Level Variations of Coplanar PCBs in Human Breast Milk at Different Times of Lactation.

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PCBs are a highly lipophilic group of global pollutants, consisting of 209 congeners which exhibit wide differences in their toxic and biological effects. The coplanar PCB (non-, mono- and di-ortho Chlorine substituted) congeners, the most toxic ones, induce similar toxic effects as 2, 3, 7, ,8 TCDD[1] .Thus for risk assessment of exposure to PCBs, the analysis of these coplanar congeners is required.

The PCB levels in human breast milk are of specific concern because of the potential health damage which may be caused to the nursing baby. The PCB levels in this sample come from previously accumulated quantities in body fat whose principal source is food, and pass directly to the nursing baby who accumulates the PCBs in adipose tissue.

The amount of total PCBs and other organochlorine compounds (OCC) in human milk at different time intervals after birth was reported earlier[2,3], but data concerning individual and coplanar PCBs are scarce in the literature. The results from some studies showed a gradual decrease of residual levels in milk and milk fat[3-5] . However, other researchs have shown differences in this respect [6-9]. These results show that OCC levels in human milk depend on several factors, such us the lactation period, number of pregnancy, dietary habits, cigarrette smoking and fat content of the milk among the more important.

The aim of the present study was to investigate the variations of 14 individual PCBs in breast milk during lactation period. We related the different concentration variations observed among the individual PCBs to their molecular structure, % fat in human breast milk and influence of parity.

MATERIALS AND METHODS.

Sampling

Milk from three individual mothers (A, B and C) were collected between 1992-1994. Mother A was 30 years old and she delivered milk samples for both her first and second child, with two years differences in age. Mothers B and C were 31 years old and they gave birth to their first and third child respectively. The milk was expressed manually, and collected every week during the first infant nursed in the morning.

Analysis

A 10 ml sample was spiked with a mixture containing 10 ng of $^{13}C_{12}$ isotopically labeled PCBs 77, 126 and 169, 153 and 180 as internal standards, followed by a 90 min incubation period at 45°C. and freeze drying for 48 h. The freeze dried milk was homogenized with 10 g of a mixture of silica gel and anhydrous sodium sulfate (1:1) mixed to become a fine powder and loaded in a column. Extraction was carried out with 100 ml of a 1/1 hexane: acetone solution. Fat was determined gravimetrically, and then dissolved in hexane and removed with concentrated sulfuric acid. The hexane layer was washed with distilled water, dried and concentrated. The concentrate was transferred to an activated Florisil column (450°C, 24 h.), and eluted with hexane and methylene chloride. One procedure blank was included for each five samples; blank levels were negligible. The recoveries for the PCB calculated by standard additions of PCB-155 and PCB-$^{13}C_{12}$ -101, added before the chromatografic inyection were between 80 and 95%. The precision of the analytical methodology was previously checked using fortified real samples with 1 ng of each PCB congeners. The average precision, expresed as coefficient variation, was equal or lower than 5%, depending of the congener. Sample extracts were analyzed by a High Resolution Gas Chromatograph (HP5890 Series II) coupled to a Low Resolution Mass Spectrometer in SIM mode. (HP5971 A).

HRGC: A 0,8 µl aliquot of the sample was injected in the splitless mode at 260°C, in a S-54 capillary column (27 m length * 0,25 mm i.d., 0,25 µm film thickness). The column temperature was programmed from 100°C (1 min) to 130°C at 50°C/min. , then to 190°C (2 min.) at a rate of 4°C/min., and finally to 230°C at 2°C/min. rate. The final temperature was maintained 15 min. Helium was used as carrier gas at a flow rate of about 5 psi.

LRMS: The eluent from the column was transferred to a quadrupole mass spectrometer with electron impact ionization and subsequent ion detection. The interface temperature was 280°C. The spectrometer was scanned from m/z 95-500 with a 1,23 sec cycle time. The source temperature was 280°C. The source was operate at 70 eV.

Two ions characteristics of each PCB homologue and the respective labeled internal quantitation standards were monitored for each analysis . Identification of individual PCB congeners was based on retention time information and the comparison of the ratios of the characteristics ions with theoretical values. Factor responses of the labeled standards to native standard congeners were calculated using the same concentration of all of them , in the PCB sample level ranges, and in the same injection. The absolute detection limits were 9.3 pg, 9.6 pg and 11 pg for PCB-77, 126 and 169 congeners, respectively.

Quality control criteria were defined by simultaneous detection of a peak for both ions monitored within the expected retention time window for each congener, ion intensity ratio of sample peaks within 20% of the mean values for calibration standards and satisfactory results for blank samples.

RESULTS AND DISCUSSION

The sum of PCBs, referring to all congeners analyzed, show variations at the different time intervals tested in all cases investigated. The general tendency was a

gradual increase from week 1 to week 10 and a slight decrease from week 10 to the end of sampling. The PCB levels found in mother A during her firts lactation period (Table I) were significantly lower than levels found during her second lactation, two years later (Table II). As other authors have found[9], the amount of PCBs are inversely correlated to the amount of lipid.

The results indicate that PCB-180, 194, 118, 153, 138 and 101 were the major contributing congeners in the breast milk during the lactation period, each making up more than 5 % of total PCBs found. PCB-77, 126, 169 and 167 have a lower contribution (< 2%), and the other congeners PCB-105, 151, 170 and 156 contributed between 2 and 5 %. These results agree with the findings of other authors[10-11] also have found that PCB-153, 118 and 180 represent the largest quantities and PCB-77, 126 and 169 represent the smallest quantities in human breast milk.

Some differences in the abundancies of the three no-ortho substituted PCB congeners were found. The PCB-77 levels found in milk from mother A, for her firts child, were always higher than PCB- 126 and PCB-169. Nevertheless, this congener was not found in samples from mother B. These results would partially explain the discrepancies found in similar studies concerning the non-ortho predominant congener in human breast milk. Thus, while some authors[10] found the PCB-126 as predominant (case of mother A, second child), others [9,12] found the PCB-169 as the most abundant.

Milk is an elimination mechanism for chemicals , which have entered into the human body by different pathways. PCB levels in milk come from previously accumulated PCBs in body fat, and from food, mainly by dairy intake . The differences found in the variations of PCB congeners in breast milk, from the same mother, along the lactation period is probably due to the summed effects of the two processes, bioacumulation in body fat and later mobilization to human breast milk. Both processes are related to the lipophilicity of the congeners and their molecular structure features. Studies concerned with the accumulation and metabolism of PCB congeners[13-15] have demonstrated that congeners with neighbor H atoms, at either meta-para or ortho-meta positions, showed the higher capacity for degradation. When neighbor H atoms are present at the ortho-meta position , the number of ortho chlorines influences the kinetic behavior of the congeners; lowered concentrations were observed only for mono-ortho congeners, while the di-ortho and tri-ortho substituted congeners behaved like non metabolized PCBs.

The differences and similarities in the molecular structure of the PCB congeners investigated were related to their behavior in the mobilism process. The 14 individual PCB congeners studied have been grouped in four categories, according to their concentration variations in the breast milk during the lactation. The PCB congeners of each group have similar molecular structures. Thus, group I formed by PCB-77, 118 and 126 (have chlorine at the meta-para positions, and neighbor H atoms are present at the ortho-meta positions. PCB-138 and 153 (group II) have chlorines at 2, 4, 5 positions and two chlorines at the ortho position. Congeners 153 and 105 (group II) have chlorines at positions 2, 3, 4 on the second ring, and PCB138 and 105 (group II) have neighbor H atoms at the ortho meta positions (group II). PCB-167 and 156 (group III) are hexachloro substituted, they have chlorines at 3, 4, 5 positions, and one ortho chlorine . Finally PCB 180, 170 and 194 (group IV), which are hepta- and octa-chlorosubstituted, have chlorines at 2,

3, 4, 5 positions, no neighbor H atoms, as well as being di-ortho substituted. On the other hand, the lipophilicity (n-octanol/water partition coefficient, Kow) are also related to the bioacumulation factor of a chemical and with the mobilism from lipid adipose tissue to human breast milk. . The log Kow of the 14 PCB congeners studied varies between 6.36 for PCB-77 and 7.80 for PCB 194 [16]. The PCB lipophilicity generally increases with the number of chlorines in their aromatic rings, but it is also related to their molecular structure. The log of Kow calculated for the 14 PCB congeners increased in the following order: PCB 77, 101, 151, 105, 118, 138, 126, 153, 156, 167, 153, 170, 180 and 194. In general, groups I and II contained the lower lipophilic congeners, and III and IV the higher ones .

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Table I. Values of PCB congener concentrations in human milk given in ng/g fat weight, during lactation period (week 8 to 12) from mother A, first child. International Toxic Equivalent (I-TEQs) are calculated according with Safe(1990)

PCB structure	Congener IUPAC N°	Experimental t_R (min)	Week 8	Week 9	Week 10	Week 11	Week 12
3,3',4,4'- T_4CB	77	25.84	2.64	35.83	8.77	3.50	9.61
2,2',4,5,5'-P_5CB	101	23.36	75.40	31.32	22.29	72.28	141.5
2,3,3',4,4'-P_5CB	105	29.63	8.46	18.29	9.72	8.84	7.02
2,3',4,4',5-P_5CB	118	27.68	23.37	49.54	31.45	28.20	57.46
3,3',4,4',5-P_5CB	126	32.12	2.25	21.63	6.04	0.87	12.61
2,2',3,4,4',5'-HxCB	138	31.42	3.98	37.61	48.62	57.23	50.41
2,2',3,5,5',6-HxCB	151	26.65	1.71	25.09	6.25	8.35	11.06
2,2',4,4´,5,5'-HxCB	153	29.37	9.48	107.3	119.3	120.9	121.6
2,3,3',4,4'5-HxCB	156	35.31	15.37	7.07	6.82	12.80	11.09
2,3',4,4',5,5'-HxCB	167	33.60	2.57	<0.31	0.50	2.25	1.87
3,3',4,4',5,5'-HxCB	169	38.41	<0.32	4.15	<0.24	<0.34	<0.46
2,2',3,3',4,4',5-HpCB	170	36.70	13.46	84.75	20.60	12.72	9.57
2,2',3,4,4',5,5'-HpCB	180	39.20	44.86	412.1	83.70	40.62	36.42
2,2',3,3',4,4',5,5'-OCB	194	42.50	172.5	409.2	46.40	12.80	9.04
% fat			4.69	1.66	3.63	1.26	1.59
I-TEQ			0.31	2.82	0.76	0.18	1.44

Table II. Values of PCB congener concentration in human milk given in ng/g fat weight, during lactation period (week 2 to 9) from mother A, second child.

CONGENER IUPAC N°	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9
77	ND	ND	ND	ND	ND	ND	9.67	ND
101	473.47	803.33	1314.35	566.62	261.08	562.89	603.80	468.35
118	146.51	308.59	357.23	216.44	105.19	397.12	378.99	122.94
105	44.41	249.08	314.72	101.52	38.36	175.92	54.55	ND
126	ND	10.13	ND	ND	31.10	ND	46.47	ND
153	42.56	493.49	773.26	210.33	133.76	262.69	232.36	231.60
138	300.45	497.70	691.57	188.61	89.87	130.67	150.85	233.96
156	23.16	-	85.91	19.48	10.13	51.59	12.08	21.60
169	ND	-	2.44	ND	ND	ND	0.10	ND
180	199.65	205.18	168.08	169.10	135.75	161.98	144.44	251.54
194	0.82	0.21	0.07	0.78	0.35	0.46	0.23	1.15
% fat	1.47	3.21	1.82	2.31	4.60	2.73	2.39	0.69