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Organochlorine Residues in Human Blood and Biopsy Fat and Their Relationship

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The presence of organochlorine (OC) pesticide residues in human blood, serum and adipose tissue has been reported in many studies over the last two decades (Brown and Chow, 1975; Wassermann et al., 1975; Murphy and Harvey, 1985; Barquero and Costenla, 1986). Most studies were carried out using serum rather than whole blood and autopsy rather than biopsy fat samples. Although some studies determined the relationship between OC residues in serum and biopsy fat residue levels (Needham et al., 1990), few investigators indicated the use of paired samples for this purpose (Wyllie et al., 1972). In a recent study, however, Krawinkel et al. (1989) determined the levels of hexachlorocyclohexane (HCH) isomers, p,p'-DDT and p,p'-DDE in whole blood and biopsy fat samples of 25 patients, but only compared the p,p'-DDT/p,p'-DDE ratios in blood and fat.

In this paper the paired whole blood and biopsy fat samples from a selected population of British Columbia (Canada) were analyzed for various organochlorine residues and their relationship examined.

MATERIALS AND METHODS

Paired blood and biopsy fat samples were obtained from 25 patients of which 7 were male and 18 were females. The samples were collected in residue-free vials, immediately frozen and only thawed just before analysis. Most biopsy fat samples were taken from the abdominal region with one or two from the buttocks or the breast.

Glass-distilled solvents were checked for interfering residues by gas chromatography (GC) after a 300-fold concentration. All glassware, glasswool and adsorbents were washed with solvents and used as described earlier by Mes and Davies (1978), except that activated Florisil (Floridin C^{2}) was used for the biopsy fat analysis and deactivated (1.5% H_2O) Florisil for the determination of OC residues in whole blood. The need to use two different Florisil activities arose, when a new batch of Florisil was purchased. The OC standards used in this study were 97-100% pure and were gifts from the Environmental Protection Agency (Triangle Park, N.C., U.S.A.), except for the chlorinated benzenes, which were purchased from Ultra Scientific Inc. (Hope, R.I., U.S.A.).

Three standard solutions (A, B and C) were made up in hexane. Standard solution A contained 1,2-dichlorobenzene at a concentration of 130 pg/μL and other chlorinated benzenes at concentrations of 3-18 pg/μL, depending on the chlorine content. Solution B contained βHCH, (hexachlorocyclohexane) heptachlor epoxide, dieldrin and c-nonachlor at concentrations of 5-8 pg/μL, while solution C continued all other OC compounds reported in this paper at concentrations of 6 pg/μL.

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Blood samples (~ 5 g) were homogenized with 15 mL benzene, centrifuged and the top layer filtered through anhydrous Na_2SO_4 as described earlier by Mes (1987). Adipose tissue samples (~ 400-1300 mg) were extracted with 50 mL benzene: acetone (1:19 v/v), filtered through glass wool and the solvent evaporated on an all-glass rotatory evaporator (< 30°C). Traces of benzene were removed by two additions of ~ 10 mL hexane and reevaporation. The oily residue was redissolved in hexane and dried by passing through anhydrous Na_2SO_4. The filtrate was concentrated, and in case of adipose tissue transferred to a 50 mL graduated centrifuge tube and made up to a final volume of 20 mL with hexane. All manipulations with benzene were carried out in the fumehood.
The extractable lipids in blood and adipose tissue were determined gravimetrically after evaporation of the entire concentrated blood extract and 1 mL of the final adipose tissue extract in preweighed aluminum weighing dishes. The blood lipids were redissolved in hexane, transferred to a 250 mL round bottom flask and concentrated to ~ 1 mL.
The entire blood extract and an aliquot of the adipose tissue extract, representing not more than 200 mg fat, were chromatographed on Florisil, using a 12 mm (O.D.) x 200 mm glass column with Teflon (Dupont C°) stopcock and 50 mL reservoir. The various residues in the blood extract were eluted from deactivated Florisil (4.5 g) in 35, 40 and 40 mL of hexane, 20% CH_2Cl_2 in hexane and 60% CH_2Cl_2 in hexane respectively, while those in the adipose tissue extract were eluted from activated Florisil (6.5 g) in 50, 60 and 60 mL of the same solvents respectively. All fractions were concentrated to an appropriate volume (0.2-5 mL) for GC analysis.

The first Florisil fraction of the blood extract, containing the chlorinated benzenes and other non-polar residues, was chromatographed on a DB-5 (J & W Scientific) capillary column (30 m x 0.24 mm I.D..; film thickness 0.25 μm), using a Varian 3500 GC with splitless injector and a ^{63}Ni electron capture detector (ECD). The injector temperature was programmed from 80°C (0.5 min) to 240°C at 160°/min. The column temperature was programmed from 130°C (7 min) to 190°C and finally to 230°C at 4° and 3°/min respectively. For adipose tissue, however, the initial column temperature gradient was 70°C (1 min) to 130°C at 50°/min, followed by the same temperature program as above. The detector temperature was set at 300°C. The second fraction of the Florisil column, containing most OC residues, was chromatographed on the same column as above and the same temperature program. Aliquots (1 μL) of standard solution A and samples were injected in the order of one standard before and after every 3 sample runs. In a similar manner solution C was used for the second fraction. The third fraction, containing all dieldrin, heptachlor epoxide and some βHCH and c-nonachlor residues, was chromatographed using a Varian 3700 GC with a glass column (0.6 x 183 cm), packed with 6% OV-210 + 4% SE-30 on chromosorb W (AW), 60/80 and an ECD detector. Injector, column and detector temperatures were 240°, 218°, and 300°C respectively. The nitrogen carrier gas flow was ~ 40 ml/min. Aliquots (1 μL) of standard solution B and samples were injected in the order indicated above.
Identification of the GC peaks was automated using a Varian Vista 402 data system. Quantitation was based on comparing peak heights of standard and sample.
The OC residues identified by GC/ECD were confirmed in pooled fractions by GC/MS using the same capillary column under similar conditions as above and a VG Analytical ZAB-2F mass spectrometer. The MS conditions together with the multiple ion monitoring process were described in detail by Mes et al. (1990).

Two samples of whole blood (5 g each) were fortified with hexachlorobenzene (HCB), βHCH, t-nonachlor, dieldrin and p,p'-DDT at levels of 0.3-3.0 ng/g, while two adipose tissue samples (1 g each) were fortified with the same OC pesticides at levels of 100-1000 ng/g. Quadruplicate and triplicate determinations of a blood and adipose tissue sample respectively were used to establish reproducibility of the method. Appropriate solvent blanks were run through the entire analytical procedure.

RESULTS AND DISCUSSION

The limit of detection for the various OC residues reported in this study are given in Table 1. These values are based on 3x the standard deviation of the blank background or if no background was encountered on the amount of standard needed to give the threshold value of peakheight counts. The reproducibility of the residue levels in blood, tested by a quadruplicate analysis and expressed as the coefficient of variation (CV), was < 10%, except for 1,2,3,5-tetrachlorobenzene (14%) and α chlordane (20%). Triplicate analysis of an adipose tissue sample gave a CV of < 10% for most OC residues, except 1,2-dichlorobenzene (19%), 1,2,3-trichlorobenzene (20%), pentachlorobenzene (17%), c-nonachlor (11%) and dieldrin (20%), pentachlorobenzene (17%), c-nonachlor (11%) and dieldrin (20%). Mean recoveries of the selected OC pesticides from fortified whole blood and adipose tissue were 80 (range 75-90%) and 86% (range 79-95%) respectively. The mean lipid contents of the blood and fat samples were 0.09 and 76.4% respectively.

Table 1. Minimum detectable levels (MDL) of OC residues in blood and adipose tissue

Chemical residue	MDL (ng/g)		Chemical residue	MDL (ng/g)	
	Whole blood	Adipose tissue		Whole blood	Adipose tissue
1,2-Dichlorobenzene	3.12	2.28	α Chlordane	0.02	0.01
1,2,3-Trichlorobenzene	1.17	1.68	γ Chlordane	0.01	0.01
1,3,5-Trichlorobenzene	4.02	0.90	t-Nonachlor	0.03	0.01
1,2,3,5-Tetrachlorobenzene	5.1	0.41	c-Nonachlor	0.01	0.01
Pentachlorobenzene	0.02	0.45	p,p'-DDE	0.05	0.01
Hexachlorobenzene	0.01	0.12	o,p'-DDT	0.13	0.01
α HCH	0.04	0.01	p,p'-DDT	0.01	0.01
β HCH	0.02	0.01	Dieldrin	0.01	0.01
Octachlorostyrene	0.01	0.01	Heptachlor epoxide	2.35	0.01
Oxychlordane	0.06	0.01	Mirex	0.04	0.24

The results of the whole blood and adipose tissue analysis are given in Table 2 on a wet tissue basis. The median values of several OC residues in blood were below the limit of detection (LOD) as specified in Table 1. The presence of other OC residues were confirmed by GC/MS. Although levels of 1,2,3-and 1,3,5-trichlorobenzenes were < LOD, traces were observed by GC/MS in both whole blood and adipose tissue. No α HCH, octachlorostyrene (OCS), dieldrin or heptachlor epoxide were detected in whole blood. In adipose tissue all median levels of OC residues were > LOD and their presence was also confirmed by GC/MS. The ratio of 17.0 for p,p'-DDE/p,p'-DDT found in adipose tissue in this study was the same as that derived from the data by Mes et al. (1990) in a nation-wide survey of the Canadian population. No comparable blood values were available, but the p,p'-DDE/p,p'-DDT ratio in blood of 29.0 found in this study was considerably higher than for adipose tissue, which may reflect recent exposure, since little p,p'-DDT is metabolized to p,p'-DDE in the liver.

The relationship between some OC residues in whole blood and adipose tissue is given in Table 3, in terms of adipose tissue/blood ratios and correlation coefficients. The ratios varied considerably depending on the residue, while for each individual residue a wide range in ratio values was observed. Although Krawinkel et al. (1989) also observed a wide range for β HCH, p,p'-DDE and p,p'-DDT, the adipose tissue/blood ratios derived from their data appear to be more consistent (~ 1000:1).

Table 2. OC residues in human blood and biopsy fatty tissue

Chemical residue	ng/g			
	Whole blood		Biopsy fatty tissue	
	Median	Maximum	Median	Maximum
1,2-Dichlorobenzene	< 3.12	14.29	28.1	154.5
1,2,3-Trichlorobenzene	< 1.17	< 1.17	1.9	9.1
1,3,5-Trichlorobenzene	< 4.02	< 4.02	1.1	3.7
1,2,3,5-Tetrachlorobenzene	0.06	0.22	0.8	15.7
Pentachlorobenzene	< 0.02	0.02	0.7	3.5
Hexachlorobenzene	0.11	0.34	18.8	87.0
α Hexachlorocyclohexane	< 0.04	< 0.04	1.1	9.6
β Hexachlorocyclohexane	0.13	2.60	18.0	748.6
Octachlorostyrene	< 0.06	< 0.06	0.5	1.3
Oxychlordane	0.07	0.16	16.5	44.9
α Chlordane	0.02	0.05	0.3	2.1
γ Chlordane	0.01	0.05	0.5	2.2
t-Nonachlor	< 0.03	0.04	22.6	74.0
c-Nonachlor	< 0.01	< 0.01	2.6	11.0
p,p'-DDE	0.87	6.05	429.2	9804.0
o,p'-DDT	< 0.13	0.27	4.7	9.1
p,p'-DDT	0.03	0.88	25.3	4390.6
Dieldrin	< 0.01	< 0.01	10.6	41.1
Heptachlor epoxide	< 2.35	< 2.35	12.5	80.9
Mirex	< 0.04	0.05	4.7	15.7

Table 3. Relationship between some chlorinated hydrocarbons in whole blood and adipose tissue

Chemical residue	N[a]	Residue ratio (wet basis) Adipose tissue/whole blood			Correlation coefficient (R)
		Mean	(CV)	Range	
1,2,3,5-Tetrachlorobenzene	14	42	(135)	5-19	0.016
Hexachlorobenzene	25	192	(25)	98-256	0.827
β Hexachlorocyclohexane	25	179	(41)	43-355	0.991
Oxychlordane	17	289	(43)	142-561	0.369
α Chlordane	15	21	(81)	5-70	0.270
γ Chlordane	21	66	(86)	12-220	0.260
p,p'-DDE	25	653	(49)	281-1621	0.957
p,p'-DDT	25	1742	(117)	355-2130	0.432

[a] Number of paired blood and fat samples.

Relationships in residue levels between adipose tissue and blood, with at least a 99% confidence level, were established for HCB, β HCH, oxychlordane and p,p'-DDE. No relationship was observed for α and γ chlordane. In a most recent publication, Sasaki et al. (1991) established a similar relationship for β HCH, p,p'-DDE and oxchlordane in blood and biopsy fat. However, in those cases where relationships were found the extrapolation from blood to adipose tissue levels should be exercised with some caution as indicated by the relatively high CV's for the mean levels of these compounds. Only

Table 4. OC residues in the lipids of human blood and adipose tissue

Chemical residue	ng/g			
	Blood lipids		Adipose lipids	
	Median	Maximum	Median	Maximum
1,2-Dichlorobenzene	< 3	20005	38	194
1,2,3-Trichlorobenzene	< 1	< 1	2	11
1,3,5-Trichlorobenzene	< 4	< 4	1	4
1,2,3,5-Tetrachlorobenzene	69	411	1	192
Pentachlorobenzene	< 1	33	1	4
Hexachlorobenzene	134	480	26	87
α Hexachlorocyclohexane	< 1	< 1	2	12
β Hexachlorocyclohexane	147	2723	28	967
Octachlorostyrene	< 1	< 1	1	1
Oxychlordane	87	246	24	49
α Chlordane	23	66	1	2
γ Chlordane	17	56	1	2
t-Nonachlor	< 1	105	32	80
c-Nonachlor	< 1	< 1	4	12
p,p'-DDE	1249	6343	585	12660
o,p'-DDT	< 1	288	6	12
p,p'-DDT	31	861	32	5670
Dieldrin	< 1	< 1	17	80
Heptachlor epoxide	< 2	< 2	16	81
Mirex	< 1	55	6	19

Table 5. Relationship between some chlorinated hydrocarbons in blood and adipose tissue lipids

Chemical residue	N[a]	Residue ratio (lipid basis)			Correlation coefficient (R)
		Adipose tissue lipids/blood lipids			
		Mean	(CV)	Range	
1,2,3,5-Tetrachlorobenzene	11	0.037	145	0.004-0.176	0.036
Hexachlorobenzene	25	0.227	53	0.085-0.645	0.788
β Hexachlorocyclohexane	25	0.215	43	0.061-0.407	0.989
Oxychlordane	16	0.305	30	0.197-0.542	0.656
α Chlordane	13	0.022	78	0.007-0.058	0.230
γ Chlordane	16	0.055	62	0.013-0.106	0.032
p,p'-DDE	25	0.735	48	0.353-1.996	0.880
p,p'-DDT	25	2.275	94	0.033-8.901	0.430

in the case of HCB would such an extrapolation give rise to a reasonable approximation of the body burden.

For comparison, the results were also expressed on a lipid basis as shown in Table 4. In many instances there was a considerable discrepancy between residue levels in blood and adipose tissue lipids, except for p,p'-DDT. Table 5 compares the adipose/blood ratios for the various residues, calculated on a lipid basis, and their correlation coefficients. In most cases the R values are slightly lower than those calculated on a whole tissue basis. This may be partly due to the imprecision of the lipid determination in small blood samples as recently reported by Mes et al. (in press). If the correlation

coefficient for p,p'-DDT in Table 5 was calculated by eliminating three extremely low datapairs, a relationship with a 99% confidence level was observed with a R-value of 0.989.

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