

Determination of Organochlorine Pesticide Content in Human Milk and Infant Formulas Using Solid Phase Extraction and Capillary Gas Chromatography

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The differences in the organochlorine pesticide contents of human milk and infant formulas are examined. Twenty-six organochlorine pesticides were routinely extracted from milk by means of a solid phase system into *n*-hexane. An aliquot of *n*-hexane was injected into a GC equipped with an electron capture or mass spectrometry detector. Advantages of this method include high sensitivity, analysis time of 45 min after a single extraction, and high recovery. Fifteen human milk samples and 45 infant formula samples, which cover the whole range of products offered in the market (starting formula, follow-up formula, adapted formula, and specialized formulas developed for premature or low birth weight infants, infants intolerant to milk proteins or lactose, infants with fat absorption problems, and infants with inborn errors of metabolism). The organochlorine pesticide content found in human milk was higher than in infant formulas.

Keywords: *Solid phase extraction; organochlorine pesticides; human milk; infant formulas; gas chromatography; mass spectrometry*

INTRODUCTION

During the past few decades, one of the most important topics in ecotoxicology has been the transference of pollutants along the trophic chains (Ramade, 1990). In the early 1960s, a study showed that DDT concentrations in organisms dramatically increase up the trophic chains. Then such "biomagnification" was confirmed for other organochlorine pesticides (Murphy, 1986). Owing to their lipophilic properties, they are primarily stored in fat-rich tissues and fluids of human and animals (Machemer, 1986; Kurtz et al., 1991). Nowadays this constitutes a hazard mainly for nursing children whose only food is milk-based compounds. This is one reason monitoring programs are needed to check the presence of organochlorine pesticides (OCPs) in infant food (Jelinnek, 1985; Renner, 1989; Hendricks, 1990).

The development of simple, efficient, and inexpensive analytical methods is crucial for monitoring and evaluating milk contamination (Bowman, 1984; Conacher, 1987). The most commonly used techniques for analyzing organochlorine contaminants, such as liquid-liquid partitioning, supercritical fluid extraction, head space, normal solid phase extraction, and many others, are effective but have their limitations (CMTTA, 1988; Seymour et al., 1987; Karen-Chang and Taylor, 1990; Adachi et al., 1983; Di Muccio et al., 1988; Noren and Sjöval, 1987; Hogendoorn et al., 1989; Luke et al., 1989).

Recently, reversed solid phase extraction (RSPE) has been developed (Barcarolo et al., 1988; Redondo et al., 1991; Mañes et al., 1993). The time efficiency, portability, precision, and detection limit, as well as the low cost of the RSPE technique, have been significantly improved in comparison with traditional solid phase extractions.

In the present study we have determined the organochlorine pesticide content of 15 human milk samples

and 45 infant formulas, which cover the whole range of products on the market, using the method based on RSPE. The use of gas chromatography-mass spectrometry (GC-MS) to confirm the organochlorine residue identity with the extracts obtained by RSPE has been tested. The results have demonstrated the applicability of the method for a residue monitoring program to supervise the safety of the food consumed by children.

MATERIALS AND METHODS

Apparatus and Chromatography. The GC analysis was carried out on a Konik 3000 chromatograph equipped with a ⁶³Ni electron-capture detector and a splitless injector. The following capillary columns were used: (a) 30 m × 0.25 mm i.d. cross-linked with BP-5 (film thickness 0.32 μm) from Supelco and (b) 30 m × 0.24 mm i.d. cross-linked with DB-17 (film thickness 0.32 μm) from J&W Scientific. The carrier gas was helium at a flow rate of 1 mL/min. The temperature program was 0.8 min at 50 °C, increased at 30 °C/min to 140 °C, which was held for 2 min, then at 5 °C/min to 280 °C. The final temperature was held for 10 min. The injector and detector temperatures were maintained at 285 and 300 °C, respectively. The injection was in the splitless mode, keeping the split valve closed for 0.7 min. All of the samples were chromatographed on the two columns. Only the compounds identified on both columns were subsequently evaluated by GC-MS.

The selected samples were analyzed by GC-MS using a HP 5890 Series II gas chromatograph coupled to a HP 5970 mass-selective detector equipped with a HP 300 data system and splitless injector. The mass-selective detector was used in the selected ion monitoring (SIM) mode, and the temperatures were as follows: transfer line, 300 °C; ion source, 200 °C; and analyzer, 230 °C. Helium was used as the carrier gas, and the other chromatographic conditions were the same as described above.

Reagents. Methanol and *n*-hexane were of pesticide grade from Merck. Preparative octadecylsilica (55-105 μm) was obtained from Waters-Millipore.

Organochlorine pesticide standards—aldrin, captafol, captan, *o,p'*-DDD, *p,p'*-DDD, *o,p'*-DDE, *p,p'*-DDE, *o,p'*-DDT, *p,p'*-DDT, dicofol, dieldrin, α-endosulfan, β-endosulfan, endosulfan sulfate, endrin, heptachlor, heptachlor epoxide, α-HCH, β-HCH,

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Table 1. Recovery and Relative Standard Deviation ($\bar{x} \pm \text{RSD}$, $n = 5$) of Organochlorine Pesticides in Human Milk and Different Kinds of Infant Formulas

pesticide	level ($\mu\text{g/L}$)	human milk	starting formula	follow-up formula	adapted formula	soy proteins	lactose intolerant
aldrin	5.1	93 \pm 8	75 \pm 8	79 \pm 8	88 \pm 7	84 \pm 8	78 \pm 5
captafol	4.1	39 \pm 12	34 \pm 11	31 \pm 10	33 \pm 13	54 \pm 7	39 \pm 10
captan	7.0	44 \pm 10	45 \pm 8	42 \pm 9	44 \pm 10	45 \pm 5	44 \pm 12
<i>o,p'</i> -DDD	6.1	90 \pm 9	84 \pm 9	87 \pm 7	85 \pm 6	87 \pm 7	79 \pm 10
<i>p,p'</i> -DDD	9.1	81 \pm 5	80 \pm 8	90 \pm 9	83 \pm 8	93 \pm 7	90 \pm 9
<i>o,p'</i> -DDE	7.0	85 \pm 6	82 \pm 6	79 \pm 9	78 \pm 11	94 \pm 8	87 \pm 4
<i>p,p'</i> -DDE	7.0	85 \pm 7	83 \pm 6	84 \pm 7	80 \pm 9	89 \pm 7	92 \pm 6
<i>o,p'</i> -DDT	9.0	89 \pm 7	90 \pm 8	92 \pm 8	87 \pm 6	95 \pm 8	92 \pm 8
<i>p,p'</i> -DDT	9.0	79 \pm 7	85 \pm 9	82 \pm 5	83 \pm 8	94 \pm 7	75 \pm 6
dicofol	4.0	69 \pm 9	65 \pm 6	70 \pm 8	71 \pm 9	79 \pm 7	79 \pm 8
dieldrin	6.0	97 \pm 7	95 \pm 9	96 \pm 9	92 \pm 10	96 \pm 8	95 \pm 9
α -endosulfan	14.6	83 \pm 9	82 \pm 8	88 \pm 7	80 \pm 10	98 \pm 9	96 \pm 8
β -endosulfan	6.6	80 \pm 7	79 \pm 9	78 \pm 6	84 \pm 7	88 \pm 6	87 \pm 7
endosulfan sulfate	10.5	89 \pm 8	90 \pm 9	90 \pm 8	86 \pm 7	93 \pm 6	95 \pm 6
endrin	12.5	91 \pm 9	90 \pm 10	89 \pm 9	88 \pm 8	100 \pm 5	96 \pm 6
HCB	3.0	106 \pm 9	99 \pm 9	100 \pm 9	102 \pm 10	102 \pm 8	102 \pm 6
α -HCH	5.0	90 \pm 9	89 \pm 8	89 \pm 9	88 \pm 10	100 \pm 8	92 \pm 7
β -HCH	5.0	93 \pm 8	90 \pm 9	89 \pm 10	92 \pm 9	96 \pm 8	91 \pm 6
δ -HCH	5.0	92 \pm 6	95 \pm 9	94 \pm 9	90 \pm 7	101 \pm 7	90 \pm 7
γ -HCH	3.0	101 \pm 9	78 \pm 8	98 \pm 8	102 \pm 9	102 \pm 9	95 \pm 6
heptachlor	5.1	94 \pm 9	93 \pm 8	98 \pm 6	93 \pm 9	83 \pm 6	81 \pm 7
heptachlor epoxide	5.0	76 \pm 10	92 \pm 7	77 \pm 10	78 \pm 6	99 \pm 9	87 \pm 6
isodrin	10.1	78 \pm 9	76 \pm 10	75 \pm 8	74 \pm 8	96 \pm 8	97 \pm 6
methoxychlor	16.4	89 \pm 8	91 \pm 9	88 \pm 6	90 \pm 9	90 \pm 7	88 \pm 6
mirex	8.2	53 \pm 10	50 \pm 6	49 \pm 10	48 \pm 8	70 \pm 9	55 \pm 7
tetradifon	0.8	89 \pm 6	84 \pm 10	82 \pm 7	80 \pm 9	82 \pm 7	80 \pm 6

δ -HCH, γ -HCH, HCB, isodrin, methoxychlor, mirex, and tetradifon—purchased from Promochem, Riedel de H en, and Aldrich. Stock solutions of standards were made by weighing and dissolving them in ethyl acetate.

Sample Preparation. Human milk samples were collected in the Spanish Mediterranean coastal area. Sample donors were chosen from rural and small urban locations where Mediterranean eating habits are still maintained. Human milk samples came from 15 primiparae and multiparae (ages 29–40 years). They were collected during the winter of 1993. Milk sampling was done by manual expression into glass containers (ca. 40 mL), and samples were kept at 4 °C.

The infant formulas were usually marketed as spray-dried powders in tin containers covered with aluminum or an aluminum bag inside a cardboard package. However, liquid ready-to-feed formulas in glass bottles were also common. The infant formulas analyzed included 11 starting formulas, 11 follow-up formulas, 4 adapted infant formulas, and 17 specialized formulas. The powdered infant formulas were reconstituted with distilled water.

Procedure. The extraction method proposed for commercial milk by Ma es et al. (1993) was used: 1 g of octadecylsilica was transferred into a 100 mm \times 9 mm i.d. glass column fitted with a coarse frit (no. 3) and covered with a plug of silanized glass wool. The column was treated with 10 mL of methanol and 10 mL of distilled water.

In a 20 mL Erlenmeyer flask a 5 mL aliquot of milk was added with 5 mL of water and 10 mL of methanol and mixed by sonication for 10 min. The mixture obtained was forced to pass through the column at flow rates of 1–20 mL/min, applying vacuum. The column was washed twice with 10 mL of distilled water, and the washings were discarded. The adsorbed residues were then eluted with 10 mL of hexane. The extract was concentrated at 45 °C to 0.5 mL, and 3 μL was injected into the gas chromatograph.

Recovery Experiments. Human milk samples (5 mL) were transferred into a glass bottle and fortified at the levels shown in Table 1 by adding 20 μL of the stock solution directly into the milk, and the mixture was left to stand overnight at 4 °C. The sample was equilibrated at room temperature before the above procedure was continued.

RESULTS AND DISCUSSION

The use of RSPE for the determination of pesticides from cows' milk has been reported previously. Com-

Table 2. Limits of Detection (Signal-to-Noise Ratio = 3) Obtained by ECD and MS-SIM

pesticide	ECD LD ($\mu\text{g/L}$)	monitored ion (m/z)	SIM LD ($\mu\text{g/L}$)	qualifier ion (m/z)
aldrin	0.25	263	0.50	261, 265
captafol	2.27	311	10.62	183, 107, 150
captan	2.32	149	10.85	117, 119
<i>o,p'</i> -DDD	0.80	235	1.00	165, 199
<i>p,p'</i> -DDD	0.45	235	1.00	165, 199
<i>o,p'</i> -DDE	0.20	246	1.00	316, 318, 322
<i>p,p'</i> -DDE	0.35	246	1.00	316, 318, 322
<i>o,p'</i> -DDT	0.30	235	1.00	270, 272, 276
<i>p,p'</i> -DDT	0.39	235	1.00	270, 272, 276
dicofol	6.36	139	2.50	111, 250
dieldrin	0.23	263	0.50	261, 265
α -endosulfan	0.30	195	1.25	241, 207
β -endosulfan	0.22	195	1.25	237, 207
endosulfan sulfate	0.34	272	10.75	229, 387
endrin	0.26	263	0.40	281, 245
HCB	0.14	284	0.35	282, 286, 288
α -HCH	0.14	181	0.25	219, 109
β -HCH	0.20	109	0.25	181, 219
δ -HCH	0.34	181	0.25	109, 219
γ -HCH	0.14	109	0.25	181, 219
heptachlor	0.25	100	0.50	272, 274
heptachlor epoxide	0.25	353	0.50	263
isodrin	0.25	193	0.40	261, 263, 265
methoxychlor	0.17	227	1.00	274
mirex	0.70	272	1.25	235, 237
tetradifon	0.50	159	1.00	111, 227

pounds evaluated according to this procedure include organochlorine pesticides (Barcarolo et al., 1988; Redondo et al., 1991; Ma es et al., 1993) and triazine herbicides (Barcarolo et al., 1988). Although the recovery efficiency of these compounds by RSPE from cows' milk has been well established, no information is available on organochlorine contaminants in human milk or infant formulas, which have a different quantitative and qualitative composition.

The recovery and the within-run precision of the proposed method (see Table 1) demonstrate that the method is useful for analyzing human milk and infant formulas. Only captan, captafol, and mirex gave low recoveries, probably because of their low solubility in the desorption solvent (Ma es et al., 1993).

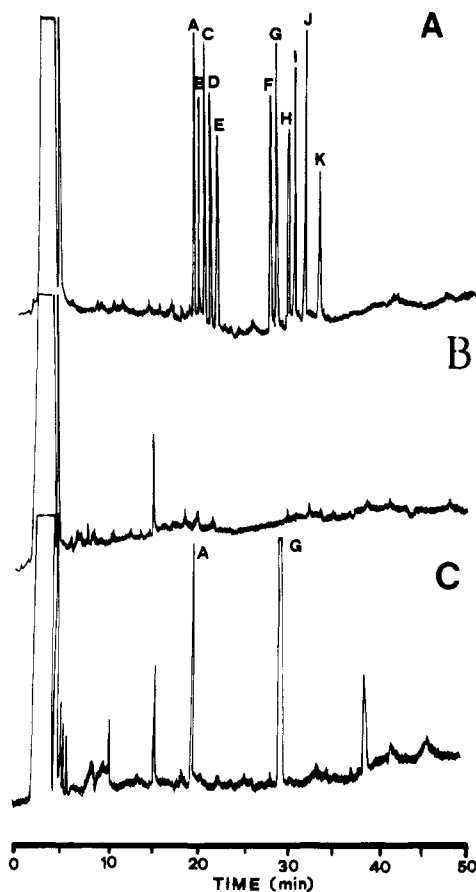


Figure 1. GC-ECD chromatogram obtained with the BP-5 column of (A) 1 μ L injection of 11 OCP standards (concentration μ g/L) [HCB (A, 30); α -HCH (B, 50); β -HCH (C, 50); γ -HCH (D, 50); δ -HCH (E, 30); *o,p'*-DDE (F, 70); *p,p'*-DDE (G, 70); *o,p'*-DDD (H, 61); *p,p'*-DDD (I, 91); *o,p'*-DDT (J, 90); *p,p'*-DDT (K, 90)]; (B) blank, and (C) sample 4.

The limits of detection obtained (see Table 2) allow quantification of the organochlorine compounds at the sub parts per billion level in all of the kinds of milk studied using either ECD or MSD in the SIM mode. To confirm that there was no interference with the target compounds, the qualifier ions in Table 2 were also monitored in several milk samples.

Typical chromatograms of a mixture of pesticides, a blank, and a human milk extract using the BP-5 column and the ECD are shown in Figure 1. Peaks were identified in chromatogram C by their retention times by comparison with known standards of chromatogram A. The same chromatograms in the DB-17 column are shown in Figure 2. In chromatogram C, corresponding to a human milk sample, HCB and *p,p'*-DDE peaks were also identified. Two chromatograms, one of a mixture of HCB and *p,p'*-DDE standards and the other of a sample obtained by GC-MS-SIM, are illustrated in Figure 3. The time program of the monitoring ions was m/z 282, 284, 286, and 288 for 10–14 min and m/z 246, 316, 318, and 322 for 14–45 min.

All of these characteristics demonstrate that the RSPE method proposed is sensitive and specific and make it possible for several commonly used organochlorine pesticides to be determined simultaneously with a GC system and a uniform set of extraction conditions.

Table 3 shows the results obtained by analyzing the human milk samples for the 26 pesticides. Concentrations are expressed per volume of milk.

All of the human milk samples contain HCB and *p,p'*-DDE. Both HCB and DDT (the parent compound of

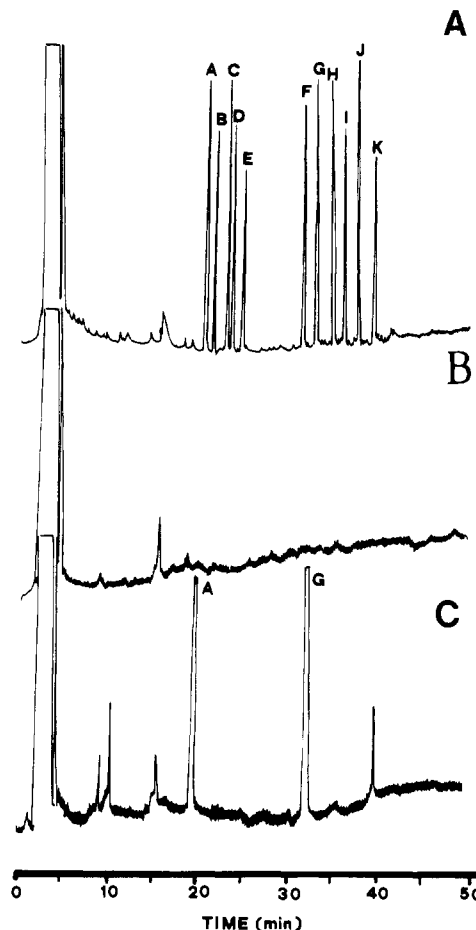


Figure 2. GC-ECD chromatogram obtained with the DB-17 column of (A) 1 μ L injection of 11 OCP standards, (B) blank, and (C) sample 4. For details see Figure 1.

p,p'-DDE) have been extensively used in agriculture and to control cattle ectoparasites. The employ of these substances in agriculture was completely outlawed in 1975 (Ministerio de Agricultura, 1975).

Although *p,p'*-DDE seems to be ubiquitous, the presence of *p,p'*-DDD and *p,p'*-DDT was detected only in one sample, the donor of which was in contact with DDT after its prohibition. *p,p'*-DDT is known to degrade slowly into *p,p'*-DDD and *p,p'*-DDE, the latter being its main metabolite stored in humans (Matue et al., 1992).

The HCH isomers and heptachlor were also detected in some of the human milk samples studied, giving positive sample frequencies of 33.3% and 5%, respectively. Heptachlor epoxide, which is the product of the heptachlor metabolism in the liver, was not found. This could indicate that the contamination of the milk by heptachlor is recent and the compound has still not been metabolized.

The other pesticides studied were not detected in the human milk samples analyzed.

Comparing the results reported here with those obtained by other authors, the mean level of sum of DDT found in this study is 10 times lower than that found in 20 human milk from Spain in 1982. The levels of HCH and heptachlor are similar (Baluja et al., 1982). Levels reported in this study for HCB are lower than those reported in 408 human milk samples in Spain from 1988 to 1991 (Conde et al., 1993a,b), and other compounds related to pesticides such as PCBs were not detected (Conde et al., 1993).

These results show that the pollution of human milk in the Spanish Mediterranean coastal area is similar

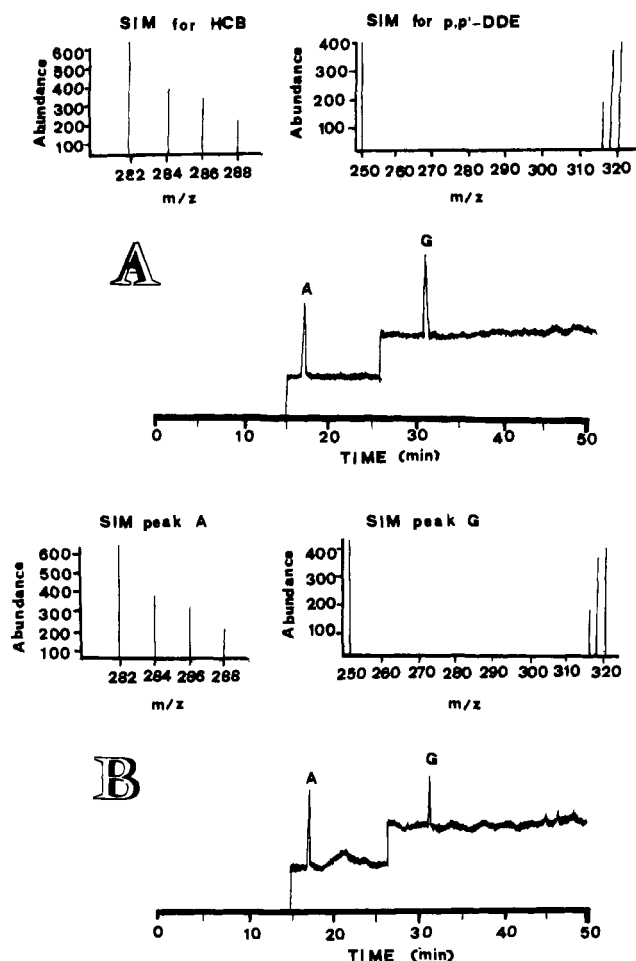


Figure 3. GC-MS mode SIM chromatograms obtained with BP-5 column and the abundance of monitored ions of (A) 1 μ L standard of HCB and *o,p'*-DDE and (B) sample 4. Peak assignment is as in Figure 1.

to that found in other European countries (Noren and Sjövall, 1987; Kanja et al., 1992; Krauthacker, 1983; Greve and Van Zoonen, 1990).

In the human milk samples, the individual variations in the residue level were not unexpected since, as is well-known, many factors may influence the results, e.g., exposure, age, and weight of the mothers, dietary habits, and parity of the mothers. In the studies of Conde (Conde et al., 1993a,b), for example, the samples were taken during the third week postpartum and this could explain the differences in the values found by these authors and the values reported in the present work. The fat content of the milk, which is also dependent on the time of sampling (during the meal, the day, and the lactation period) and seasonal variations, may also influence the results (Kanja et al., 1992).

A diminution in the concentrations of organochlorine pesticides in samples of the same donor taken along the lactation period (Table 3, samples 4 and 7) was observed; this fact is in agreement with the findings of Greve and Van Zoonen (1990), who found that the concentrations decrease even up to 6 months of lactation.

The data in Table 3 can be used to predict the daily intakes of *p,p'*-DDE and HCB by breast-fed babies, assuming that a child consumes 150 mL of milk/kg of body weight. The daily intakes were calculated from the median and maximal concentrations of the compounds. The values obtained were 2.03 and 3.64 μ g/kg of body weight for HCB and 2.28 and 5.43 μ g/kg of body weight for DDT.

HCB values are higher than acceptable daily intakes suggested by the World Health Organization (WHO), which for HCB and DDT are 0.6 and 5 μ g/kg of body weight, respectively. However, the DDT values are within the limit of tolerance.

The infant formulas are manufactured by modifying the composition of cows' milk so that it resembles human milk. This involves mainly an adaptation of the

Table 3. Concentration of OCPs in Human Milk

sample	days after birth	compd found	μ g/L	sample	days after birth	compd found	μ g/L
1 primiparae	28	HCB	6.3	8 multiparae	60	HCB	22.7
		<i>p,p'</i> -DDE	31.6			β -HCH	2.6
2 primiparae	22	HCB	10.5	9 primiparae	60	τ -HCH	2.2
		<i>p,p'</i> -DDE	25.1			<i>p,p'</i> -DDE	11.6
3 primiparae	30	HCB	23.4	10 primiparae	60	HCB	18.2
		<i>p,p'</i> -DDE	20.2			<i>p,p'</i> -DDE	36.2
4 multiparae	60	HCB	6.9	11 multiparae	41	<i>p,p'</i> -DDD	2.6
	120	<i>p,p'</i> -DDE	10.2			<i>p,p'</i> -DDT	7.0
5 primiparae	60	HCB	17.0	12 multiparae	60	HCB	15.6
		heptachlor	5.4			<i>p,p'</i> -DDE	6.9
		<i>p,p'</i> -DDE	16.3			HCB	5.7
6 multiparae	60	HCB	0.3	13 primiparae	29	<i>p,p'</i> -DDE	0.8
		τ -HCH	1.2			HCB	17.5
		<i>p,p'</i> -DDE	0.6			τ -HCH	4.3
7 multiparae	60	HCB	5.0	14 multiparae	30	<i>p,p'</i> -DDE	32.5
	90	<i>p,p'</i> -DDE	10.6			HCB	7.9
	120	HCB	0.5			<i>p,p'</i> -DDE	0.8
		<i>p,p'</i> -DDE	1.6	15 primiparae	30	HCB	22.1
		HCB	0.4			τ -HCH	3.2
		<i>p,p'</i> -DDE	1.1			<i>p,p'</i> -DDE	13.6

Table 4. Concentration of OCPs in Infant Formulas

sample characteristics	compd found	$\mu\text{g/L}$
adapted formula	HCB	1.0
	heptachlor	13.5
	endrin	8.5
	<i>p,p'</i> -DDE	11.5
	<i>p,p'</i> -DDD	4.0
hypoallergic formula	γ -HCH	1.0
	aldrin	11.5
	<i>p,p'</i> -DDE	2.0
	<i>p,p'</i> -DDD	1.4
follow-up formula	HCB	4.0
	aldrin	3.8
	aldrin	3.8

total levels of protein, fat, and carbohydrates in cows' milk. Most formulas are based on skim milk with or without demineralized whey, vegetable oils, or vegetable oils/milk fat mixtures.

Data on infant formulas are shown in Table 4. Of 45 samples analyzed, organochlorine pesticides were found only in 4 samples. Their concentrations and frequencies are lower than in human milk. This could be explained by the biomagnification of these pollutants through the trophic chains, in which human beings constitute the last link.

The presence of pesticides was not detected in infant formulas, in which cows' milk fat is replaced by vegetable fat or a mixture of vegetable fat and cows' milk fat or tallow.

There are only a few data on the occurrence of organochlorine pesticides in infant formulas. Pozo Lora et al. (1979) published a study in which 55 samples were analyzed and dieldrin, DDT, HCH isomers, endrin, and heptachlor were detected. Their work reports levels and frequencies of DDT, dieldrin, and endrin similar to those obtained in the present study. However, HCH and heptachlor levels were 10 times higher than the values obtained in the present study.

CONCLUSIONS

In the infant formulas analyzed, the RSPE method described gave good recoveries (>80%) for the organochlorine pesticides studied, except for captan, captafol, and mirex, which are not very soluble in hexane.

The RSPE method offers important savings in terms of time and glass material used in comparison with conventional extraction and partition steps, as well as highly sophisticated cleanup procedures.

Organochlorine pesticides were found in both human milk and infant formulas in which cows' milk fat is not replaced. The concentrations and frequency of contamination found in human milk are higher than in the infant formulas.

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