Bull. Environm. Contam. Toxicol. 19, 564-570 (1978)

Traces of Mirex in Some Canadian Human Milk Samples

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Introduction

Mirex (1,2,3,4,5,5,6,7,8,9,10,10-dodecachlorooctahydro-1,3,4-metheno-2H-cyclobuta (c,d) pentalene) has mainly been associated with the control of the Fire ant in the Southeastern U.S.A. (ALLEY 1973), where it was subsequently found in non-target organisms (COLLINS et al. 1973, WOJCIK et al. 1975) including man (ANONYMOUS 1976). Recently Mirex has been found in Canada in fish from Lake Ontario (KAISER 1974) and in eggs of double crested cormorants (ZITKO 1976). This paper reports on a limited study of Mirex in human milk samples from across Canada, using U.V. irradiation to limit interference from polychlorinated biphenyls (PCBs).

Sampling

Fourteen human milk samples were selected from samples acquired during a recent human milk survey of polychlorinated biphenyls. Five samples came from Ontario, 2 from the Eastern (New Brunswick, Nova Scotia), 3 from the Central (Manitoba, Saskatchewan) and 4 from the Western (Alberta, British Columbia) region.
The selection was based on observed Aroclor 1260 patterns, where a proportionally large peak 13 (REYNOLDS, 1969) could indicate the presence of Mirex under the gas chromatographic (GC) conditions used. The samples were collected, during the first 4 to 5 days of lactation, in previously washed, heated (350°C) and hexane rinsed bottles with aluminum lined caps. Sample weights ranged from 18.5 to 114.0g.

Analytical Methods

Glass-distilled, residue free solvents were used. Aroclor 1260 and Mirex (99.9%, pure) were obtained from Monsanto Chemical Co. and EPA-Research Triangle Park N.C. respectively. Silicic acid (Mallinckrodt 2847), Florisil, anhydrous Na_2SO_4 and Celite (545,

0007-4861/78/0019-0564 $1.40

AW) were soxhlet extracted with CH_2Cl_2 until free of interfering residues, and heated at 130°, 300°, 90° and 80°C respectively. Silicic acid was deactivated with 4.4 % and Florisil with 2% H_2O.

Extraction and cleanup. The total milk sample was weighed and centrifuged at 1200 rpm for 20 min in a Squibb separatory funnel. The fat layer was saved and extracted with 100 ml of 5% benzene in acetone (v/v) for 2 min. Any precipitate was removed by a Buchner funnel and the acetone evaporated on a glass rotatory evaporator (<30°C). The wet residue was redissolved in hexane and filtered through ~ 2.5 cm of anhydrous Na_2SO_4. The hexane was evaporated as above and the residue redissolved in acetone for precipitation of the fat at - 78°C (MCLEOD et al. 1972).

Separation. A slurry of 4.5 g silicic acid and 1 g Celite in hexane was poured into a glass column (12 mm O.D. x 40 cm) with a 50 ml reservoir and teflon stopcock and packed under N_2 pressure. Excess hexane was eluted until a few mm from the top of the adsorbent. Dry Florisil (4.5 g) was added to fill the remaining column space. The fat free residue was applied in hexane to the Florisil-silicic acid column. Mirex and PCBs were collected under pressure in 35 ml of hexane at a flow rate of 2-2.5 ml/min.

Mass spectrometry (MS). A Varian Mat 311A coupled to a Varian 1440 GC by a Watson-Biemann separator was used.

The hexane fraction from the column was evaporated under N_2 and redissolved in 100 µl CH_2Cl_2. Single ion monitoring was carried out with 5 µl injections of sample and standard (2 ng/µl) at m/e = 545.6 and m/e = 271.8. Mirex was estimated using the molecular ion peak (m/e = 545.6).

U.V. irradiation. Standard solutions were prepared in 5% diethylamine (DEA) in hexane (Table 1). Two

TABLE 1

Standard solution	Concentration in ug/ul Arochlor 1260	Mirex	Ratio PCB/Mirex
A		0.96×10^{-4}	
B	9.4×10^{-4}		
C	9.4×10^{-4}	0.96×10^{-4}	10
D	9.4×10^{-3}	0.19×10^{-4}	50
E	3.1×10^{-3}	0.96×10^{-4}	32
F	6.3×10^{-3}	0.96×10^{-4}	66

ml of solutions A and B were irradiated according to LEWIS et al. (1976) in a quartz cuvette with cork stopper at 8 cm distance from the U.V. light source (General Electric germicidal lamp 615T8, 15W).

Recoveries were checked at 20, 25, 30, 45 and 60 min. A 2 ml aliquot of solutions C to F was irradiated for 30 min to determine Mirex recovery.
The hexane fraction remaining after MS analysis was concentrated, transferred to a quartz cuvette, evaporated and redissolved in 1 ml of 5% DEA in hexane. Samples were irradiated for 30 min.
Gas chromatography. After irradiation 10 µl out of 250 µl were injected into a Varian 1400 with Scandium Tritide electron capture detector, under the following conditions:
Column: 0.6 x 183 cm, glass, packed with 3% OV-210 + 3% SE-30 on chromosorb W(AW) 60/80 (0.3 g of each phase on 10 g solid support).
Temperatures: Injection : 222°C; Column : 183°C; Detector : 262°C. Under these conditions the absolute retention time of Mirex was approximately 60 min.
Ten microliter injections of solutions A and B were made before and after every two sample injections. Peak heights were used for quantification.
Controls. Two human milk samples, spiked with 0.1 and 0.01 ppm Mirex, and one blank were subjected to the entire analytical procedure including the U.V. irradiation.

Results and Discussion

The calculated peak height ratios of peaks 13/8 in aroclor 1260 in human milk samples were 0.56 and 0.40 for a 3% OV-210 + 3% SE-30 and 5% OV-210 GC column respectively. Standard aroclor 1260 gave ratios of 1.03 and 0.65 for the same columns. The ratios of most of the samples used in this investigation were considerably > 1.00 (Fig. 1). The PCBs of these samples were chromatographed under conditions where Mirex and peak 13 would give identical retention times. The unusual ratios could have been caused by the presence of Mirex.
The length of time Mirex was irradiated did not seem to affect its recovery (Table 2), while the overall effect on PCB recovery was not considered large enough to irradiate more than 30 min.

TABLE 2

Effect of duration of U.V. irradiation on Mirex and PCB recoveries

Time (min)	% Mirex recovered	% Peak 13 of aroclor 1260 recovered
20	90	12
25	87	8
30	86	10
45	91	7
60	90	5

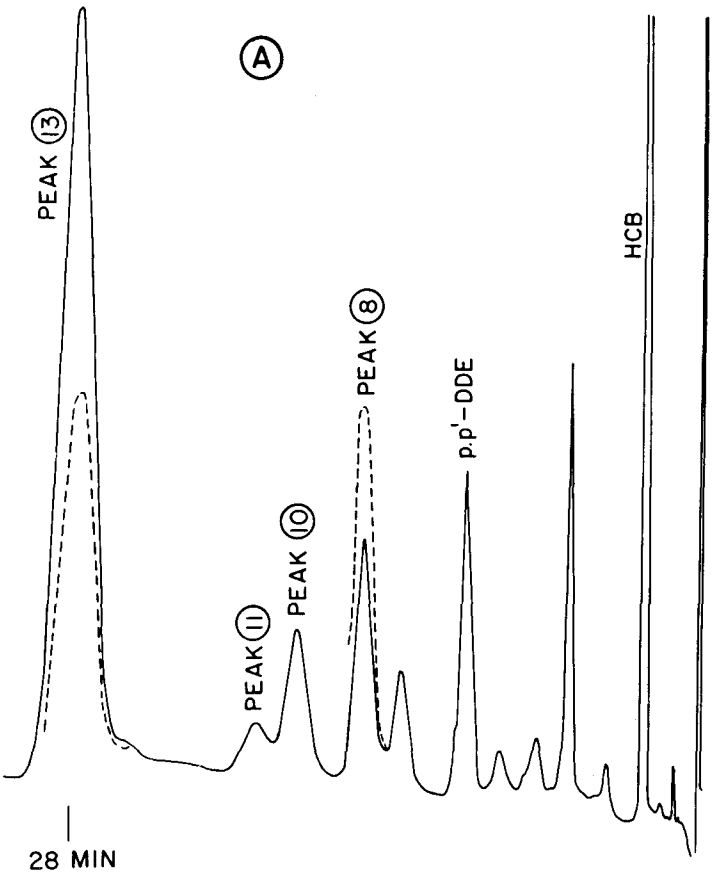

Fig. 1. Chromatogram A represents a typical PCB elution pattern of human milk used in this investigation. The broken line indicates relative response of peak 8 and 13 in standard aroclor 1260 under the same conditions.

In mixtures of Mirex and PCB, varying the mirex content did not seem to affect recovery of the latter (Table 3).

TABLE 3

The effect of varying the PCB/Mirex ratio during irradiation on Mirex recovery

Ratio PCB/Mirex	Quantity kept constant	% Mirex recovered[a]
10	PCB	90.9 ± 7.4
50	PCB	92.7 ± 5.0
32	Mirex	78.3 ± 1.5
66	Mirex	82.6 ± 3.7

[a] 3 determinations.

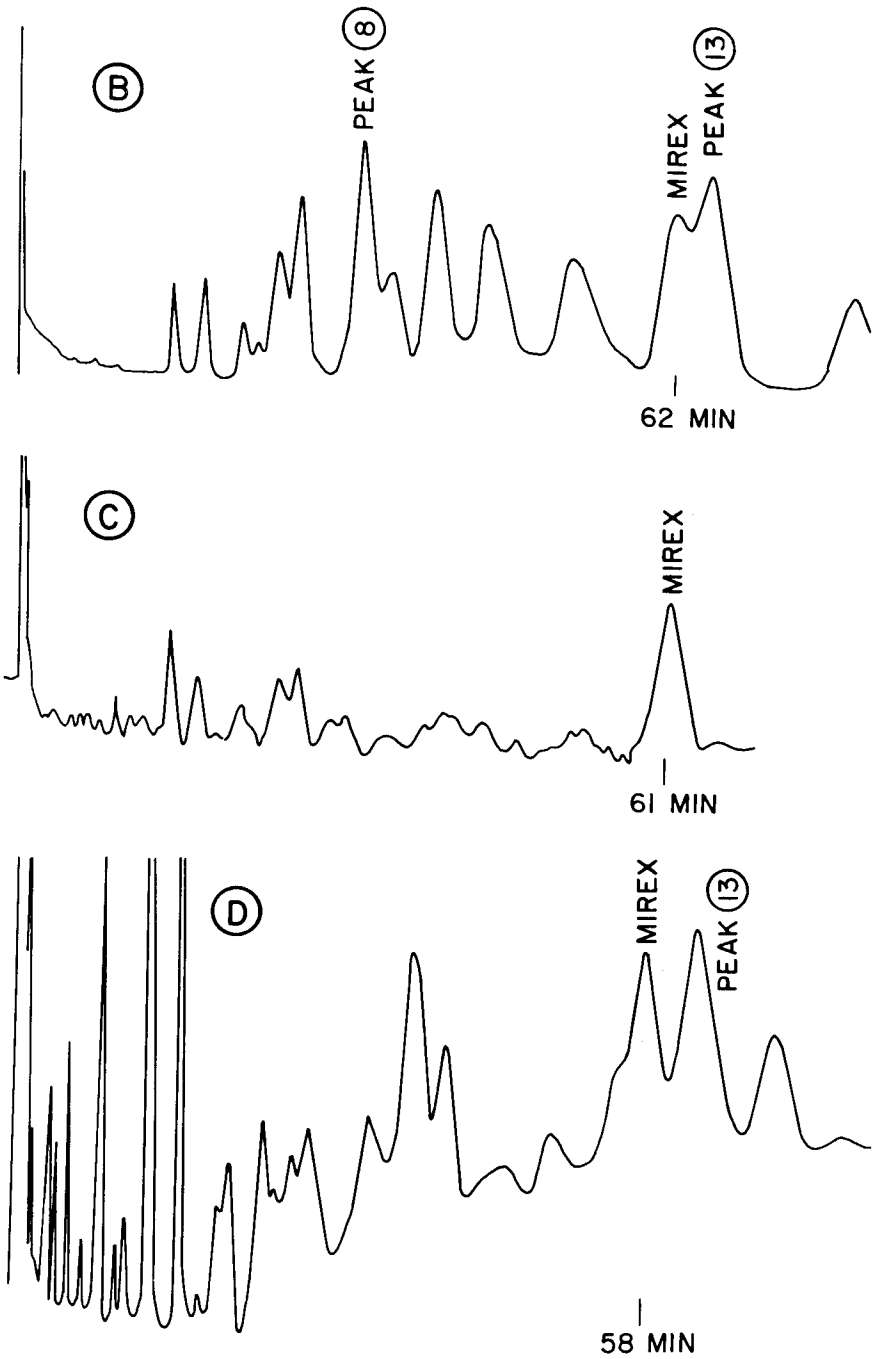

Fig. 2. The gas chromatograms B and C are elution patterns of standard solution C before and after irradition. Chromatogram D represents an irradiated human milk sample.

Increasing the PCB content reduced Mirex recovery by approximately 10%. Examples of before and after U.V. irradiation are given in Fig. 2, chromatograms B and C.
The recovery of Mirex from spiked samples at the 0.1 and 0.01 ppm level were 88 and 77% respectively. The blank was free of interfering residues.
Mirex was found in 3 out of the 14 samples analysed (Fig. 3). Although Mirex can be identified by GC,

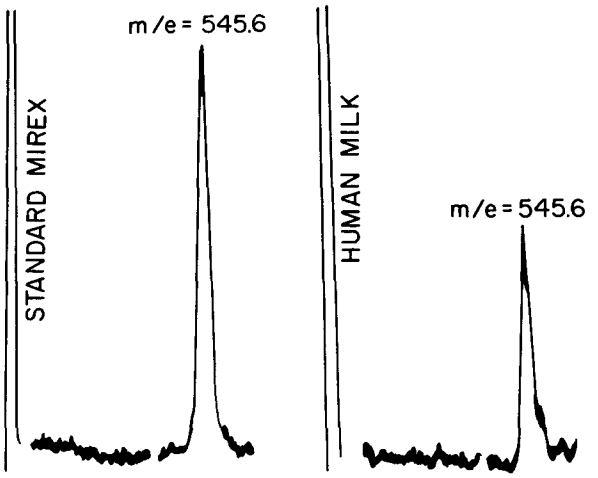

Fig. 3. Mass spectrogram of Mirex in human milk at $M/\Delta M = 2000$.

using irradiation to limit PCB interference and its presence confirmed by MS, quantitative results at these low levels obtained by GC remain questionable as indicated by the poor agreement between MS and GC data. The data in Table 4 are more an indication of the presence of Mirex than their absolute levels.

TABLE 4

Mirex in human milk samples

Sample	ng/g			
	MS		GC	
No.	wet weight	fat	wet weight	fat
1	0.6	15.3	0.4	9.3
2	0.3	21.5	0.2	11.1
3	0.2	6.4	0.1	2.3

All positive samples came from cities on Lake Ontario and Erie, where the Mirex problem was first identified (KAISER, 1974).
Chromatogram D in Fig 2 shows a human milk sample containing Mirex. At the <1 ppb level interference is still considerable.
The small amounts of Mirex do not account for the large ratio differences found in the human milk samples selected for this study compared to others previously analysed for PCB. This still remains an interesting question.

Acknowledgement

The authors wish to thank Drs. T. Panalaks and J. Lawrence for reviewing this paper.

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