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Organochlorine Residues in Human Milk in Spain. Polychlorinated Biphenyls (PCBs) from 1988 to 1991

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Polychlorinated biphenyls (PCBs) are a class of chlorinated aromatic hydrocarbons that are synthesized by the direct chlorination of biphenyl. Commercial PCBs are fat soluble, environmentally persistent, non-inflammable and electric insulating. PCBs have had many applications in manufacturing hydraulic and dielectric fluids for capacitors and transformers, plastics, wrapping paper, carbon paper,printing ink, plasticizers and stabilizers in pesticide sprays, etc.. Due to their widespread use and environmental stability, the PCBs are the most ubiquitous industrial pollutants in the ecosystem. The well known accidents of Yusho (1968) and Taiwan (1979) first drew attention to the hazards of the PCBs accumulation in the environment. Since then, the use of PCBs has gradually decreased through international restrictions which, nowadays, even affect their use in closed electrical systems.

The organochlorine compounds are persistent lipophilic pollutants and the biological monitoring of the human body burden by these contaminants is based on methods using adipose tissue taken from necropsies. The human milk offers a more feasible medium for assessing levels of body contamination by lipophilic residues in different areas within or between countries. It is known that no significant differences exist between the levels of organochlorine residues in the subcutaneous tissue and the milk fat of individual donors (Skaare 1988). However, as adipose tissue is taken from deceased people who were generally older than the nursing mothers, the mean contaminant levels by this method are somewhat higher than those obtained in milk samplings.
The donors were selected using very narrow limits for the parameters of age, weight, diet, smoking habits and previous nursing time, etc. so that the variations in the mean PCBs levels of the different groups could only be attributed to the environmental conditions of the

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areas selected for this study.

MATERIAL AND METHODS

All glassware was washed, heated to 300°C and rinsed with acetone and petroleum ether before use. All solvents were glass distilled and free from interfering residues when tested by gas chromatography (concentration 250:1). The water was distilled twice in a glass apparatus and shaken with petroleum ether. Sodium sulphate anhydrous was heated at 600°C for 6 hours, stored in desiccator and heated to 130°C before use. Aluminum oxide (Merck 60, active, basic, 70-320 mesh) was activated at 200°C overnight and subsequently deactivated with 5% water. Silica gel (Kieselgel Merck 60, 70-230 mesh) was activated at 140°C overnight and subsequently deactivated with 0.5% water.

BZ No. 8,52,101,118,138 and 180 PCB isomers (obtained from the Reference Bureau of the European Community) and Aroclor 1260, were used as references. PCB isomer BZ nº 185 and Heptachlor (Serva) were used as Internal Standards (I.S.) (heptachlor was not detected in the Spanish human milk in a previous study of organo-chlorine pesticides).

The milk was expressed manually with complete emptying of one breast into a graduated glass cylinder. An aliquot of 20ml was transferred to a vial with a screw cap and stored at -20°C until analyzed.

1 vol milk (10-20ml) was mixed with 100 mg potassium oxalate and shaken with 1 vol ethanol 95%. The mixture was shaken again with 1 vol diethyl ether-petroleum ether (1+1 v/v) and phases separated by centrifugation for 10 min at 1000rpm. The upper phase was transferred to a glass cylinder and the aqueous residue was extracted twice with 1/2 vol diethyl ether-petroleum ether. The combined extracts were washed with water, passed through anhydrous Na2So4, and dried to 1-2ml under nitrogen in a 35-40ºC water bath. The volume was adjusted to 10ml with petroleum ether. An aliquot was taken for lipid determination.
Clean up was carried out as described by De Vooght et al (1986). Essentially, extracts amounting to 60mg of lipid were chromatographed on a combined aluminum oxide-silica column using petroleum ether as eluting solvent. A volume of 40ml was collected. The eluate was concentrated to 5ml. An aliquot of 200μl was taken for measuring the HCB and the rest was used for PCB determination. 50μl of I.S. (100 pg/μl) was added and the mixture was carefully concentrated to a final volume of 50μl.

GC 5890 Gas chromatograph Hewlett Packard with electron

capture detector. Splitless injector. Crosslinked methylsilicone column 25m x 0.20mm x 0.11 μm. GC/MS. Hewlett Packard MS 5985 System coupled with a Hewlett Packard GC 5440A with a splitless injector.

The column temperature was programmed as follows: after initially being maintained at 100°C for 1 minute, it was heated to 163°C in 9 minutes, and then heated at a rate of 3°C minute to a final temperature of 260°C.
Qualitative GC/MS was carried out using chromatographic conditions similar to those as above. Multiple ion detection analysis was performed with an ion source temperature of 250°C, an electron energy at 70ev and resolution 900. The sample was monitored for the following ions m/z: 324 and 326 for penta-, 360 for hexa-, 394 and 396 for hepta- and 248 for octacholobiphenyls.

Six aliquots of an infant formula having a 6.2% lipid content were fortified with a mixture of isomers BZ nº 8, 52, 101, 118, 153, 138, and 180 to a final concentration of approximately 30ng per gram of milk fat. Petroleum ether was used as the isomer solvent. The extraction and clean up procedures were identical to those used for the human milk samples. Unfortified samples from the same infant formula were run concurrently with the fortified samples during the entire analytic procedure. The mean recovery values ranged from 61% (nº 8) to 89% (nº 180) with an overall mean of 80.7%.
Reproducibility: The mean PCB value of 8 aliquots from a pool of human milk was 0.996 ppm (in Aroclor 1260) with a coefficient of variation of 11.8%

An analysis of variance was first applied to sets of three independent samples (Levene). In a second stage, the means were compared by sets of two samples (Bonferroni and Tukey). When the variances were different, the Brown-Forsythe test was used to compare the means. Non parametric tests (Kruskal-Wallis test) were also performed. All the statistical analysis were done with the BMPD packet, using the BMPD3D,BMPD7D and BMPD3S programs.

The following four quantification methods were applied to a set of 50 samples collected in Madrid. Method one: using Aroclor 1260 as the reference standard. The PCB concentration was calculated by comparing the sum of the integrated areas of all the peaks observed in the reference standard and in the milk sample. With this method, an average PCB level of 0.87±0.44 ppm was found. Method two: the concentrations of the three isomers 153, 138 and 180 were determined by using their respective integrated areas, and were compared to their known concentration in Aroclor 1260 "three peaks method". The average PCB level obtained by this method was 1.44±0.51

TABLE I. PCBs in Spanish Human Milk (μg/g fat milk) from 1988 to 1991.

	Nª	PCBs(ppm)
Madrid. Urban area 1989	50	0.88±0.34
1991	52	0.90±0.40
1990-1991		
Rural areas in cereal producing provinces	84	0.59±0.23
Urban areas in cereal producing provinces	39	0.68±0.29
Rural areas in irrigation farming provinces	54	0.78±0.48
Urban areas in irrigating farming provinces	38	0.78±0.46
Industrial areas	52	0.78±0.42
Plastic manufacturing area	10	0.82±0.31
Coastal fishing areas	29	1.28±0.58

ppm. The conversion factor from method 2 to method 1 was 0.64 and the correlation between both methods was r=0.991. Method three: the sum of the three peaks 153, 138 and 180 amounts to about 61% of the total PCBs in human milk in Western Europe. Assuming that this is also the case for the Spanish samples pattern, the PCB content was calculated by multiplying the sum of the measured concentration of these three congeners by a factor of 1.64 (100/61). The average PCB level obtained by this method was 0.76±0.25 ppm. A fourth approach was attempted by applying the 0.5 factor of Schulte et al (1984) who used Clophen 60A as a standard, to our "three peak values." By this method, a mean value of 0.72±0.23 ppm was obtained. It was decided to adopt Method One for this study.

RESULTS AND DISCUSSION

From November 1988 to October 1991, a total of 408 milk samples from individual mothers were analyzed. The samples were taken at the 3rd week post-partum. Sixty percent of the mothers were nursing their first infant, and the rest their second one. They had been living in their respective areas for at least eight years before the sampling and were in good nutritional condition. The mean age of the donors was 26±3 years. Answers to questions regarding dietary and smoking habits, weight and weight loss as well as possible occupational exposure were obtained from the mothers. The PCB results are summarized in Table 1.

The communities living in the coastal fishing areas showed the highest PCB levels average (1.2 ppm), whereas the lowest levels (0.59 and 0.68 ppm) were obtained in the urban and rural areas of the provinces with a predominance of grain agriculture and far from industrial areas. The rest of the groups showed average levels between 0.7 and 0.9 ppm.
The levels found in Madrid were significantly higher than those in the rural communities ($p<0.001$) and significantly lower than those in the coastal areas

($p<0.001$). About 50% of the samples from the coastal zone showed values over 1.5ppm, the highest permissible level for milk from a nutritional point of view. Thirteen samples from different areas showed values between 3 and 7 ppm. One of the donors (6ppm) was a gypsy whose husband was a livestock dealer. Another donor (4.6ppm) was married to a car repair worker and lived in the workshop. A third mother (6ppm) lived in an industrial building. As for the rest of the samples, no apparent explanation was found in their reports. These 13 cases were not included in the calculations of their respective groups. The ten mothers who lived in the community in which there was a plastic manufacturing industry all worked in it. Nine donors from the rural areas worked in green houses. Their average PCB levels were no different from those found in their respective provinces.

Our values can not be compared to those reported by Baluja et al (1982) in Madrid as they are expressed on a whole milk basis. Gomez et al (1991) reported average PCB levels of 1.13 and 0.97 ppm with two different methods of quantification on samples of adipose tissue from autopsies obtained in Barcelona.

The higher mean level found in the coastal zone is remarkable. Even if the figures are not alarming, they are significantly higher than those in the rest of the country. The donors belong to a prosperous Basque fishing community whose population also includes more fresh fish in their diet than any other community in Spain. This finding, but at a much lower level, is similar to that reported by Devailly et al (1989) who found PCB levels five times higher in the milk of eskimo women (3.3ppm) than in that of white women living in the south of Quebec (0.77ppm) and attributed this difference to the high content of contaminated fish in the diet of the eskimos. Another explanation could be that, in the seventies, when restrictive measures for the use of PCBs were imposed in the industrialized countries, Spain was undergoing a period of political and economical transition in which no restrictive measures could be positively implanted. By then, the industrialization of the Basque Community was very superior to that of the rest of Spain.
The mean PCB values found in the present study are not very different from those reported in other industrialized countries when these prospective studies were carried out in the early eighties. Noren (1983), Skaare (1981), Wicstrom et al (1983), Rogirst et al (1983), Collins et al (1982), Wickizer et al (1981), Mes et al (1984). This suggests that, for the time being, the international restrictions for the use of PCBs are being observed in Spain.

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