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Presence of Polychlorinated Biphenyl and Organochlorine Pesticide Residues and the Absence of Polychlorinated Terphenyls in Canadian Human Milk Samples

Jos Mes and David J. Davies

Department of National Health and Welfare, Health Protection Branch, Food Directorate, Tunney's Pasture, Ottawa, Canada

Polychlorinated biphenyls (PCBs) and organochlorine (OC) pesticides in human milk have been reported both in Canada (RITCEY 1972, MUSIAL 1974) and elsewhere (SIYALI 1973, SAVAGE 1973, GRACA 1974, PESENDORFER 1975). Concurrently, Japanese reports indicated the presence of polychlorinated terphenyls (PCTs) in human tissues (DOGUCHI 1973) and milk (NISHIMOTO 1973). This paper reports on a nation-wide survey of PCBs, PCTs and OC pesticides in the milk of Canadian mothers as part of a continuing program to follow trends in the levels of pesticides and industrial contaminants.

MATERIALS AND METHODS

Sampling

Samples were collected as previously described (MES et al. 1978). Since manual expression of the milk could not be guaranteed in all cases, instructions were to immediately transfer the milk from the breastpump to the residue free bottle, to avoid prolonged contact with any plastic or rubber parts.
Out of a total of 100 samples, 10 came from the Eastern region (Newfoundland, Prince Edward Island, Nova Scotia, New Brunswick), 25 from Québec, 35 from Ontario, 10 from the Central (Manitoba, Saskatchewan) and 20 from the Western (Alberta, British Columbia) regions.

Analytical Methods

All solvents were glass-distilled and free of interfering residues. Adsorbents were prepared as earlier reported (MES et al. in press). Oxychlordane and α-hexachlorocyclohexane (HCH) were 98% pure; Aroclor 1260 was of a technical grade and all other standards were 99% pure.
Extraction and cleanup. Fifty gram samples were extracted and their fat content determined according to MES et al. (in press). The fat was removed from the extract by low temperature precipitation (MCLEOD and WALES 1972).

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Separation. The fat free extract was chromatographed on a semi-micro silicic acid column (MES et al. 1976), which was extended with 4.5 g Florisil and topped with some anhydrous Na_2SO_4. The following 3 fractions were collected:

Fraction	Eluting solvent	Eluted compounds
I	35 ml hexane	PCB, HCB, some PCT, and p,p'-DDE
II	40 ml of 20% CH_2Cl_2 in hexane	PCT, p,p'-DDE, DDTs and chlordanes
III	40 ml of 60% CH_2Cl_2 in hexane	β-HCH, dieldrin, heptachlor epoxide

The fractions were carefully evaporated on a glass rotatory evaporator (< 30°C), the residues transferred in hexane to 15-ml centrifuge tubes and the volume adjusted to 1 ml.

Identification and quantification. Gas chromatography (GC) and quantification of PCBs and most OC pesticides was carried out on a 6% OV-210 + 4% SE-30 column (MES and CAMPBELL 1976). The β- and γ-HCH isomers were quantitated on a 5% OV-210 column (MES et al. 1977). Fractions I and II were also chromatographed on a Varian 1400 with Scandium Tritide electron capture detector for the determination of PCTs in every 5th sample, under the following conditions:

Column: 0.3 x 183 cm glass, packed with 3% OV-210 + 3% SE-30 on 60/80 Chromosorb W(AW) (0.3 g of each phase on 10 g solid support).

Temperatures: Injection: 255°C; Column: 264°C; Detector 268°C.

Aroclor 5460 (2.5×10^{-3} µg/5 µl injection) was completely eluted after 50 min at a flow rate of 45 ml N_2/min.

Confirmation of PCB. The fractions containing PCB (I) of every other 10 samples, exclusive of every 10th, were pooled to give ~ 4.5 µg of estimated PCB/pooled sample.

Thin layer chromatography (TLC) was carried out on precoated, aluminum oxide (type E) F_{254}, 20 x 20 cm plates (Brinkman Ltd., Canada), activated at 110°C for 1 hr. Half 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 plates were developed in 1% acetone in hexane (v/v) and the reference spots visualized 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 60% CH_2Cl_2 in

hexane and gas chromatographed as above.
The remaining fractions I, which were not pooled, were re-chromatographed on a 5% OV-210 column to confirm the identity of HCB, p,p'-DDE and PCB and then perchlorinated according to ARMOUR (1973) and the decachlorobiphenyl chromatographed on a 0.6 x 60 cm glass column packed with 4% SE-30 on 60/80 Chromosorb W(AW). The PCB fractions from TLC were analysed by mass spectrometry (MS) using a Varian Mat 311A coupled to a Varian 1400 Series gas chromatograph by a two-stage Watson-Biemann Separator and monitored for a single ion at m/e = 359.8.
Confirmation of OC pesticides. Components in fractions II and III were confirmed on 5% OV-210 (MES et al. 1976). Every other 10 samples were pooled and chromatographed (1-30 µg of estimated pesticide/spot) on TLC plates with appropriate standards (5-10 µg of individual pesticide/spot), eluted and re-chromatographed as above on two different GC columns.
Single ion monitoring MS was carried out on HCB (m/e = 283.8), β-HCH (m/e = 218.9), oxychlordane (m/e = 386.8), trans-nonachlor (m/e = 408.8), heptachlor epoxide (m/e = 272.0), dieldrin (m/e = 380.0), p,p'-DDE (m/e = 317.9), and p,p'-DDT (m/e = 235.0).
Controls. Every 20th sample was repeated by fortifying it at the 0.1 ppm level with every compound analysed for, except γ-HCH and trans-nonachlor. At different times during the survey blanks were run through the complete analytical procedure.

RESULTS AND DISCUSSION

The data in Table 1 show the average residue levels in the milk of Canadian mothers, as collected in 1975.

TABLE 1

Organochlorine residues in Canadian human milk

Compound	ng/g whole milk		% of samples with residues ≥ 1 ng/g whole milk
	Average[a]	Max. observed	
PCB, as Aroclor 1260	12	68	98
HCB	2	21	81
β-HCH	2	21	91
Heptachlor epoxide	1	3	69
Oxychlordane	1	2	77
trans-Nonachlor	1	2	68
p,p'-DDE	35	144	100
Dieldrin	2	6	84
o,p'-DDT	3	48	32
p,p'-DDT	6	21	100

[a]Averages are based on the number of samples with residues ≥ 1 ng/g whole milk.

Polychlorinated biphenyls, hexachlorobenzene (HCB), β-HCH, p,p'-DDE, dieldrin and p,p'-DDT were present in more than 80% of the samples. All samples contained p,p'-DDE and p,p'-DDT. All samples, which did not register residues of oxychlordane and trans-nonachlor ≥ 1 ppb, did indicate what appeared to be trace (< 1 ppb) amounts of these two compounds.
Compared to previous surveys on Canadian human milk by RITCEY et al. (1972) and unpublished data from this laboratory, heptachlor epoxide, p,p'-DDE, dieldrin, o,p'-DDT and p,p'-DDT levels all decreased over an eight year period.

TABLE 2

Trends of organochlorine residues in Canadian human milk

	Average ng/g whole milk		
	Year of sample collection		
Compound	1967	1970[a]	1975
PCB, as Aroclor 1260		6	12
HCB			2
β-HCH			2
γ-HCH	3	2	
Heptachlor epoxide	3	4	1
Oxychlordane			1
trans-Nonachlor			1
p,p'-DDE	103	56	35
Dieldrin	5	5	2
o,p'-DDT	5	3	3
p,p'-TDE	4	3	
p,p'-DDT	33	15	6

[a] Unpublished data

At the same time other compounds have been detected such as HCB, β-HCH, oxychlordane and trans-nonachlor. Lindane (γ-HCH) was only found in 8 samples at levels ≥ 1 ppb and all in Ontario. Nevertheless 71% of the samples apparently still contained traces of lindane, which could not technically be confirmed by MS.
Whether or not the PCB increase between 1970 and 1975 was significant will be difficult to determine with the limited data. It was observed, however, that perchlorination accounted for only 38% of the PCBs as calculated on the basis of GC results. A duplicate sample fortified before the perchlorination step with Aroclor 1260 at 0.2 ppm gave 75.7% recovery with a standard deviation of ±0.5. A single perchlorination of 10 µg Aroclor 1260 gave 110% recovery. If this apparent discrepancy is indeed valid, the average 1975 PCB level

could well be lower than the 1970 one. Consequently, at the same time, this could indicate the presence of unknown contaminants in the PCB fraction of Canadian human milk. The authors earlier described (MES et al. in press) the unusual ratios of two PCB peaks in 14% of the milk samples, which could not be attributed to Mirex.

Table 3 shows the geographic distribution of the residues in the latest survey. The highest average PCB, p,p'-DDE and p,p'-DDT levels were found in Ontario

TABLE 3

Regional distribution of organochlorine residues in Canadian human milk

Compound	Regional averages[a] in ng/g whole milk				
	Eastern	Quebec	Ontario	Central	Western
PCB, as Aroclor 1260	8	10	17	8	15
HCB	1	1	2	1	4
β-HCH	1	1	3	2	2
Heptachlor epoxide	1	1	1	1	2
Oxychlordane	1	1	1	1	1
trans-Nonachlor	1	1	1	1	1
p,p'-DDE	29	34	34	21	59
Dieldrin	2	1	2	1	2
o,p'-DDT	3	1	6	-[b]	1
p,p'-DDT	5	7	6	5	8

[a] Averages based on the number of samples with residues $\geq$ 1 ng/g whole milk.

[b] None detected.

and the Western region. The Central region had the lowest residue levels. The latter was also observed in data from human adipose tissue (MES et al. 1977).

Figure 1 shows two unknown peaks in fraction III, chromatographed on two different columns. It is plausible that peaks x and y in chromatogram A represents the same compounds as peaks A and B in chromatogram B, but further investigation is needed. These two unknown peaks appear in most samples and only differ in their magnitudes.

All reported compounds were confirmed by MS. Recoveries from fortified samples ranged from 84-98% for all pesticides, except HCB and PCB, which were recovered at 78 and 70%, respectively. The low PCB recovery will be further investigated, since preliminary work had indicated >95% recovery from Florisil-silicic acid column. The data in Table 1 were not corrected for these low recoveries.

The average lipid content of the human milk samples was 2.20% with a standard deviation of ± 1.35.

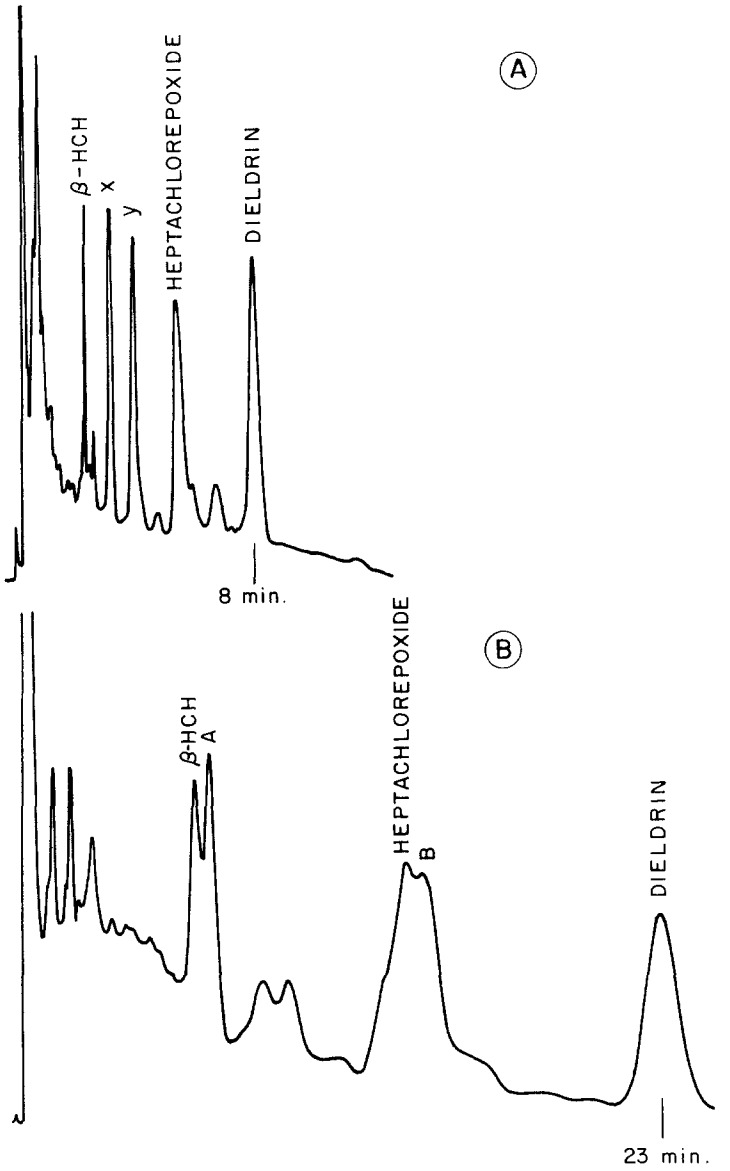

FIG. 1 Chromatogram A represents a GC elution pattern of a particular fraction III on 6% OV-210 + 4% SE-30. Chromatogram B represents the same fraction but on 5% OV-210.

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