036 ± 0.017	0.053 ± 0.028	0.049 ± 0.026	0.056 ± 0.048	0.045 ± 0.022	0.036 ± 0.010
p,p'-DDE	0.965 ± 0.697	1.764 ± 1.248	1.531 ± 1.215	2.268 ± 1.704	1.663 ± 1.590	1.915 ± 1.167
o,p'-DDT	0.017 ± 0.010	0.028 ± 0.020	0.034 ± 0.041	0.031 ± 0.014	0.051 ± 0.113	0.032 ± 0.000
p,p'-DDT	0.118 ± 0.030	0.308 ± 0.328	0.225 ± 0.146	0.332 ± 0.323	0.404 ± 0.819	0.261 ± 0.064

[a] Prince Edward Island and Nova Scotia

[b] Manitoba and Saskatchewan

[c] Alberta and British Columbia.

TABLE 4

Chlorinated hydrocarbon residues in adipose tissue of Canadians as related to sex

Compound	Average µg/g wet weight ± S.D.	
	Male (N[a] = 53)	Female (N = 45)
PCB, as Aroclor 1260	0.887 ± 0.689	1.007 ± 1.114
PCB, as Aroclor 1242	0.321 ± 0.335	0.291 ± 0.178
HCBD	0.004 ± 0.000	0.003 ± 0.000
PCBz	0.002 ± 0.000	0.002 ± 0.000
HCB	0.066 ± 0.037	0.130 ± 0.141
α HCH	0.005 ± 0.000	0.004 ± 0.000
β HCH	0.183 ± 0.612	0.116 ± 0.166
γ HCH	0.003 ± 0.000	0.003 ± 0.000
Oxychlordane	0.053 ± 0.020	0.057 ± 0.033
t-Nonachlor	0.058 ± 0.033	0.054 ± 0.030
Heptachlor epoxide	0.035 ± 0.028	0.040 ± 0.059
Dieldrin	0.050 ± 0.024	0.047 ± 0.035
p,p'-DDE	1.726 ± 1.497	1.726 ± 1.284
o,p'-DDT	0.039 ± 0.078	0.025 ± 0.017
p,p'-DDT	0.355 ± 0.614	0.264 ± 0.300

[a] Number of samples

TABLE 5

Chlorinated hydrocarbon residues in adipose tissue of Canadians as related to age

Compound	Average µg/g wet weight ± S.D.		
	0-25 years (N = 33)	26-50 yrs (N = 41)	51 + yrs (N = 24)
PCB, as Aroclor 1260	0.697 ± 0.640	0.926 ± 0.567	1.233 ± 1.426
PCB, as Aroclor 1242	0.237 ± 0.215	0.319 ± 0.178	0.384 ± 0.428
HCBD	0.003 ± 0.000	0.004 ± 0.000	0.004 ± 0.000
PCBz	0.002 ± 0.000	0.002 ± 0.000	0.002 ± 0.000
HCB	0.051 ± 0.026	0.094 ± 0.079	0.160 ± 0.164
α HCH	0.005 ± 0.000	0.004 ± 0.000	0.004 ± 0.000
β HCH	0.067 ± 0.051	0.260 ± 0.698	0.082 ± 0.037
γ HCH	0.002 ± 0.000	0.003 ± 0.000	0.004 ± 0.000
Oxychlordane	0.045 ± 0.020	0.056 ± 0.024	0.067 ± 0.037
t-Nonachlor	0.049 ± 0.039	0.059 ± 0.024	0.063 ± 0.030
Heptachlor epoxide	0.021 ± 0.000	0.047 ± 0.066	0.042 ± 0.024
Dieldrin	0.033 ± 0.010	0.056 ± 0.028	0.059 ± 0.039
p,p'-DDE	1.052 ± 1.292	2.024 ± 1.318	2.139 ± 1.377
o,p'-DDT	0.036 ± 0.098	0.029 ± 0.017	0.034 ± 0.024
p,p'-DDT	0.195 ± 0.339	0.412 ± 0.661	0.301 ± 0.276

HCB, oxychlordane, heptachlorepoxide and p,p'-DDE and for dieldrin in the 0-25 year age group, which was significantly lower than in the other two groups.

Sixteen samples from the Eastern region and Quebec were analysed for Mirex. An average Mirex residue level of 6 ppb was found. The range was 2-19 ppb. Figure 2 shows a typical GC elution pattern of PCBs and Mirex on a capillary column. The presence of Mirex in the total pooled sample was confirmed by MS.

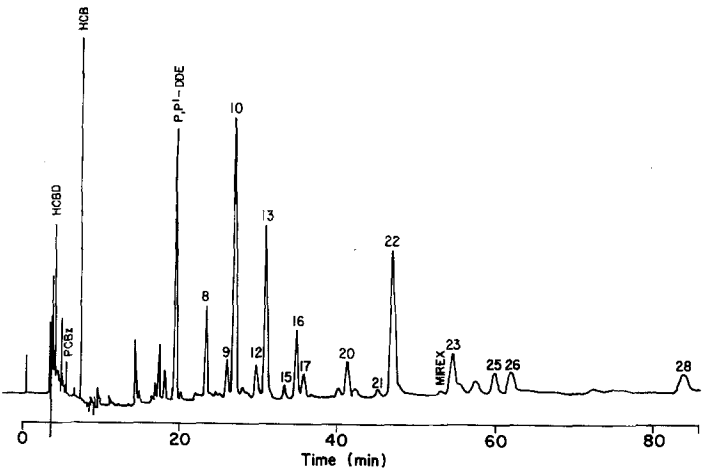

FIG. 2. A typical GC elution pattern of PCBs in human adipose tissue by capillary gas liquid chromatography. The numbers are not related to those in fig. 1, but are from an arbitrary numbering system for capillary GC, used in our laboratory.

ACKNOWLEDGEMENTS

The authors wish to thank the Field Operations Directorate for their assistance in procuring the samples and H. Newsome and D. Grant for reviewing this manuscript. We also wish to thank Pui-Yan Lau and D. Lewis for the MS and B. Collins for the statistical analysis.

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