Polychlorinated Biphenyl and Organochlorine Pesticide Residues in Adipose Tissue of Canadians

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Introduction

Organochlorine (OC) insecticide residues in adipose tissue of Canadians have earlier been reported by RITCEY et al (1973) in a first nation-wide survey. Since the initiation of this survey, more chlorinated environmental pollutants have been found in human adipose tissue, such as polychlorinated biphenyl (PCB) (PESENDORFER 1973), hexachlorobenzene (HCB) (BRADY 1972) and oxychlordane (BIROS 1973). At the same time some of the most persistent organochlorine pesticides such as p,p'-DDT have been restricted in their use. The data reported here are part of a continuing monitoring program of chlorinated hydrocarbons in adipose tissue of Canadians in order to determine a possible trend of both the disappearance of restricted OC pesticides as well as the appearance of new environmental contaminants.

Sampling

Regional sample contribution was as follows: 16 from the Eastern region (Newfoundland, Prince Edward Island, Nova Scotia and New Brunswick), 50 from Quebec, 57 from Ontario, 22 and 27 from the Central (Manitoba and Saskatchewan) and Western (Alberta and British Columbia) regions respectively. Samples were collected as described earlier (MES et al. 1974).

Analytical Methods

All solvents were glass-distilled and checked for purity by a fifty fold concentration. Pesticides were 99% pure, except for technical hexachlorocyclohexane (HCH) (90%).

Extraction and cleanup. Five gram samples were extracted in a mixture of benzene and acetone (1:19 v/v) and their fat content determined according to MES and CAMPBELL (1976). The fat was removed from the extract by low temperature precipitation (MCLEOD and WALES 1972).

Separation. The fat free extract was applied to a

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Florisil column, deactivated with 2% water after heating for 8 hrs at 300°C (MCLEOD and RITCEY 1973) and eluted into 2 fractions, I and II. Fractions I (PCBs and most OC pesticides) and II (dieldrin and heptachlor-epoxide) were obtained by eluting with hexane containing 5% and 30% methylene chloride respectively. Both fractions were carefully evaporated to dryness on an all-glass rotatory evaporator (<30°C). One quarter of fraction I was rechromatographed on a microsilicic acid column (MES and CAMPBELL 1976) and the PCB and OC pesticide fractions designated as III and IV respectively.

Identification and quantification. Fractions II and III were adjusted to 1 ml of hexane; fraction IV was evaporated as above and redissolved in 1 ml of hexane. Dilutions were made where necessary.

Gas chromatography (GC) was carried out on a 6% OV-210 + 4% SE-30 column (MES and CAMPBELL 1976). Residue levels were calculated by using integrated peak areas or peak heights. The PCB peaks 8, 10 and 11-15 in aroclor 1260 (REYNOLDS 1969) were used for quantification. The β and γ HCH isomers were quantitated from chromatograms obtained on a 5% OV-210 column as described below.

Confirmation of PCB. The PCB fractions (III) of every other 20 samples, exclusive of the 20th, were pooled to give approximately 11 µg of estimated PCB/pooled sample.

Thin layer chromatography (TLC) was carried out on precoated aluminum oxide (type E) F_{254} 20x20 cm plates (Brinkman Ltd., Canada), activated at 110°C for 1 hr. One fifth of the pooled sample was applied per spot. Reference spots of aroclor 1260, p,p'-DDE and HCB at 5, 2.5 and 2.5 µg/spot respectively were applied at each end of the line of origin together with a blank spot. The plate was developed in 1% acetone in hexane (v/v) and the spots visualised with $AgNO_3$ (MCLEOD and RITCEY 1973). Adsorbent from the areas of the sample and blank corresponding to those of the respective standards were scraped, eluted with hexane and gas chromatographed as above.

Those PCB fractions not included in the pooled samples were re-gaschromatographed on a 5% OV-210 column to confirm the identity of HCB and p,p'-DDE in the PCB fraction. The same PCB fractions were subsequently subjected to perchlorination (ARMOUR 1973) and identified as the decachlorobiphenyl on a 0.6 x 60 cm glass column packed with 4% SE-30 on chromosorb W(AW) 60/80. All PCB fractions from TLC were combined and analysed by mass spectrometry (MS) using a Varian MAT 311A coupled to a Varian 1440 Series gas chromatograph by a two-stage Watson-Biemann separator and monitored for a single ion (m/e = 291.919 ± 0.058).

Confirmation of pesticides. Fractions II and IV were confirmed on a 0.6 x 183 cm glass column packed with 5% OV-210 on chromosorb W(AW) 60/80 (0.5 g OV-210 + 10 g solid support) at injection, column and detector temperatures of 200°, 160° and 220°C respectively. Every other 20 samples of fractions II and IV were pooled and 1-20 µg/spot of estimated pesticide chromatographed on TLC plates with appropriate standards (5-10 µg/spot of individual pesticide), eluted and regaschromatographed as above.
Single ion monitoring MS was carried out on HCB (m/e = 283.810 ± 0.057), α, β and γ HCH (m/e = 218.912 ± 0.044), oxychlordane (m/e = 386.805 ± 0.077), trans-nonachlor (m/e = 406.787 ± 0.081), heptachlorepoxide (m/e = 352.844 ± 0.071), dieldrin (m/e = 344.899 ± 0.069), p,p'-DDE (m/e = 317.935 ± 0.064), o,p'-DDT and p,p'-DDT (m/e = 235.008 ± 0.047).
Controls. Samples were spiked by adding 5 µg of each pesticide and aroclor 1260 to 5 g of tissue before extraction. Samples were not spiked with trans-nonachlor. At different times during the survey blanks were run through the complete analytical procedure.

Results and Discussion

The results in Table 1 show the occurrence and high incidence of OC pesticide and PCB residues in adipose tissue of Canadians as collected in 1972. Compared to

TABLE 1

Chlorinated hydrocarbon residues in lipid extracts of human tissues in Canada.

Compound	µg/g wet weight		% of samples containing residues
	mean[a] ± S.D.[b]	range	
PCB as aroclor 1260	0.907 ± 0.817	0.106 - 6.603	100
HCB	0.062 ± 0.155	0.001 - 0.520	100
α HCH	0.004 ± 0.005	0.001 - 0.036	88
β HCH	0.054 ± 0.144	0.001 - 1.790	88
γ HCH	0.007 ± 0.014	0.001 - 0.136	78
Oxychlordane	0.055 ± 0.047	0.003 - 0.336	97
trans-Nonachlor	0.065 ± 0.054	0.010 - 0.367	99
Heptachlor-epoxide	0.043 ± 0.043	0.003 - 0.477	100
Dieldrin	0.069 ± 0.055	0.001 - 0.353	100
p,p'-DDE	2.095 ± 1.697	0.054 - 15.534	100
o,p'-DDT	0.031 ± 0.036	0.001 - 0.229	63
p,p'-TDE	0.006 ± 0.036	0.002 - 0.402	26
p,p'-DDT	0.439 ± 0.332	0.018 - 2.057	100

[a]Average of 168 samples. Four samples were lost or lacked proper information. [b]S.D. = standard deviation.

a previous survey by RITCEY et al. (1973) in 1969 the average levels of lindane, dieldrin, p,p'-DDE, p,p'-DDT and total DDT are lower, except for heptachlorepoxide, as indicated in Table 2. The lower DDT level could be attributed to the restricted use of this pesticide in Canada since 1969. However PCBs were not determined in the previous survey and could have influenced the 1969 DDT data.

TABLE 2

OC pesticide residues in Canadian human adipose tissue.

Compound	µg/g wet weight	
	RITCEY et al. (1973)	This survey
γ HCH	0.015	0.007
Heptachlorepoxide	0.040	0.043
Dieldrin	0.122	0.069
p,p'-DDE	3.430	2.095
p,p'-DDT	1.017	0.439
Total DDT	4.543	2.571

The present survey also revealed the presence of α and β HCH, oxychlordane and trans-nonachlor. The HCH isomers have earlier been reported by Wassermann et al. (1972), while BIROS and ENOS (1973) first reported oxychlordane residues in human adipose tissue.
A typical GC elution pattern of OC pesticides in human adipose tissue is presented in Fig. 1. The retention time of trans-nonachlor is very close to that of trans-chlordane on the 5% OV-210 column, but close to cis-chlordane on the mixed stationary phase. The unknown peak in chromatogram B was not further identified.
The regional distribution of the various residues in human adipose tissue is given in Table 3. A significant ($P< 0.05$) regional difference in the levels of α and γ HCH was detected and attributed to Quebec having higher levels than the rest of Canada. Also a significant regional difference in the levels of oxychlordane was observed.
Considerable higher values for p,p'-DDE and p,p'-DDT were reported by KADIS et al (1970) from a study on Alberta residents. The lower values reported in this paper for the Western region may reinforce the observed trend of decreasing DDT levels for the whole of Canada, especially since the above author reported the absence of PCB.
The accumulation of residues in adipose tissues of the two sexes is given in Table 4. A significant difference in the levels of trans-nonachlor was found between the two sexes. Although the overall residue levels in females were lower than in males, this difference was not statistically significant.
Table 5 shows residue levels in relation to age. The

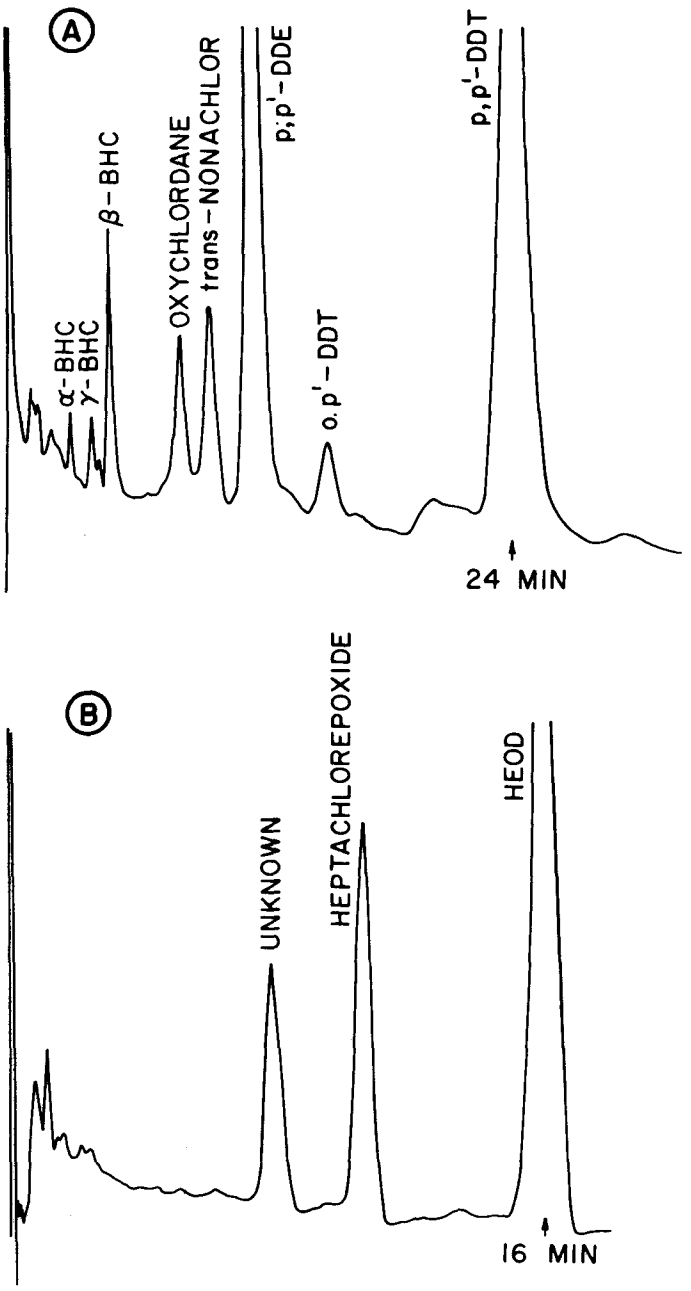

FIG. 1. Chromatogram A and B represent a typical GC elution pattern of pesticides found in fractions IV and II respectively on 5% OV-210.

TABLE 3

Regional distribution of chlorinated hydrocarbon residues extracted from human tissues

Compound	Average µg/g wet weight ± S.D.				
	Region				
	Eastern	Quebec	Ontario	Central	Western
PCB, as aroclor 1260	0.727±0.362	0.969±1.070	1.070±0.658	0.499±0.321	0.898±0.995
HCB	0.025±0.017	0.107±0.267	0.060±0.075	0.015±0.021	0.043±0.032
α HCH	0.003±0.009	0.006±0.004	0.004±0.005	0.002±0.002	0.005±0.002
β HCH	0.022±0.020	0.064±0.090	0.075±0.227	0.018±0.016	0.039±0.020
γ HCH	0.003±0.009	0.011±0.014	0.009±0.019	0.001±0.002	0.002±0.001
Oxychlordane	0.038±0.023	0.039±0.016	0.070±0.063	0.039±0.020	0.079±0.067
trans-Nonachlor	0.031±0.020	0.057±0.026	0.083±0.077	0.044±0.022	0.079±0.058
Hepta-chlorepoxide	0.031±0.022	0.027±0.018	0.046±0.065	0.052±0.032	0.063±0.037
Dieldrin	0.064±0.052	0.060±0.055	0.085±0.062	0.053±0.041	0.070±0.044
p,p'-DDE	2.098±1.113	2.011±0.970	2.008±2.027	1.706±0.917	2.764±2.580
o,p'-DDT	0.028±0.056	0.039±0.030	0.032±0.035	0.022±0.044	0.023±0.028
p,p'-TDE	N.D.[a]	0.006±0.023	0.010±0.056	0.002±0.007	0.001±0.005
p,p'-DDT	0.392±0.301	0.485±0.262	0.372±0.256	0.398±0.250	0.555±0.599

[a] N.D. = not detected

TABLE 4

Chlorinated hydrocarbon residues in human adipose tissue as related to sex.

Compound	Average µg/g wet tissue ± S.D.	
	Male (N[a]=111)	Female (N=57)
PCB, as anochlor 1260	1.020±0.957	0.685±0.353
HCB	0.052±0.067	0.082±0.259
α HCH	0.004±0.004	0.005±0.005
β HCH	0.042±0.029	0.078±0.253
γ HCH	0.008±0.015	0.006±0.012
Oxychlordane	0.064±0.055	0.038±0.024
trans-Nonachlor	0.076±0.062	0.044±0.029
Hepta-chlorepoxide	0.043±0.029	0.041±0.065
Dieldrin	0.078±0.061	0.051±0.037
p,p'-DDE	2.332±1.854	1.633±1.289
o,p'-DDT	0.033±0.037	0.027±0.035
p,p'-TDE	0.006±0.040	0.005±0.023
p,p'-DDT	0.450±0.307	0.416±0.381

[a] N = number of samples.

TABLE 5

Chlorinated hydrocarbon residue levels as related to age groups.

Compound	Average µg/g wet weight ± S.D.		
	0-25 yrs (N=49)	26-50 yrs (N=65)	51+ yrs (N=54)
PCB, as aroclor 1260	0.662±0.323	1.040±0.763	0.968±1.135
HCB	0.076±0.268	0.045±0.050	0.070±0.090
α HCH	0.005±0.003	0.004±0.005	0.004±0.005
β HCH	0.071±0.249	0.048±0.081	0.046±0.034
γ HCH	0.008±0.012	0.008±0.018	0.005±0.008
Oxy-chlordane	0.044±0.036	0.062±0.044	0.058±0.059
trans-Nonachlor	0.049±0.035	0.077±0.050	0.066±0.069
Heptachlor-epoxide	0.037±0.068	0.044±0.029	0.046±0.030
Dieldrin	0.057±0.057	0.070±0.048	0.080±0.066
p,p'-DDE	1.479±0.852	2.127±1.119	2.615±2.629
o,p'-DDT	0.037±0.042	0.028±0.028	0.030±0.039
p,p'-TDE	0.007±0.025	0.008±0.052	0.002±0.005
p,p'-DDT	0.382±0.217	0.444±0.366	0.485±0.371

only significant difference was in the levels of p,p'-DDE. The latter also increased with increase in age group. Although the same is true for p,p'-DDT, there was no significant difference between age groups. Wassermann et al. (1972) observed higher levels of OC pesticides in the age group of 25-44 years.
All residues reported in this paper were confirmed by GC, TLC, MS or derivation either in individual or pooled samples. Recoveries of spiked samples were between 70-90%, except for HCB (40%).
The average % lipid in adipose tissue samples was 81.4±11.0.

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