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Chlorobenzene Residues in Human Fat and Milk

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Chlorobenzenes (CBs) are similar in chemical structure, properties and toxicity to organochlorine pesticides and polychlorinated biphenyls (PCBs). The occurence of last two in populations all over the world has been intensively investigated (WASSERMANN et al. 1975, 1979); but little information is available on CB levels in the population, especially of those isomers with less than six chloro atoms on the benzene nucleus (MORITA 1977, MORITA & OHI 1978). CBs are used widely (FISHBEIN 1979) in the chemical industry as solvents and as intermediates in the production of other chemicals. Secondary sources of CBs include reaction products from pesticide (e.g.HCH) metabolism processes (ENGST et al.1979) and probably formation during incineration (CHOUDHRY & HUTZINGER, 1982) of organic matter and chloro compounds. In the aqueous environment (OLIVER & BOTHEN 1980) and in water living organisms (JAN & MALNERŠIČ 1980) they appear frequently. They tend to accumulate in biological material rich in lipids, such as fatty tissue and milk. This study was performed in order to investigate CB residues in man. For their determination high resolution glass capillary GL chromatography was used, after a simple clean-up procedure as described for organochlorine pesticides (VEIEROV & AHARONSON 1980).

EXPERIMENTAL

Samples. Samples of adipose tissues were received from Ljubljana and Maribor hospitals (Republic of Slovenia, N.W.Yugoslavia) from persons of both sexes between 20-60 years of age, who died in traffic accidents in 1979-80. Human milk samples were obtained 3-5 days after parturition between September-October, 1981, from mothers in the Ljubljana Maternity Clinic. The samples were kept in a deep freeze prior to analysis.

Procedure

Adipose tissue: To 1 g adipose tissue fat dissolved in 10 mL of n-hexane were added 20 mL of conc.sulfuric acid. The mixture was shaken in a separatory funnel. The cleaning of the hexane extract was repeated twice with 10 mL of conc.sulfuric acid. The hexane extract was passed through a Florisil column 10 cm in height with an i.d.

of 1 cm (Florisil 60-100 mesh, Fluka, was Soxhlet extracted from impurities with benzene, then treated for 3 h at 550 °C and maintained at 130 °C prior to use), having 0.5 cm of anhydrous Na_2SO_4 on the top, and eluted with 25 mL of 6 % diethyl ether in hexane. The eluate was concentrated to 2 mL at 50 °C in a flow of nitrogen and chromatographed.

Human milk: 20 g of milk and 10 mL of n-hexane for extraction were cooled below 0 °C. 20 mL of conc.sulfuric acid were slowly added with stirring. After standing overnight at room temperature, the hexane layer was separated and cleaned up twice with 10 mL of conc. sulfuric acid and through the Florisil column, and the eluate evaporated and analysed by GL chromatography. An additional clean-up was done by hydrolysis (YOUNG & BURKE 1972): to the hexane eluate evaporated to 1 mL, 1 mL of 2 % ethanolic KOH was added, this was then sealed in a glass vial and hydrolysed 1 h at 95 °C. After cooling, water was added to the reaction mixture, extracted with hexane and cleaned-up through conc. sulfuric acid and Florisil as above and chromatographed. For quantitative assessment, the increased trichlorobenzene levels produced from hydrolysis of HCH isomers were taken into consideration.

Gas liquid chromatography: A Varian GC mod.3700 equipped with an electron capture detector (^{63}Ni) was used with 20 m glass capillary columns of an i.d. of 0.32 mm coated with SE-30, as well as with an i.d. of 0.30 mm coated with OV-1. The chromatographic conditions were as follows: the temperature of the injector was 210 °C, of the detector 240 °C. The initial oven temperature was 60 °C held for 40 s (during the purge activation time) and then raised to 70 °C in 20 s and programmed from 70 to 180 °C at 4 °C/min. Splitless injection was used, the volume injected being 2 and 3 µL, the purge activation time 40 s, and nitrogen flow was for the column 0.9 mL/min., injector purge 120 mL/min. and make up 25 mL/min.

The identification of compounds found was carried out by comparing their retention time with that of the test substances: 1,3-dichlorobenzene (1,3-DCB), 1,4-dichlorobenzene (1,4-DCB), 1,2-dichlorobenzene (1,2-DCB), 1,2,3-trichlorobenzene (1,2,3-TCB), 1,2,4-trichlorobenzene (1,2,4-TCB), 1,3,5-trichlorobenzene (1,3,5-TCB), 1,2,3,4-tetrachlorobenzene (1,2,3,4-TeCB), 1,2,3,5-tetrachlorobenzene (1,2,3,5-TeCB), 1,2,4,5-tetrachlorobenzene (1,2,4,5-TeCB), pentachlorobenzene (PeCB) and hexachlorobenzene (HCB), all supplied by Fluka.

To lower the blank value level, the glassware was rigorously cleaned with methanol and heated at 300 °C for 1 h. Recoveries of CBs were determined in edible oil spiked with CBs. Recoveries were over 80 %. The milk fat level was determined in a parallel sample.

TABLE 1

Mean levels of chlorobenzenes (CBs) in human milk and adipose tissue (μg/kg). Samples collected in Slovenia, Yugoslavia

Sample \ Compound	DCB			TCB			TeCB		PeCB	HCB	Total HCH[a]
	1,3-	1,4-	1,2	1,3,5-	1,2,4-	1,2,3-	1,2,3,5- and/or 1,2,4,5-	1,2,3,4-			
Human milk (n^b=12) "as in" basis	tr[d]	25 (5-35)	9 (5-12)	1 (nd-3)	1 (nd-4)	5 (2-10)	2 (nd-5)	1 (nd-3)	0.7 (nd-3)	2.1 (1-7)	9.7 (5-18)
fat basis[c]		640	230	25	25	128	50	25	18	53	250
Human adipose tissue n=15 fat basis	nd[e]	146 (nd-200)	13 (nd-20)	16 (8-20)	9 (2-15)	103 (20-150)	16 (2-20)	1 (nd-1)	1.2 (nd-3)	55 (20-170)	270 (170-700)

Concentrations are expressed as arithmetic means. Figures within parenthesses show ranges.

a the sum of alpha-, beta-, gamma-hexachlorocyclohexane isomers

b number of samples

c calculated. The fat content of the milk was 3.9 % (1.6-5.1)

d <5 μg/kg whole milk basis. The lower electron capture detector sensitivity of DCB (approx. 10^2 lower than HCB) increases the limit of detection

e not detected.

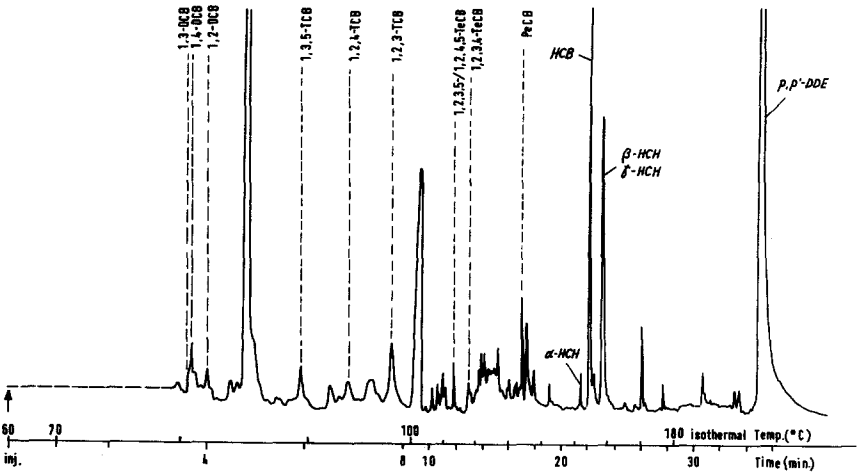

Fig.1. Chromatogram on the SE-30 glass capillary column of the human milk extract. For abbrevations, see the text.

RESULTS AND DISCUSSION

The mean levels of CBs in human milk and adipose tissue are summarized in Table 1; a comparison with HCH (hexachlorocyclohexane) pesticide levels is included. In Fig.1 a chromatogram of a human milk extract is presented.

The concentration of total CBs in human tissue (the sum of di-, tri-, tetra-, penta-, and hexa-chlorobenzene isomers) is of the same order of magnitude as those of PCBs and organochlorine pesticides in some European contries (WASSERMANN et al. 1975, 1979). The acute oral toxicity of CB, expressed as the LD 50 in the rat, is 500 mg/kg for 1,4 - DCB (LEHMAN 1952), and similar to that of organochlorine pesticides (125, 500, and 6000 mg/kg for gamma-, alpha-, and beta- HCH isomers, respectively; CHRISTENSEN et al. 1976). For the infant, the potentially harmful effect of CBs from the daily consumption of human milk is important. Based on the assumption that the average infant (5 kg of weight) consumes daily approximately 0.5 kg of breast milk, the daily intake of total CBs can be estimated as 4.6 µg/kg body weight. For the breast fed infant, the calculated intake of CBs is approximately equal to the daily intake of organochlorine pesticides and PCBs in some contries (HOFVANDER et al. 1981).

The distribution of some CB isomers in adipose fatty tissue and milk is different. If we take into consideration only 1,4-DCB and HCB we could explain the lower

concentration of 1,4-DCB in fatty tissue by its less lipophilic character. This reasoning is supported from data on the n-octanol/water partition coefficient (KÖNEMANN & LEEUWEN 1980), where log $P_{oct.}$ is 3.53 and 6.44, as well as from the bioconcentration factor (TULP & HUTZINGER 1978); here log BF is 2.33 and 3.89 for 1,4-DCB and HCB, respectively.

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