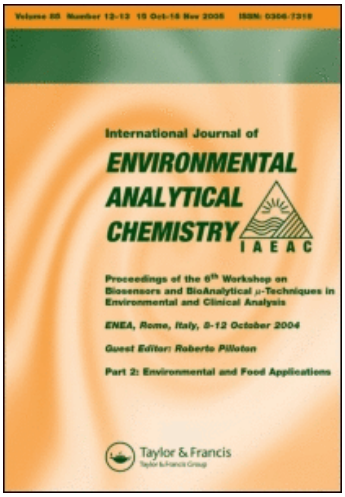

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Experiences in Human Milk Analysis for Halogenated Hydrocarbon Residues

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Experiences in Human Milk Analysis for Halogenated Hydrocarbon Residues

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Factors affecting the different stages of human milk analysis for halogenated hydrocarbon residues are explored. The variation in milk fat during breast feeding and its consequences for sampling are outlined. Extraction efficiency is discussed in terms of fat content, residue recovery and sample size.

The importance of clean-up procedures is illustrated by the removal of fat from the extract and unknown contaminants from adsorbents. Polychlorinated biphenyls (PCBs) are partially separated from other halogenated hydrocarbon residues on a Florisil-silicic acid column.

Both packed and capillary gas chromatographic columns can be used for identification and quantification of residues in human milk.

Confirmation by chemical derivation and mass spectrometry is illustrated by the perchlorination of PCBs and PCB isomer distribution respectively. Fortification of samples and the use of a collaborative study are described as a means for method evaluation.

KEY WORDS: Human milk, residue, recovery, effect.

INTRODUCTION

Halogenated hydrocarbon residues in human milk, such as polychlorinated and polybrominated biphenyls, Mirex, polychlorinated terphenyls and many organochlorine pesticides, have been the subject of many studies.[1-9] The purpose of most of these studies was to gather residue data to determine the extent of infant exposure and to evaluate the potential health hazard. Few studies dealt with the analytical problems and limitations of analyzing halogenated hydrocarbon residues in human milk. Nevertheless, the residue data from such monitoring studies are often used to help to determine an acceptable level of infant exposure.

In this paper some of the analytical aspects of human milk analysis will be considered from the point of view of sampling, extraction, clean-up, separation, identification, quantification, confirmation and evaluation.

SAMPLING

Sampling is an important part of any analytical procedure, but especially so in the case of biological samples. A representative sample of human milk is difficult to obtain, both because of its limitation in sample size and possible variation in fat composition. It has been reported, that the fat content of human milk not only increases during the feeding of the infant, but changes from feeding to feeding during the day.[8] Hytten reported a difference in the fat content of the milk between the right and left breast.[10] This author also observed, that the fat content was at its lowest and highest at the 6 and 10 a.m. feedings, respectively. Table I shows some of

TABLE I

Variation in fat content of human milk during feeding

Sample code[a]	% Fat	
	Donor 1[b]	Donor 2[c]
M1	2.21	0.97
M2	1.86	0.54
M3	2.55	0.66
M4	3.80	0.55
M5		0.73
M6		0.63
MD1	0.55	1.10
MD2	0.89	1.09
MD3	1.93	1.34
MD4	3.55	1.52
MD5	4.69	1.85
MD6		1.48
E1	1.35	3.19
E2	1.59	3.21
E3	2.47	3.43
E4	3.78	3.63
Average	2.40	1.62

[a] M = morning feeding.
MD = mid-day feeding.
E = evening feeding.
[b] ~ 20 ml fractions.
[c] ~ 10 ml fractions.

this variation in the fat content of human milk. Both donors expressed milk from the right breast only and did not feed the infant on that breast between morning and evening milk expressions. Donor 1 expressed milk at 7 a.m. and 1 and 6.30 p.m. on her 126th day of lactation, while donor 2 expressed milk at 5.30 a.m. and 11.30 and 5.30 p.m. on her 177th day of lactation. The importance of the fat content, for the determination of halogenated hydrocarbons, lies in the lipophilic nature of these contaminants and its use for calculating residue levels on a fat basis. Therefore, the timing in milk sampling is important. At the present state of our knowledge it seems prudent to take a random sample.

The mother should be asked to express her milk at different times during a particular feeding, at all feedings during the day and alternating between right and left breast. The amount of sample to be collected depends on the requirements of the analytical procedure, expected residue levels and availability. A 50-ml sample, which represents approximately less than 10% of the total volume of milk available per day, would be ample, but not necessarily representative. The milk should be collected without external contamination.

In our laboratory a special kit is prepared, consisting of a 4 oz brown bottle with Teflon-lined cap, a 16-oz large-mouth jar, a 50-mm glass funnel and instruction sheet with questionnaire. All glassware in the kit is washed and heated at 350°C for 1 hr and finally rinsed 5× with acetone and 3× with hexane. The funnel is wrapped in aluminum foil, of which the non-shiny side (in contact with the funnel) has been rinsed with hexane. The coded questionnaire covers such questions as age of mother, fish eating habits, pesticide exposure, menstrual irregularities, number of babies breast fed, etc. The mother is requested to express the milk manually into the large mouth jar, transfer it to the brown bottle via the glass funnel and keep the sample refrigerated between collections. After the final collection the milk should be frozen, and kept that way during transport and storage until analysed.

EXTRACTION

Halogenated hydrocarbon residues are lipophilic and may be isolated by separation or extraction of the milk fat. This can be done by direct solvent extraction of the human milk or by a combination of physical separation of fat from milk and solvent extraction as shown in Figure 1. The choice of extraction method will depend on the extraction efficiency of a particular residue or residues, required accuracy and available time and equipment. In our laboratory, PCBs in human milk have ben analyzed, using several extraction techniques and solvents, including saponification

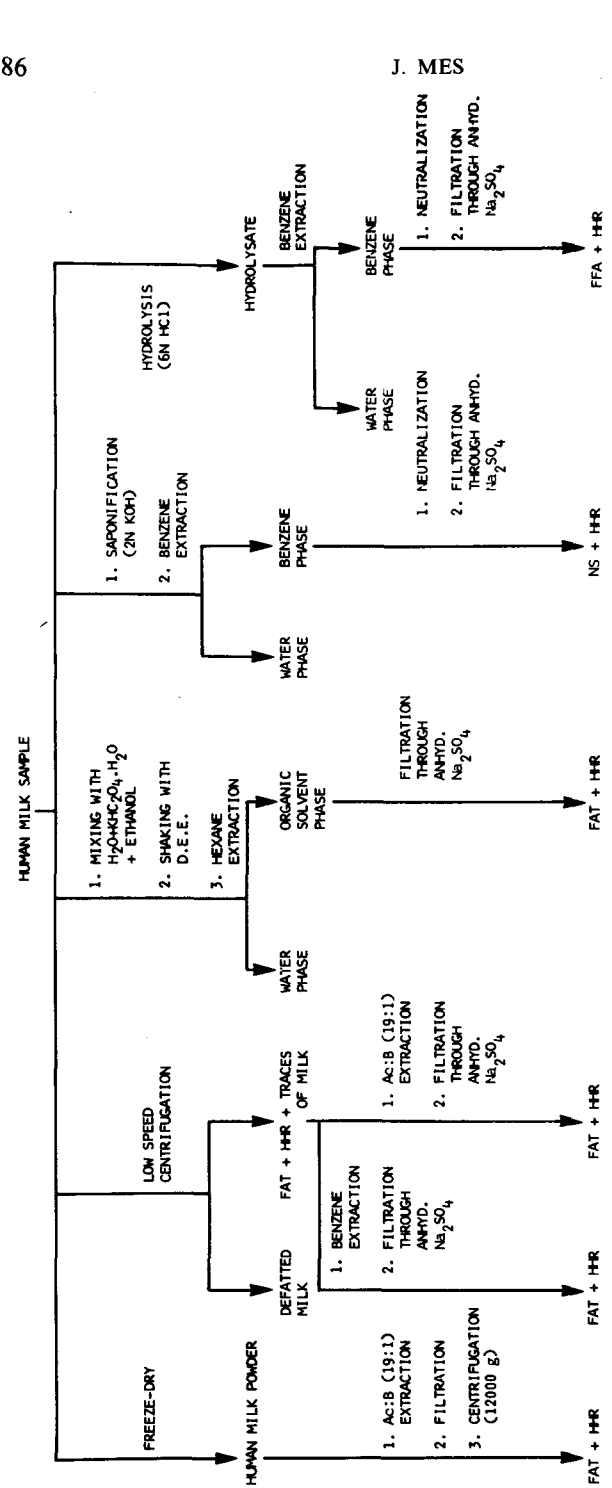

FIGURE 1 Scheme for the extraction of halogenated hydrocarbons from human milk by various methods.

and acid hydrolysis. The latter two procedures have the advantage that halogenated hydrocarbon residues can be extracted with considerable less lipid material, but the disadvantage that some residues may be destroyed or altered.

PCBs however are an important exception. In the saponification method, the fat content can be determined by acidifying the alkaline water layer after benzene extraction. The results in Table II are based on 25 g samples, but for hydrolysis and saponification only 10 g samples were used. Although the "direct extraction" method gave the highest PCB level, it also showed the greatest variation (coefficient of variation = ~30).[11]

Small samples (<2 ml) would eliminate the need to physically separate the fat by centrifugation prior to extraction.[12]

TABLE II

The effect of residue isolation techniques on the fat and PCB content of human milk

Method	Extraction solvent	% fat	C.V.	ng PCB per g whole milk[a]	C.V.
Freeze-drying	Ac:C_6H_6 (19:1)[d]	1.22	0.8	7.0	6.2
Centrifugation	C_6H_6	1.17	3.8	7.4	2.3
Centrifugation	Ac:C_6H_6 (19:1)	1.13	0.5	7.2	2.9
Direct extraction	EtOH-DEE[c]-Hex	1.34	2.2	8.6	32.6
Acid hydrolysis	C_6H_6	N.A.		6.6[b]	3.2
Saponification	C_6H_6	N.D.		7.0[b]	7.1

[a]Triplicate determinations.
[b]Duplicate determinations.
[c]Ethanol-diethylether.
[d]Acetone.
N.A. = not applicable.
N.D. = not determined.
C.V. = coefficient of variation.

Table III compares the fat and PCB content obtained by using the macro (25 g) and micro (2 g) methods. There seems to be little difference in the fat content. The lower values for the micromethod may have partly been caused by losses incurred during the fat determination step, where the entire extract is transferred to and evaporated in an aluminum weighing dish before column chromatography. In the macromethod, only an aliquot of the extract is removed for fat determination. However, both macro- and micro-values may well fall within the expected variation at these low residue levels.

TABLE III

The effect of sample size on the extraction efficiency of fat and PCBs from human milk

Extraction method[a]	Extraction solvent	% Fat[b]				ng PCB/g whole milk[a]			
		Macro	C.V.	Micro	C.V.	Macro	C.V.	Micro	C.V.
1	Ac:C_6H_6 (19:1)	1.22	0.8	0.92	0.0	7.0	6.2	6.9	4.3
2	C_6H_6	1.17	3.8	0.77	5.1	7.4	2.3	4.4	4.5
3	Ac:C_6H_6 (19:1)	1.13	0.5	1.13	5.3	7.2	2.9	5.1	5.9
4	DEE-Hex	1.34	2.2	1.25	0.8	8.6	32.6	6.7	6.0

[a]1 = freeze-drying for both macro- and micromethod.
2 and 3 = centrifugation and homogenization for macro- and micromethod, respectively.
4 = direct extraction for both macro- and micromethod.
[b]Triplicate determinations.

What is not apparent from Table III is the background problem during gas chromatography. Figure 2 compares chromatograms obtained with the macro and micromethods. The negative background differed in magnitude. From this example it is evident, that quantification poses a problem.

However, using a capillary GC system good agreement and acceptable chromatograms were obtained comparing macro and micromethods (Table IV and Figure 3). Extraction solvents were not the same: acetone:benzene (19:1 v/v) was used in the micro and benzene in the macromethod. The differences between results in Table III and IV may lie in the magnitude of the residue levels measured. The lower the residue level the more difficult the determination will be.

Although there is no strong indication, which of the two methods is superior, the higher concentration of residues obtained with the macromethod would give less background problems in GC analyses.

CLEAN-UP

Clear-up procedures are very important, when minute quantities of residues have to be analyzed. Both the sample itself and all other media with which the sample comes in contact should be free of interfering contaminants. In human milk the fat and extraneous matter, such as colors, have to be removed in order to clearly identify the residues by GC. In our laboratory two approaches have been used: precipitation of the fat at $-72°C$ (freeze-out)[12] and/or retention on a Florisil column. Table V shows to what extent fat can be removed on a semi-micro combined Florisil-silicic acid column. The latter can take up to 350 mg of fat before contaminating the PCB fraction (35 ml Hexane). A combination

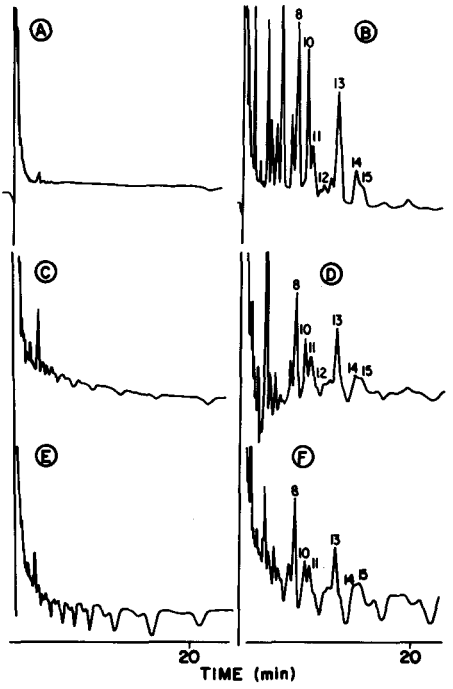

FIGURE 2 The GC elution pattern of PCBs in human milk (B, D, F) and blanks (A, C, E) on packed column (183 × 0.6 cm; 6% OV-210 + 4% SE-30 on Chromosorb W (AW) 100/120; column temperature 208°C). Chromatograms A and B: macromethod (freeze-dried sample); C and D: micromethod (freeze-dried sample); E and F: micromethod (centrifuged sample).

of the freeze-out and column techniques is required for large samples with high lipid content.

Adsorbents and solvents are two of the most important contributors to sample contamination. Glass distilled solvents, although free of interfering residues under normal conditions (concentration 250:1), show contaminants when large volumes are concentrated to 1 ml or less. Adsorbents, such as Florisil and silicic acid should be cleaned to remove all traces of contamination, that could interfere at the level of sample concentration expected during the analysis.

Figure 4 shows a slightly modified soxhlet extraction set-up for washing adsorbents. This modification allows the collection of extraction solvent (CH_2Cl_2) during the siphoning cycle via a two-way stopcock. A 50-ml aliquot is collected and concentrated to 0.2 ml. Figure 5 shows contaminants found in successive washings.

TABLE IV

A comparison of the PCB levels in human milk between the macro- and micromethods

Sample no.	ng PCB/g whole milk		Ratio
	Macromethod	Micromethod	Macro/micro
1HM/7	32	32	1.00
1HM/14	25	29	0.86
1HM/28	24	24	1.00
1HM/42	14	13	1.08
1HM/70	13	15	0.87
2HM/14	36	29	1.24
2HM/28	26	32	0.81
3HM/7	6	14	0.43
5HM/14	26	27	0.96
5HM/28	20	30	0.67

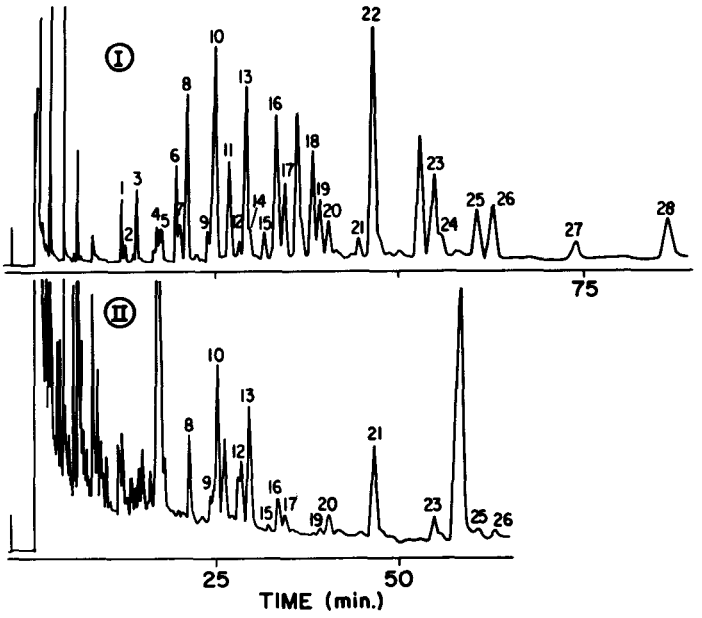

FIGURE 3 The GC elution patterns of standard Aroclor 1260 (I) and PCBs in human milk (II) on capillary column (36 m × 0.5 mm; SP 2100; column temperature 224°C).

TABLE V

Retention of rapeseed oil on a combined semi-micro Florisil-silicic acid column

	% Recovery[b]					
	Lipid load					
Fraction[a]	50 mg	C.V.	100 mg	C.V.	200 mg	C.V.
I	0.2	0	N.D.		N.D.	
II	0.3	33	0.1	0	0.1	0
III	0.2	0	0.5	20	0.8	13
IV	7.0	37	12.1	12	53.9	15
V	83.0	3	80.9	2	41.6	19
VI	N.C.		8.4	2	5.2	104

[a]I = 35 ml hexane; II to V = 40 ml of 20, 40, 60 and 80% $CHCl_3$ in hexane respectively; VI = 40 ml of 20% ethylacetate in CH_2Cl_2.

[b]Average of 3 determinations.

N.D. = not detected; N.C. = not collected.

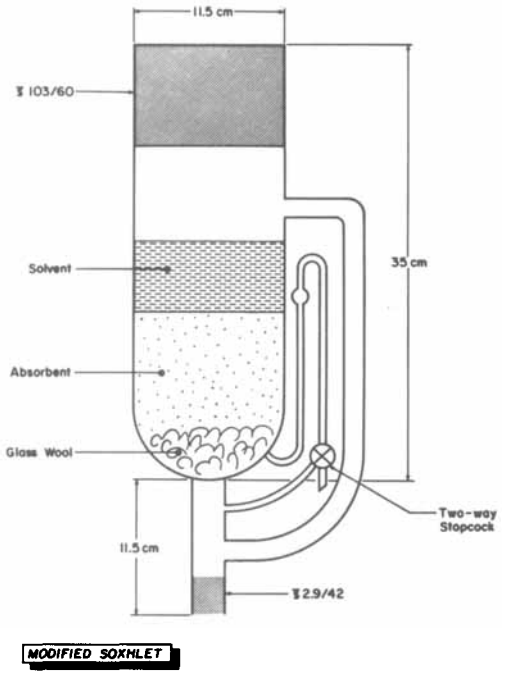

FIGURE 4 Modified soxhlet apparatus for washing adsorbents.

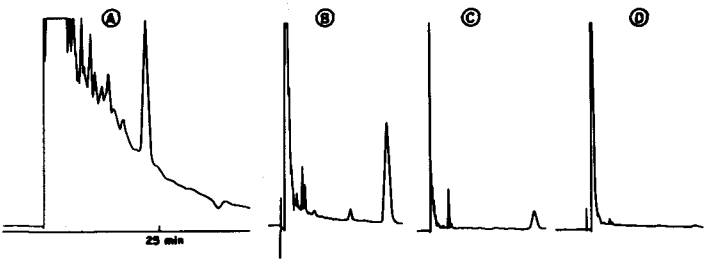

FIGURE 5 Contamination in Florisil washings (250:1). Chromatogram A = after 1st siphoning; B = after 4 hrs; C = after 8 hrs; D = after 12 hr.

SEPARATION

Human milk is often monitored for residues of different polarity such as PCBs, DDTs, Dieldrin and Heptachlorepoxide. A pre-gas chromatographic separation is not only desirable, but often necessary. In our laboratory a combined semi-micro Florisil-silicic acid column is used to separate halogenated hydrocarbons into several fractions. Table VI shows the elution pattern of a selected number of halogenated hydrocarbons, many of them appearing in human milk. It is evident from Table VI, that many compounds elute in more than one fraction, especially Aroclors. It is fortunate, that the PCB pattern in human milk closely resembles that of Aroclor 1260, which elutes 95% in one fraction. Recently however, lower chlorinated biphenyls have been reported in human milk.[13] These lower chlorinated biphenyls would elute in more than one fraction. Nevertheless, many other compounds can still interfere with the PCB analysis, such as p,p'-DDE and Mirex.

IDENTIFICATION AND QUANTIFICATION

Individual compounds can be identified by gas chromatography. The type of column and/or stationary phase to be used depends on the analytical requirements and available equipment. For example, PCBs can be identified on both packed and capillary columns. Figure 6 shows PCBs in human milk chromatographed both ways. In our laboratory PCBs in human milk are estimated on a packed column, using peaks 8 and 10–15, according to the numbering system of Reynolds[14] and Jensen and Widmark.[15] On the capillary column, peaks 8–13, 15–17, 19, 20, 22, 23, 25, 26 and 28 were used according to our own numbering system. Quantification will only be as accurate as the separation is good. No

doubt the capillary column gives better separation than the packed column, but from experience in our laboratory there was no quantitative difference. This could indicate, that compounds other than PCBs were not interfering on the packed column. Capillary gas chromatography holds the

TABLE VI

Elution pattern of some halogenated hydrocarbons from a combined Florisil-silicic acid column

Compound (2.5 μg each)	% Recovery									
	Fraction[a]									
	I	C.V.	II	C.V.	IV	C.V.	VI	C.V.	Total	C.V.
Aroclor 1016	62	5	43	3	—		—		105	3
Aroclor 1242	54	9	47	10	—		—		101	4
Aroclor 1254	88	2	16	21	—		—		104	4
Aroclor 1260	95	1	7	14	—		—		102	1
Aroclor 5460	36	4	74	6	—		—		110	5
Mirex	88	8	*t*		2	140	—		90+	9
Photomirex	107	3	3	13	—		—		110	3
p,p′-DDE	19	1	88	3	2	5	*t*		109	2
pentachlorobenzene	107	3	1	50	—		—		108	2
hexachlorobenzene	95	2	*t*		—		—		95+	2
heptachlor	22	94	79	43	1	140	—		102	11
Polybrominated biphenyl	84	6	22	57	4	68	—		110	9
Methylated pentachlorophenol	—		101	6	*t*		—		101+	6
heptachlor epoxide	—		1	40	87	3	*t*		88+	2
o,p′-DDT	—		102	6	—		—		102	6
p,p′-DDT	—		107	7	—		—		107	7
oxychlordane	—		113	5	*t*		—		113+	5
γ chlordane	—		90	2	*t*		—		90+	2
α hexachlorocyclohexane	—		96	3	*t*		*t*		96+	3
β hexachlorocyclohexane	—		26	7	74	3	*t*		100+	<1
γ hexachlorocyclohexane	—		93	3	7	16	*t*		100+	3
toxaphene	—		90	2	8	13	*t*		98+	3
dieldrin	—		—		96	2	*t*		96+	2
methoxychlor	—		—		94	7	12	45	106	2
octachlorodibenzo-p-dioxin	—		—		5	36	85	7	90	9
octachlorodibenzofuran	—		—		8	6	94	4	102	4

[a]Fractions are the same as in Table V.

[b]*t* = trace (< 1 %).

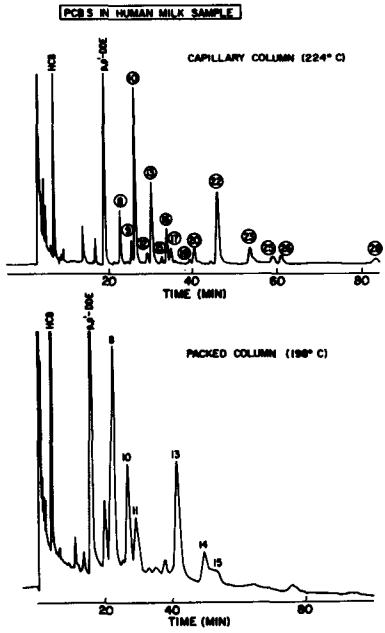

FIGURE 6 GC elution patterns of PCBs in human milk on capillary and packed columns.

possibility of eliminating pre-gas chromatographic fractionation, because it can separate many more compounds at a given time. The simultaneous separation of 17 organochlorine pesticides is shown in Figure 7. This figure also illustrates that caution is needed in identification, since column temperature can affect elution time on capillary columns, as indicated by p,p'-DDE and dieldrin.

CONFIRMATION

At present PCBs are the most important industrial contaminant in human milk and when identified, their presence should be confirmed, if at all possible. In a single sample the residue level is often low for mass spectrometry or chemical derivation, and pooling of samples may be necessary. The determination of PCBs by conversion to the decachlorobiphenyl by means of perchlorination, seems to compare favourably to direct measurement of the PCB pattern by GC (Table VII).

The success of this derivation technique may partly depend on the amount of higher chlorinated biphenyls in the human milk. The lower chlorinated biphenyls are more volatile and easily lost, and recently,

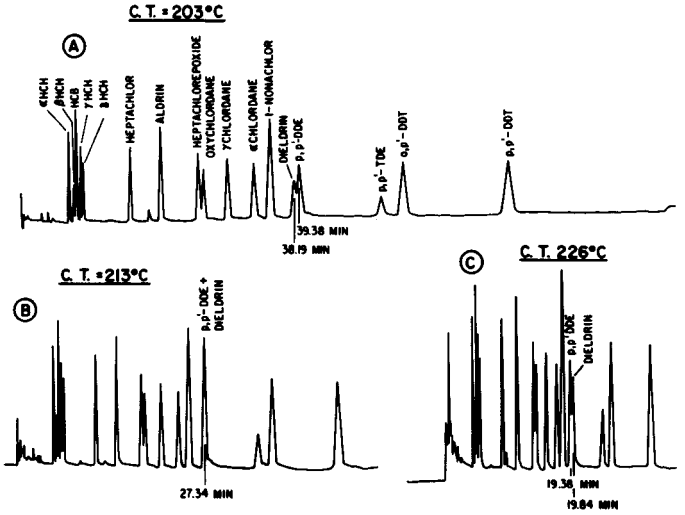

FIGURE 7 The effect of capillary column temperature on the elution time of p,p'-DDE and dieldrin.

TABLE VII

Comparison of PCB quantification in human milk between gas chromatographic and perchlorination techniques

Sample code	ng PCB/g whole milk		
	PC[a]	GC[b]	PC/GC
M1	5	6	0.83
M2	5	6	0.83
M3	9	6	1.50
M4	9	9	1.00
MD1	6	3	2.00
MD2	4	4	1.00
MD3	6	6	1.00
MD4		8	
MD5	16	15	1.07
E1	5	5	1.00
E2	6	5	1.20
E3	7	7	1.00
E4	8	8	1.00

[a]PC = perchlorination (corrected for blank).
[b]GC = gas liquid chromatography.

substantial quantities were found in human milk.[13] It is our experience that better results were obtained when extracts were immediately perchlorinated after the GC pattern was established.

Although the quantitative value of this technique may be questioned, the validity of the confirmatory aspect remains, if no biphenyl is present.

Preliminary results on the possible variation within the PCB isomer distribution due to extraction technique, have been reported[11] and are shown in Table VIII. These results indicate the need for further investigation into the effect of extraction technique on isomer distribution, especially since the data are based on a single MS determination. Nevertheless, the first three methods in Table VIII gave almost identical isomer composition, while two of these methods used the same extraction solvent. Aroclor 1260 contains 12% penta-, 38% hexa-, 41% hepta- and 8% octachlorobiphenyl.[16] This composition closely approximates that found for the first three methods, except for hexachlorobiphenyl.

TABLE VIII

Variation in the isomer distribution of PCBs extracted from human milk by various techniques

Extraction[a]		% [b]					
Method	Solvent	Cl_3	Cl_4	Cl_5	Cl_6	Cl_7	Cl_8
Freeze-dry	Ac:C_6H_6	7	25	12	14	35	7
Centrifugation	C_6H_6	4	26	13	14	34	8
Centrifugation	Ac:C_6H_6	4	25	12	15	36	8
Direct	EtOH:ether:hex	10	28	16	12	27	7
Acid hydrolysis	C_6H_6	7	17	10	13	44	9
Saponification	C_6H_6	4	15	11	14	49	8

[a]For details of extraction techniques, see Reference 11.

[b]Percent of tri- (Cl_3), tetra- (Cl_4), penta- (Cl_5), hexa- (Cl_6), hepta- (Cl_7) and octachlorobiphenyl (Cl_8) present.

EVALUATION

It is evident from the foregoing, that the analyst has to make many decisions before a routine analytical procedure is established. He must decide how best sampling, extraction, separation, identification and quantification should be carried out and to what extent his results needs confirmation. In some cases objective judgements cannot be easily made, e.g. does the PCB pattern in human milk resemble Aroclor 1254 or 1260? How many and which peaks to use for quantification?

The reliability of the methodology may be supported by fortification experiments, where known amounts of halogenated hydrocarbons are

added to the human milk. However, even excellent fortification results do not necessarily give an accurate representation of the residue level in the sample, or indicate the suitability of the method.[17] Nevertheless this technique is the only guide for the residue chemist in evaluating his methodology, without extensive research.

Table IX shows recoveries of chlorinated hydrocarbons from human milk fortified at the 100 ppb level. Acetone:Benzene (19:1 v/v) was used as extraction solvent and the fat removed by precipitation. Recoveries of PCB and HCB are not only lower, but also show much greater variation than for other compounds.

Recovery of PCBs at low levels of fortification (10 ppb), using a micromethod (no freeze-out procedure), was >95% as shown in Table X.

TABLE IX

Recovery of chlorinated hydrocarbons from human milk samples fortified at 100 ppb

Compound	% Recovery ± S.D. ($N = 5$)[a]
PCB (Aroclor 1260)	70.6 ± 15.9
HCB	78.4 ± 17.5
βHCH	87.3 ± 5.2
heptachlor epoxide	97.6 ± 10.0
oxychlordane	88.7 ± 5.2
p,p'-DDE	91.1 ± 11.8
dieldrin	88.1 ± 8.0
o,p'-DDT	89.3 ± 5.7
p,p'-DDT	85.9 ± 13.0

[a]S.D. = standard deviation; N = number of samples.

TABLE X

Effect of various parameters on the recovery of PCB from human milk, fortified at 10 ppb

Sample no.	Fortification		Extraction solvent	ng PCB/g whole milk[a] (as Aroclor 1260)	% Recovery[b]
	Solvent	Volume (μl)			
1	—	—	Benzene	3.1	
2	Acetone	20	Benzene	7.4	44
3	Acetone	100	Benzene	7.7	47
4	Hexane	100	Benzene	12.3	95
5	Acetone	100	Acetone:C_6H_6	13.4	106

[a]Average of 3 determinations (1 ml samples).
[b]Corrected for background.
—Not detected.

Recovery is apparently affected by the right combination of extraction and fortification solvents. This apparent increase in PCB recovery from human milk with decrease in fortification level (100 vs. 10 ppb) lies more in the evolution of analytical technique than in the level of fortification.

The reliability of a method and consequently its usefulness may be verified by a collaborative study. Table XI shows the results of a limited study of PCBs in human milk, using a modified "Direct extraction" method.[2] The extracted milk fat was mixed with 25 g of 2% deactivated Florisil, which was then added to 25 g Florisil already in a 22-mm I.D. glass column. The PCBs were eluted in 300 ml hexane. This hexane eluant was treated with 0.5 N KOH before GC analysis. The coefficient of variation for both fat and PCB level was ~30. However, the results do give a certain degree of confidence, considering the level of contamination, the problems of quantification and the inexperience of some laboratories.

TABLE XI

PCB and fat levels in a human milk check sample

Laboratory no.	% Fat	ng PCB/g whole milk
1	3.4	27
2	3.9	67
3	4.9	20
4	2.9	22
5	3.3	13
6	3.0	11
7	1.9	16
Average ± S.D.[a]	3.3 ± 0.9	17 ± 5[b]

[a]S.D. = standard deviation.
[b]Laboratory no. 2 excluded.

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References

1. W. R. Ritcey, G. Savary and K. M. McCully, *Can. J. Publ. Health* **63,** 126 (1972).
2. D. S. Siyali, *Med. J. Aust.* **2,** 815 (1973).
3. H. von Pesendorfer, *Wien. Klin. Wochenschr.* **87,** 732 (1975).
4. Z. W. Polishuk, M. Ron, M. Wassermann, S. Cucos, D. Wassermann and C. Lemesch, *Pestic. Monit. J.* **10,** 121 (1977).
5. M. van Hove Holdrinet, H. E. Braun, R. Frank, G. J. Stopps, M. S. Smaut and J. W. McWade, *Can. J. Publ. Health* **68,** 74 (1977).

6. J. Mes and D. J. Davies, *Bull. Environm. Contam. Toxicol.* **21,** 381 (1979).
7. J. Mes, D. J. Davies and W. Miles, *Bull. Environm. Contam. Toxicol.* **19,** 564 (1978).
8. J. Mes and D. J. Davies, *Chemosphere* **9,** 699 (1978).
9. *Report from the Michigan Senate Investigation Committee* **131,** July (1975).
10. F. E. Hytten, *Brit. Med. J.* **175,** Jan. (1954).
11. J. Mes and D. J. Davies, To appear in *Chemosphere* (in press).
12. H. A. McLeod and P. J. Wales, *J. Agric. Food Chem.* **20,** 624 (1972).
13. T. Yakushiji, I. Watanabe, K. Kuwabara and S. Yoshida, *J. Chromatog.* **154,** 203 (1978).
14. L. M. Reynolds, *Bull. Environm. Contam. Toxicol.* **4,** 128 (1969).
15. S. Jensen and J. Widmark, *Organization for Economic Cooperation and Development (O.E.C.D.) Report* 1966–1967.
16. O. Hutzinger, S. Safe and V. Zitko, *The Chemistry of PCBs*, CRC Press, Cleveland, U.S.A., 1974.
17. P. W. Albro, *Ann. N.Y. Acad. Sci.* **320,** 19 (1979).