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Organochlorine Insecticide and Polychlorinated Biphenyl Residues in Human Breast Milk in Madrid (Spain)

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The intensive use of organochlorine insecticides in agriculture and polychlorinated biphenyls (PCBs) in industry have lead to widespread contamination throughout the environment. Since the organochlorine compounds are lipid-soluble and tend to accumulate in the food chain and store in high concentrations in tissues and lipid-rich organs such as adipose tissue and liver. Human milk is the most important and indispensable food for the newborn; during lactation, fat mobilitation could take place from adipose tissue and therefore organochlorine compounds are mobilized and excreted together with the milk. Thus, milk secretion is the most important route of excretion of those types of compounds in women. The presence of organochlorine insecticides and PCBs in human breast milk has been reported in works from various countries during 1970-80 years, since analysis of breast milk supply information on the exposure of different populations to organochlorine contaminants. A critical review of the literature available from more than 40 countries has been published (Jensen 1983). Surveys were carried out in Spain by Pozo et al. (1979) and Baluja et al. (1982). These surveys indicated the existence of high levels of DDT compounds, isomers of hexachlorocyclohexane (HCH) and PCBs.

In this study we have attempted: a) to estimate the levels of organochlorine pollutants in human milk samples collected in Madrid (Spain) during 1991; b) to investigate whether significant alterations in levels of these contaminants have occurred since 1979-81, in Spain; c) to find out if the residue levels exceeded the limits recommended by FAO/WHO in human milk.

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MATERIALS AND METHODS

Between January and March 1991, 51 milk samples were obtained from individual mothers who lived in urban area of Madrid (Spain). Milk samples were collected 0-20 days after parturition, and were expressed directly into hexane-washed glass bottles. The age of the mothers ranged from 18 to 40 years (mean age: 29 years).

The analysis of samples was carried out essentially as previously described Tessari and Savage (1980). Thus, the samples of whole breast milk (10 g) were dispersed in sodium sulphate anhydrous and the mixture extracted with hexane-acetone. The extract was then evaporated by purified Nitrogen flush whithout external heating and accurately weighed the amount of fat. Organochlorine compounds were isolated by a liquid-liquid partitioning process and purified chromatographically on a Florisil column; cleanup was achieved using 25% diethyl ether in hexane as eluate.

Gas chromatography analysis was done on a Hewlett-Packard 5890 equipped with Ni 63 Electron Capture Detector. A 30 m long capillary column covered with RSL-200 was used. Chromatographic conditions as follow: detector 280°C; injector 300°C; temperature programme, isothermal phases at 180°C (1 min) and 250°C (30 min), with intermediate temperature increase rate of 2°C/min. Peaks were identified on the integrator by retention times; tolerance allowed was ± 0.05 min. A 50 m capillary column covered with BP-5 was used for confirmation of identity of the organochlorine pollutants. The peaks were measured by area counts given by the integrator (minimum count 500). Quantitation was done comparing the peak areas in the sample with those in corresponding standards. PCBs were quantitated by summation of peak areas from seven major peaks. Recoveries of organochlorine compounds ranged from 89-101%, except for HCB wich showed recoveries of 80%, but the residue data in the tables were not adjusted on the basis of these recoveries. The detection limit was 1 ng g^{-1}. Both levels were expressed on whole milk (ng g^{-1}) and fat basis (ng g^{-1}).

RESULTS AND DISCUSSION

The mean (whole milk and fat basis), standard deviation, range values, and percentage of positive analysis of organochlorine compounds are shown in Table 1. The levels on fat basis are considered the most appropriate for reporting concentrations of organochlorine pollutants in human milk due to the effects of variable lipids levels on data based on whole milk (Jensen 1983). The levels on whole milk are practical to esti-

Table 1. Residues of organochlorine pesticides and PCBs in human milk (ng g^{-1} whole milk) in Madrid.

	Mean	Mean[a]	Min.[b]	Max.[c]	% [d]
α-HCH	1.0	34.2	N.D.[e]	2334	68.6
β-HCH	7.2	235.0	N.D.	3226	85.7
τ-HCH	0.3	10.5	N.D.	141	64.7
HCB	0.02	0.8	N.D.	8	67.8
Hept.	0.1	4.3	N.D.	85	33.3
H.ep.	0.9	31.1	N.D.	552	92.1
Diel.	0.1	3.9	N.D.	217	11.7
DBP	1.1	37.2	N.D.	1489	74.5
DDE	18.7	604.1	17	7733	100
TDE	0.1	5.4	N.D.	174	5.9
DDT	0.4	12.5	N.D.	291	21.5
ΣDDT	20.4	659.8	17	7901	100
PCBs	61.0	1967.0	53	33631	100

[a]: Fat basis (ng g_{-1}). [b]: Minimum. [c]: Maximum. [d]: Percentage of positive samples. [e]: Not detected. Number of samples: 51.

mate infant`s daily intake.

It can be seen that α-HCH, β-HCH, τ-HCH, HCB, hep-tachlor epoxide, dichlorobenzophenone, p,p' DDE, and PCBs were presents in the greater part of the samples analyzed, whereas lower percentage of positive results were observed for heptachlor, dieldrin, p,p'TDE, and p,p'DDT. Residues of p,p'DDE and PCBs were present in all samples. A detectable amount of α-HCH was found in 68.6%, β-HCH in 85.7%, τ-HCH in 64.7%, HCB in 67.8%, heptachlor in 33.3%, heptachlor epoxide in 92.1%, dieldrin in 11.7%, dichlorobenzophenone in 74.5%, p,p`TDE in 5.9%, and p,p`DDT in 21.5% of the samples. No residues of other chlorinated insecticides included in the analytical survey were detected (aldrin, mirex and chlordane). The major insecticide present in milk samples was p,p'DDE, while only small amounts of the parent compound (p,p'DDT) and other metabolites (dichlorobenzophenone and p,p'TDE) were found. Concentrations of p,p'DDE ranged from 3.2 to 3,838 ng g^{-1} fat weight, with a mean of 604 ng g^{-1}; levels of p,p'DDT varied from not detected to 409 ng g^{-1} fat weight, with a mean of 12.5 ng g^{-1}; concentrations of p,p'TDE ranged from not detected to 194 ng g^{-1} fat weight, with a mean of 5.4 ng g^{-1}; levels of dichlorobenzophenone varied from not detected to 173 ng g^{-1} fat weight, with a mean of 37.2 ng g^{-1}. Isomers of HCH also ranged considerably in relative concentration: the most important compound is β-HCH with a mean con-

centration of 235 ng g^{-1} fat weight (range not detected to 1,289 ng g^{-1}). Levels of α-HCH and τ-HCH are lower than β-HCH, with a mean concentration of 34.1 and 10.5 ng g^{-1} fat weight (range, not detected to 607.9 and not detected to 62.5 ng g^{-1}) respectively. High levels of β-HCH shown the use of lindane, still commercialized in Spain, since β-HCH is an impurity of technical lindane; in effect, β-HCH is a persistent contaminant as shown in experimental tests, β-HCH had to 10 to 30 fold bigger capacity to accumulate in fat tissues (Heeschen 1980) and a five fold slower rate of elimination in comparison to τ-HCH (Pfeilsticker 1973). Cyclodiene insecticides varied in relative concentration: the most conspicuous compound is heptachlor epoxide with a mean concentration of 31 ng g^{-1} fat weight (range, not detected to 180 ng g^{-1}). Levels of heptachlor and dieldrin are lower, with a mean concentration of 4.2 and 3.9 ng g^{-1} fat weight (range, not detected to 75 and not detected to 59 ng g^{-1}), respectively. HCB concentrations are the smallest, with a mean concentration of 0.8 ng g^{-1} fat weight (range, not detected to 3.2 ng g^{-1}). Finally, PCBs were presents in all samples, with a mean concentration of 1,967 ng g^{-1} fat weight (range, 63 to 19,380 ng g^{-1}).

Comparison of the results of this study with the data from the Spanish investigations in 1979 (year publication) (Pozo et al. 1979) and 1981 (Baluja et al. 1982) shown in Table 2, indicates that organochlorine compounds levels are decreasing, although laboratory differences in methodology and sample origin exist and the results must be compared with precaution. With this proviso, only τ-HCH remains stable, whereas the rest of organochlorine compounds in this study shown a decrease in the average values in comparison to the data from Pozo and Baluja: α-HCH 1.3 and 63 times lower, respectively, heptachlor 3 and 10, heptachlor epoxide 39 and 3, dieldrin 30 and 5, p,p`DDE 6 and 9, p,p`TDE 6 and 18.7, p,p`DDT 3.1 and 4, and PCBs 4. Thus, the comparison of the present investigation with those of previous surveys in Spain indicates the effectiveness of regulatory actions adopted, since the agricultural use of the organochlorine insecticides were first restricted and then banned in Spain in 1977, with exception of τ-HCH.

WHO and FAO (1985) haye proposed as acceptable daily intake (ADI) 20 μg kg^{-1} body weight for total DDT compounds, 10 μg kg^{-1} b.w. for τ-HCH and HCB, 0.1 μg kg^{-1} b.w. for aldrin + dieldrin, and 0.5 μg kg^{-1} for chlordane and heptachlor + heptachlor epoxide. For PCBs Galetin-Smith (1990) and Cordle and Kolbye (1979) recommended as acceptable daily intake 1 μg kg^{-1} b.w. If these ADI values are applied and assuming a mean intake of 0.8 L human milk per day by infant with a mean

Table 2. Comparison between results obtained in different surveys in Spain (ng g^{-1} whole milk).

	Pozo et al. (1979) (45)[a]	Baluja et al. (1982) (20)	This study (51)
α-HCH	1	1	1
τ-HCH	0.4	19	0.3
Hept.	0.3	1	0.1
H.ep.	39	3	1
Diel.	0.5	3	0.1
DDE	114	170	18.7
TDE	1	3	0.1
DDT	65	83	0.4
PCBs	N.A.[b]	250	61

[a]: Number of samples. [b]: Not analyzed.

body weight of 5 kg, then the mean concentrations not to be exceeded are 125 μg kg^{-1} whole milk for total DDT compounds, 62.5 μg kg^{-1} w.m. for τ-HCH and HCB, 0.62 μg kg^{-1} w.m. for aldrin + dieldrin, 3.12 μg kg^{-1} w.m. for chlordane and heptachlor + heptachlor epoxide, and 6.25 μg kg^{-1} w.m. for PCBs. None of mean levels of organochlorine insecticides investigated in this study exceeded the WHO/FAO recommendations. Mean value of PCBs exceeded the ADI value proposed by Galetin-Smith and Cordle and Kolbye. However, in this study, 11.7% of human milk samples contained amounts of heptachlor + heptachlor epoxide exceeding the WHO/FAO recommendations, as did 9.8% of samples for aldrin + dieldrin, and none for τ-HCH, HCB, chlordane and total DDT compounds. For PCBs, 88% of the human milk samples analyzed exceeded the ADI recommended. The use of PCBs has been reduced in Spain, but is probably early to observe the effect of restriction measures on the concentrations in human breast milk, given that the ubiquity and the persistence of these compounds is big.

In Table 3 the levels of organochlorine compounds in human milk recorded in the present work were compared with those of other countries during 1982-89. By comparison with published data from other countries, levels of HCH from Spain are intermediate between the moderate HCH values found in Poland, Greece, Canada, Yugoslavia, Italy, and Finland and the high values found in Turkey, Israel, Hong-Kong, and India. Probably, the explanation is lindane has not been completely banned in Spain.

The total DDT compound levels in our study are lower than levels reported in other countries during 1982-89, with the exception of Italy. Likewise, in our study the ratio between p,p`DDE and p,p`DDT mean values is 50; this ratio is lower in all countries compared in Table

Table 3. Organochlorine compounds in human milk in various countries. Mean (ng g^{-1} fat milk)

Country	Year	[a]	α-HCH	β-HCH	γ-HCH	DDE	TDE	DDT	PCBs	DDE/DDT	Ref.
Poland	1987[b]	540	20	130	50	4950	30	460	380	10.7	1
Greece	1983	30	N.A.[c]	N.D.[d]	15	N.A.	N.A.	35	N.A.	N.A.	2
India	1986	60	N.A.	N.A.	N.A.	7280	N.A.	1597	N.A.	4.5	3
Finland	1982[b]	50	N.A.	N.A.	N.A.	850	9	36	450	23.6	4
Turkey	1987[b]	163	10	1023	10	4606	N.A.	653	N.A.	7	5
Canada	1987[b]	18	5	114	6	835	19	70	N.A.	11.9	6
Yugoslavia	1987	53	N.D.	75	N.D.	815	N.D.	N.D.	385	∞	7
Italy	1985	65	2	5	N.D.	40	N.A.	7	70	5.7	8
Finland	1985[b]	155		80[e]		610	N.A.	60	930	10.1	9
Israel	1985[b]	100	N.D.	350	40	2440	N.A.	290	540	8.4	10
Hong Kong	1989[b]	25	520	15960	60	11670	N.A.	2170	640	5.4	11
Canada	1988	72	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	2185	N.A.	12
India	1988	25	432	5550	47	1265	12	250	120	5	13
Spain	1991	51	34	235	10	604	5	12	1967	50	14

[a]: Number of samples. [b]: Year of publication. [c]: Not analyzed. [d]: Not detected. [e]: Sum of isomers. References: 1: Sitarska et al. 1987; 2: Fytianos et al. 1985; 3: Zaidi et al. 1989; Wickström et al. 1983; 5: Karakaya et al. 1987; 6: Dewailly et al. 1989; 7: Krauthacker 1991; 8: Dommarco et al. 1987; 9: Mussalo-Rauhamaa et al. 1988; 10: Weisenberg et al. 1985: 11: Ip and Phillips 1989; 12: Davies and Mes 1987; 13: Tanabe et al. 1990; 14: This study.

3, with the exception of Yugoslavia, reflecting that some countries are still consumers of p,p`DDT or that our values are later than other countries with restrictions put on the use of this compound. These results indicate the effectiveness of the regulatory actions adopted in Spain, where p,p`DDT was banned in 1977. The enforcement of p,p`DDT prohibition caused, after some years, a decrease of p,p`DDT and p,p`TDE levels in human milk, whereas p,p`DDE levels still increase through the consumption of animal food and/or p,p`DDT metabolism. Finally, PCB concentrations are in line with those of most industrialized countries, although are higher than those reported in Table 3, with the exception of Canada.

Mothers were classified arbitrarily according to their age into three groups: 18-25, 26-33, and 34-40 yr. No significant differences were observed between residue levels and the donnors age group. This may be explained by the fact that there was no significant difference in the exposure period between three age groups, since are latter to the start of use of organochlorine compounds. Likewise, in this study no correlation between residue levels and number of children was found.

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