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 C2) was used for the biopsy fat analysis and deactivated (1.5% H2O) 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$_2$SO$_4$ 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$_2$SO$_4$. 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$_3$) 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$_2$Cl$_2$ in hexane and 60% CH$_2$Cl$_2$ 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 63Ni electron capture detector (ECD). The injector temperature was programmed from 80°C (0.5 min) to 240°C at 160°C/min. The column temperature was programmed from 130°C (7 min) to 190°C and finally to 230°C at 4°C and 3°C/min respectively. For adipose tissue, however, the initial column temperature gradient was 70°C (1 min) to 130°C at 50°C/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.