Bull. Environ. Contam. Toxicol. (1995) 54:349–356

Environmental Contamination and Toxicology

Levels of Coplanar PCBs in Human Breast Milk at Different Times of Lactation

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Received: 10 April 1994/Accepted: 1 August 1994

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 (Safe, 1990) 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 (Galetin-Smith et al. 1990; Skaare and Polder 1990), but data concerning individual and coplanar PCBs are sparse in the literature. The results from some studies showed a gradual decrease of residual levels in milk and milk fat (Skaare and Polder 1990; Mes et al. 1984; Klein et al. 1986, Rogan et al. 1986). However, other research has shown differences in this respect (Curley and Kimbrough 1969; Kodoma and Ota 1980; Al Omar et al. 1986; Fooken and Butte 1987; Hori 1993).

We present here our first result concerning the concentration of 14 individual PCBs (13 coplanars) in breast milk from the same mother, during weeks 8 to 12 of lactation. We related the different concentration variations observed among the individual PCBs to their molecular structure and % fat in human breast milk.

MATERIALS AND METHODS

A 10 ml sample was spiked with a mixture containing 10 ng of $^{13}C_{12}$ isotopically labeled PCBs 77, 126 and 169, as internal standards, followed by a 90 min incubation period at 45°C. and freeze drying for 48 h. The freeze dried milk was

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Table 1. Ions monitored for coplanar PCB analyses.

Congener IUPAC N°	Mass A M/M+2	Mass B M+2/M+4	Ratios A/B
77	292.15	290.05	0.886
13C-77	302.15	204.20	0.886
126	324.00	326.05	0.713
13C-126	336.20	338.20	0.713
169	360.10	362.05	1.25
13C-169	372.20	374.25	1.25
180	394.05	396.05	
194	430.25	428.15	

homogenized with 21 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 200 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. Two procedure blanks were included; blank levels were negligible. The recoveries for the PCB calculated by standard additions 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 was lower than 4%. 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 ((table I). 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 are expressed in ppb(ng/g fat basis) and in International 2378 TCDD toxic equivalents (I-TEQs) after the so called "International formula" (Safe 1990).

RESULTS AND DISCUSSION

Table II shows the concentrations of 14 PCB congeners in breast milk, on a fat weight basis, taken from the same mother and obtained between weeks 8 and 12 after birth. The sum of PCBs, referring to all congeners analyzed, show variations at the different time intervals tested. Thus, the 376,04 ng/g found at 8 weeks after birth increased to 1243,84 ng/g (3,3 times higher) one week later. This level decreased to 522,49 ng/g (2,38 times lower), and to 381,39 ng/g (1,36 times lower) at weeks 10 and 11 of lactation. Finally, the concentration level increased again to 479,31 ng/g (1,25 times) at week 12 As other authors have found (Hori, 1993), the amount of PCBs are inversely correlated to the amount of lipid.

The results shown in table II 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 (Noren et al. 1990; Duarte-Davidson et al. 1991) who 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.

The non-ortho congeners did not contribute significantly to the sum of PCBs. PCB-77 and 126 were always more abundant than PCB-169. PCB-77 was the dominant non-ortho congener from 8 to 11 weeks of the lactation period, while PCB-126 was the dominant non-ortho congener at week 12. These results would partially explain the discrepancies found in similar studies concerning the non-ortho predominant congener in human breast milk. Thus, while Noren and Luden (1991) and Dewailly et al. (1989) found the PCB-126 as predominant, Hori (1993) and Johansen (1993) found the PCB-169 as the most abundant.

When the concentrations were calculated in terms of the 2,3,7,8 TCDD International Toxic equivalents (I-TEQ), the values fluctuate in the same way as the total PCB concentration did. The value increased from 0,31 ng TEQ/g (at 8

Table 2. Values of PCB congener concentrations in human milk given in ng/g fat weight, during lactation period (week 8 to 12) from one mother. 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

weeks) to 2,82 ng TEQ/g (at 9 weeks); it decreased to 0,47 ng TEQ/g. (at 10 weeks) and to 0,18 ng TEQ/g (at 11 weeks) and finally increased to 1,44 ng TEQ/g (at 12 weeks).

The non-ortho congeners (PCB-77, 126 and 169) were the major contributors at 9 weeks to the toxic equivalents (TEQ), calculated by the International formula (Safe, 1990) making up approximately 73 % of all measured PCBs. The mono-ortho contributed with approximately 21 %, and the di-ortho with 6 %.

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 (Bush et al. 1985; Yakushiji et al. 1984; Boon et al. 1987), 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 period (Fig 1). 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 (Hawker and Connell, 1988). 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

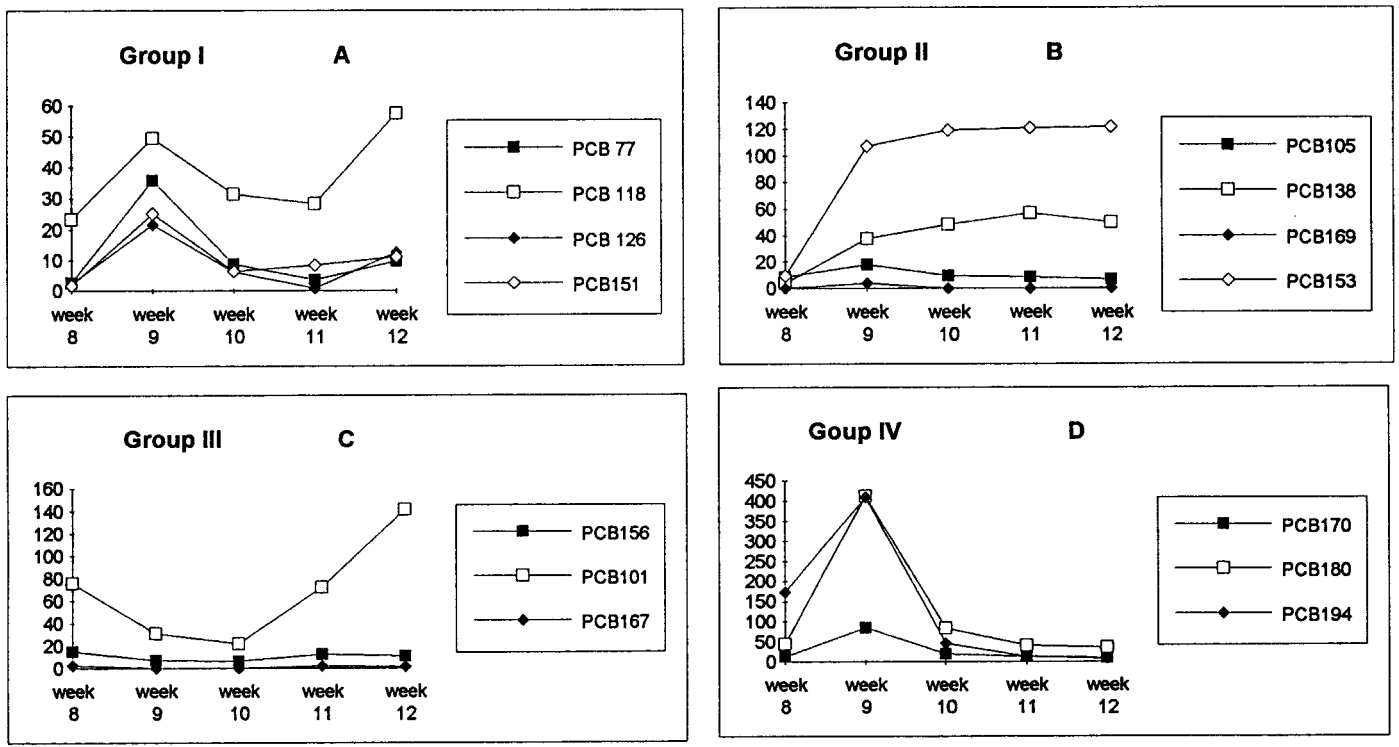

Figure 1. The variations of PCB congener concentrations (ng/g fat) in breast milk during lactation period. a) PCB-77, 118, 126 and 151, b) PCB 105, 138, 169 and 153, c) PCB-156, 101 and 167, d) PCB- 170, 180 and 194.

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 (Figure 1).

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