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Organochlorine Residues in Adipose Tissue of Canadians

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The presence of chlorinated hydrocarbon residues in adipose tissue of human populations continues to be globally demonstrated. Recently Ahmad et al. (1988) determined total DDTs and dieldrin in a segment of the Australian population. DDT and its analogs were also found by Mpofu (1986) in the fat of selected populations of Zimbabwe. In addition to DDTs, Karakaya and Özalp (1987) studied the presence of hexachlorocyclohexanes (HCH) in biopsy fat samples from patients in Ankara (Turkey). Furthermore, Vaman Rao and Banerji (1988) determined polychlorinated biphenyls (PCBs) in human fat samples from India, while Williams et al. (1988) analyzed adipose fat from cadavers in six municipalities from Ontario (Canada) for 28 chlorinated hydrocarbon residues.

The Health Protection Branch (HPB) of the Department of Health and Welfare Canada has been monitoring chlorinated hydrocarbon residues in the Canadian population since 1969 by carrying out national surveys at given time intervals. This paper reports on the results of the latest monitoring program in terms of residue levels in the body fat of Canadians and draws a comparison to similar exposures in other countries. In addition, regional differences and the effect of age and sex on the body burden of these residues will also be discussed.

MATERIALS AND METHODS

A total of 108 human autopsy fat samples were collected in 1985 as described earlier (Mes et al., 1982) and came from the following provinces: Newfoundland (4), Prince Edward Island (2), New Brunswick (8), Quebec (47), Ontario (17), Alberta (24) and British Columbia (6). No samples were obtained from the provinces of Manitoba, Saskatchewan and Nova Scotia, which could have biased the effects of regional distribution.

Solvents were glass-distilled and checked for interfering residues by gas chromatography (GC) after a 300-fold concentration. All chlorinated hydrocarbon standards (>95% pure) were gifts from the Environmental Protection Agency (USA), except for the chlorinated

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benzenes, which were purchased from Ultra Scientific (Hope, R.I., USA). Adsorbents and glass wool were washed with dichloromethane as described previously (Mes, 1981).

Samples (~1 g) were homogenized with 50 mL of benzene:acetone (1:19, v/v) for 2 min and the extracts filtered through glass wool and evaporated on an all-glass rotatory evaporator (<30°C). The residue was redissolved in hexane, filtered through anhydrous Na_2SO_4, concentrated and transferred to a 50 mL graduated centrifuge tube. After adjusting the volume to 20 mL with hexane, a 1 mL aliquot was evaporated in a preweighed aluminum weighing dish to determine the benzene:acetone soluble lipids. An aliquot containing no more than 200 mg of lipids, was then concentrated to ~1 mL for simultaneous cleanup and separation of residues. This was accomplished by using a 12 mm (od) x 200 mm glass column with 50 mL reservoir and Teflon (Dupont Co.) stopcock, packed with a glass wool plug at the bottom, followed by 6.5 g Florisil[R] (activated at 300°C) and topped with approximately 1 cm of anhydrous Na_2SO_4. The concentrated aliquot was eluted as indicated in Table 1.

Table 1. Cleanup and fractionation of residues on Florisil

Fraction	Eluting Solvent	Eluted Residues
I	50 mL Hexane	PCBs, chlorinated benzenes, octachlorostyrene (OCS), trans-nonachlor (t-NCl), p,p'-DDE, o,p'-DDT, Mirex
II	60 mL of 20% CH_2Cl_2 in hexane	HCH isomers, hexachlorobenzene (HCB), oxychlordane (OCl), α and γ chlordane, c-NCl, and p,p'-DDT
III	60 mL of 60% CH_2Cl_2 in hexane	Heptachlor epoxide, dieldrin

The fractions were concentrated as above and transferred to 15 mL graduated centrifuge tubes and either diluted or concentrated (under a gentle stream of N_2) to an appropriate volume for GC analysis.

The PCBs in fraction I and the heptachlor epoxide and dieldrin in fraction III were identified and quantitated on a Varian 3700 Series GC, equipped with a 0.6 x 183 cm glass column packed with 6% OV-210 + 4% SE-30 on chromosorb W (AW) 60/80 and a ^{63}Ni electron capture detector (ECD). The injector, column and detector temperatures were 240, 218 and 300°C, respectively. The N_2 flow rate through the column was 40 mL/min. Residues other than PCBs in fraction I were gas chromatographed on the same

[R] Floridin Co.

instrument, but using a 30 m x 0.24 mm (id) fused silica column, coated with 0.25 μm DB-5 (J&W Scientific Inc., Folsom, CA, USA). The injector temperature was programmed from 50° (0.5 min) to 240°C at 160°C/min. The initial column temperature was kept at 70°C for 1 min and then increased to 230° at 10°C/min and held at this final temperature for 33 min. Helium carrier and N_2 make-up gas flows were 38 cm/sec (at 70°C) and 30 mL/min respectively. Residues in fraction II were chromatographed on a Varian 3500 Series GC under identical conditions, except for the column temperature, which was only increased to 130° at 50°C/min after its initial temperature and then further programmed to 190 and 230°C at 4 and 3°C/min respectively. The column was kept for 18 min at its final temperature. The He carrier gas flow rate was 50 cm/sec and the make-up gas flow the same as above.

Aliquots of standard solutions (1 and 5 μl for the capillary and packed columns respectively) of Aroclor 1260 (200 pg/μl) and other chlorinated hydrocarbons (2-130 pg/μl, depending on individual response) were injected before and after every 3 or 4 sample injections where appropriate. Individual compounds were quantitated by measuring their respective peak heights. PCBs however, were estimated by summation of the peak heights as reported earlier (Mes et al., 1982).

Residues were confirmed by GC mass spectrometry (GC/MS), using the same GC capillary column and conditions as above, except that the initial temperature was 80°C (1 min), followed by an increase to 140°C in 1.2 min and a final programming to 270°C at 4°C/min. A VG Analytical ZAB-2F mass spectrometer was used for selected ion monitoring, with a dedicated ion source, an electron energy of 32 eV and a resolution of 1000. The dwell time was 80 msec, with an interscan time of 20 msec. Under these conditions fractions I to III were subjected to GC/MS analysis by using pooled fractions from each region and monitoring selected ions of the following compounds: trichlorobiphenyls (m/z = 258), tetrachlorobiphenyls (m/z = 292), pentachlorobiphenyls (m/z = 326), hexachlorobiphenyls (m/z = 358), heptachlorobiphenyls (m/z = 396), dichlorobenzenes (m/z = 148), trichlorobenzenes (m/z = 180), tetrachlorobenzenes (m/z = 216, 218), pentachlorobenzene (m/z = 250), hexachlorobenzene (m/z = 284), octachlorostyrene (m/z = 380). Mirex (m/z = 272), p,p'-DDE (m/z = 318), HCH isomers (m/z = 220), DDTs (m/z = 235), oxychlorodane (m/z = 387), t-nonachlor (m/z = 408), c-nonachlor (m/z = 408), α- and γ-chlordanes (m/z = 375), heptachlor epoxide (m/z = 388) and dieldrin (m/z = 380).

Quality control was carried out by fortification of 1 g of every 20th sample with 50 μl of a solution containing 14 ng 1,2,3-trichlorobenzene, 105 ng HCB, 49 ng t-nonachlor, 2014 ng p,p'-DDE, 257 ng β-HCH, 502 ng p,p'-DDT and 52 ng dieldrin/μl in acetone. In addition one sample was analyzed in quadruplicate to establish reproducibility, while at regular intervals solvent blanks were subjected to the entire analytical procedure to determine background interference.

RESULTS AND DISCUSSION

Average recoveries of 1,2,3-trichlorobenzene, HCB, t-nonachlor, p,p'-DDE, β-HCH, p,p'-DDT and dieldrin from fortified adipose tissue samples were 74, 87, 108, 88, 87, 84 and 99% respectively with an average coefficient of variation (CV) of 17% (range 4-27%). The highest CV (27%) was observed for 1,2,3-trichlorobenzene. The limit of detection (LOD) was defined as 3x the standard deviation of the blank (N=5) and was <16 ng/g (range 0.1-15.3) for all residues, except the chlorobenzenes (LOD ranged from 18-66 ng/g). Quadruplicate determinations of residues in one of the samples, resulted in an average CV of 9% with a range of 2-23%, except for pentachlorobenzene. At levels of <1 ng/g the reproducibility of the pentachlorobenzene was poor (CV of 33%).

Residues of PCBs, HCB, β-HCH, oxychlordane, t-nonachlor, p,p'-DDE and p,p'-DDT were found to be the major contaminants (>25 ng/g) in adipose tissue of Canadians and omnipresent (Table 2). Their levels were considerably lower than in a previous survey (Mes et al., 1982). Although most chlorobenzenes were also present at levels >25 ng/g, they were less frequently encountered.

Table 3 shows chlorinated hydrocarbon residue levels in human fat samples of several industrial nations. All residue levels reported in Table 3 were lower in Canadian fat samples than elsewhere. However, ΣDDT levels in Canada were close to those found in the United Kingdom.

Regional differences of residue levels in fatty tissue of Canadians are shown in Table 4. The total residue burden in ng/g was the highest for Quebec donors and lowest for those from the Eastern region. The residue levels from Ontario may not necessarily reflect those of the whole province, since all samples came from the Toronto area. The results were considerably lower than those reported for the city of Toronto by Williams et al. (1988). These authors however, used fewer samples (7) of older donors (average 64 yr) as compared to the analysis of 17 samples from donors with an average age of 31 yr as reported in this study. Furthermore, they did not find any tri-, tetra- or pentachlorobenzene residues.

Statistically significant differences ($p<0.025$) were observed for PCB and p,p'-DDT residues of Quebec and the Western region, and for 1,2,3-trichlorobenzene residues from Quebec and Ontario. Although several residues showed numerical differences between the regions, these were not statistically significant as for example in the case of p,p'-DDE.

No significant differences were observed in the residue levels of males and females (Table 5), although in several instances the mean residue levels were somewhat higher in males than in females. This observation does not support earlier work by Mes et al. (1982), who found higher levels of HCB in females, or a recent study by Williams et al., (1988) who in addition to HCB reported

Table 2. Chlorinated hydrocarbon residues in Canadian adipose tissue

Compound	ng Residue/g Wet tissue Mean[a] (N=108)	(CV)	Maximum observed	Frequency of residue (%)
PCBs (as Aroclor 1260)	410	(100)	2101	100
1,2-Dichlorobenzene	136	(152)	1032	27
1,2,3-Trichlorobenzene	44	(113)	329	61
1,2,4-Trichlorobenzene	103	(54)	358	26
1,2,5-Trichlorobenzene	126	(61)	362	19
1,2,3,4-Trichlorobenzene	67	(67)	253	36
1,2,3,5-Trichlorobenzene	30	(50)	80	12
Pentachlorobenzene	<1		3	84
Hexachlorobenzene	25	(101)	118	100
β-Hexachlorocyclohexane	31	(302)	910	100
Octachlorostyrene	1	(432)	44	98
α-Chlordane	2	(92)	6	72
γ-Chlordane	2	(79)	5	100
Oxychlordane	33	(65)	103	100
t-Nonachlor	50	(76)	182	100
c-Nonachlor	8	(97)	39	100
Heptachlor epoxide	8	(229)	73	83
Dieldrin	4	(422)	23	13
p,p'-DDE	811	(210)	6070	100
o,p'-DDT	4	(174)	30	44
p,p'-DDT	48	(114)	250	100
Mirex	7	(183)	72	100

[a] Geometric mean of positive values only.

higher levels of β-HCH and DDTs in females from Ontario. This apparent discrepancy may be partly caused by the use of geometric rather than arithmetic means for statistical evaluation of the results.

Table 3. A comparison of residue levels in adipose tissue from different countries.

Residue	Mean residue level in mg/kg Canada (This survey 1985)	Italy (Focardi et al. 1986)	Poland (Szymczynski et al. 1986)	United Kingdom[a] (Abbott et al. 1985)	Spain (Camps et al. 1989)
HCB	0.025	2.26	0.221	0.10	2.99
β-HCH	0.031		0.211	0.25	3.06
Dieldrin	0.004			0.06	0.072
p,p'-DDE	0.811	7.35	14.095	1.2	6.27
p,p'-DDT	0.048	0.83	1.772	0.08	1.50
ε DDT[b]	0.863	8.18	16.300	1.28	7.849
PCBs	0.410	1.75		0.8	1.68

[a] Median values. [b] Total DDTs include small amounts of other DDT analogs not reported separately in this table.

Table 4. Regional distribution of residues in adipose tissue of Canadians

Residue	Mean[a] residue level in ng/g wet tissue			
	Region			
	Eastern[b] (N=14)	Quebec (N=47)	Ontario (N=17)	Western[c] (N=30)
PCBs	520	534	316	281
1,2-Dichlorobenzene	73	116	220	181
1,2,3-Trichlorobenzene	21	30	46	85
1,2,4-Trichlorobenzene	-[d]	112	93	110
1,3,5-Trichlorobenzene	-	99	105	153
1,2,3,4-Tetrachlorobenzene	-	55	79	64
1,2,3,5-Tetrachlorobenzene	-	80[f]	26	29
Pentachlorobenzene	<1	<1	ND[e]	<1
Hexachlorobenzene	25	26	17	28
β-Hexachlorocyclohexane	27	31	24	35
Octachlorostyrene	1	1	1	2
α-Chlordane	1	2	1	ND
γ-Chlordane	1	2	2	1
Oxychlordane	32	34	33	32
t-Nonachlor	56	53	45	45
c-Nonachlor	9	8	7	7
Heptachlor epoxide	4	7	4	13
Dieldrin	4	ND	ND	ND
p,p'-DDE	759	1118	561	625
o,p'-DDT	ND	ND	4	4
p,p'-DDT	41	66	36	37
Mirex	8	8	7	6

[a] Geometric [b] Newfoundland, Prince Edward Island, and New Brunswick [c] Alberta and British Columbia [d] Individual samples were <LOD [e] ND = not detected by GC/MS [f] Single value only.

Table 6 compares residue levels between arbitrarily selected age groups. Residue levels of oxychlordane, increased significantly ($p<0.025$) with selected age groups. Significant differences in residue levels were also found for HCB between those over 51 yr and the other two age groups. No such significant differences were observed for the tri- and tetrachlorobenzenes. In addition, significantly higher levels of PCBs, β-HCH, cis- and trans-nonachlor, p,p'-DDE and p,p'-DDT were found in those older than 51 yr. Similar increases of residue levels with selected age groups, as reported in Table 6, have also been observed earlier for identical age groups, by Mes et al. (1982) for HCB, oxychlordane and p,p'-DDE. Although the dieldrin level was considerably higher in the 26-50 yr old group, it was not significantly different from the 0-25 yr old group.

Table 5. Residue levels in adipose tissue of Canadians as related to sex

Residue	Mean residue level in ng/g wet tissue	
	Male (N=81)	Female (N=27)
PCBs	432	352
1,2-Dichlorobenzene	116	210
1,2,3-Trichlorobenzene	43	41
1,2,4-Trichlorobenzene	104	101
1,3,5-Trichlorobenzene	120	137
1,2,3,4-Tetrachlorobenzene	68	65
1,2,3,5-Tetrachlorobenzene	32	27
Pentachlorobenzene	<1	<1
Hexachlorobenzene	23	30
β-Hexachlorocyclohexane	28	40
Octachlorostyrene	1	2
α-Chlordane	2	2
γ-Chlordane	2	1
Oxychlordane	34	31
t-Nonachlor	52	44
c-Nonachlor	8	8
Heptachlor epoxide	8	6
Dieldrin	4	ND
p,p'-DDE	844	722
o,p'-DDT	5	2
p,p'-DDT	48	47
Mirex	8	6

Table 6. Residues in adipose tissue of Canadians as related to age

Residue	Mean residue level in ng/g wet tissue		
	0-25 yr (N=28)	26-50 yr (N=41)	>51 yr (N=39)
PCBs	306	411	504
1,2-Dichlorobenzene	188	84	147
1,2,3-Trichlorobenzene	50	46	40
1,2,4-Trichlorobenzene	89	123	99
1,3,5-Trichlorobenzene	121	127	130
1,2,3,4-Tetrachlorobenzene	68	55	85
1,2,3,5-Tetrachlorobenzene	27	29	32
Pentachlorobenzene	<1	<1	<1
Hexachlorobenzene	16	21	39
β-Hexachorocyclohexane	25	26	41
Octachlorostyrene	1	1	1
α-Chlordane	1	2	2
γ-Chlordane	1	1	2
Oxychlordane	24	32	43
t-Nonachlor	37	50	61
c-Nonachlor	6	8	10
Heptachlorepoxide	5	10	17
Dieldrin	1	10	ND
p,p'-DDE	427	702	1497
o,p'-DDT	5	4	4
p,p'-DDT	31	48	66
Mirex	7	7	8

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