Surveillance of Polychlorinated Biphenyl Congeneric Patterns in Human Breast Milk from 1973 to 2000 in Osaka, Japan

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Abstract

Objective: Because the analysis of polychlorinated biphenyls (PCBs) in 1970's was performed using a packed column gas chromatograph with an electron capture detector to determine total-PCB concentration, analytical data obtained by this analysis do not meet the requirement for the risk assessment of PCB congeners. In this context, the present study was carried out to reevaluate the congeneric analysis data by analyzing breast milk fat specimens that have been kept frozen.

Methods: PCB congeners in human breast milk were analyzed by high-resolution gas chromatography/high-resolution mass spectrometry (HRGC/HRMS) using a capillary column for the selected ion monitoring (SIM) of PCBs.

Results: Twelve major PCB congeners were detected in breast milk. The concentrations of all congeners of PCBs in breast milk were found to decrease annually, and their changes over time differed greatly for each congener. Between 1973 and 2000, almost no changes in the proportions of the 12 congeners of heptachlorinated biphenyls (HpCBs) and hexachlorinated biphenyls (HxCBs) were found whereas a marked and a slight decrease in the proportion of pentachlorinated biphenyls (PeCBs) and tetrachlorinated biphenyls (TeCBs), respectively, were observed.

Conclusions: The PCB contamination of the Japanese population is estimated to be largely caused by seafood intake, but the proportions of PCB congeners in fish commodities were different from those in breast milk. The absorption, metabolism and therefore the accumulation of PCBs in the human body differ greatly depending on the congener.

Key words: polychlorinated biphenyl, human breast milk, congener, contamination

Introduction

Many chemical substances released into the environment have been reported to have serious impacts on the natural ecosystems of flora and fauna, particularly on endocrine systems, and thus affect humans. In 1996, the Healthy Earth Research Program Promotion Research Project reported research on such endocrine disruptors (1). This research project report warned that polychlorinated biphenyls (PCBs) are endocrine disruptors with extremely potent effects similar to those of estrogens.

From 1972 until the present, the Health Promotion Section of the Osaka Prefectural Health Division has served as the

coordinating body for the Osaka Prefecture Breast Feeding Promotion Project, in cooperation with prefectural health centers, the Environment and Public Health Bureau of Osaka City, the Sanitation Section of Sakai City, and the Health Sanitation Division of Higashiosaka City. The Osaka Prefectural Institute of Public Health has been measuring organochlorine compounds such as PCBs in the breast milk of lactating mothers living in Osaka Prefecture from one to three months postpartum (2, 3). Every year, the coordinating body has convened meetings of the Osaka Prefecture Breast Feeding Promotion Project Review Board, which conducts comprehensive evaluations regarding the safety of breast-feeding and related issues. To date, there have been no reports of dangerously high levels of contaminants in breast milk for infants. For lactating women in general, the government has concluded that in consideration of the nutritional advantages of breast milk, there is no reason not to encourage breast-feeding. The authors have previously reported on changes in the levels of *beta*-hexachlorocyclohexane (*beta*-HCH), dichlorodiphenyltrichloroethane (DDT), chlordane and PCB contamination in human

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breast milk from 1972 to 1998 (4). PCB analysis at that time was conducted using Kanechlor-500 (chlorine content=about 54.6%) as the standard apparatus. The total amount of PCBs was measured by GC-ECD with a packed column, and the calculation method is based on the total height of the seven major chromatographic peaks for which the gas chromatographic retention times are greater than that of *p,p'*-DDE. In recent years, however, the toxicity assessment of individual PCB congeners has become an issue. Toxicities such as endocrine disruption differ among congeners, making it necessary to analyze individual PCB congeners. This led us to measure PCB congeners in breast milk, using milk fat that has been in frozen storage since the original studies.

Subjects and Methods

Breast milk specimens were collected from women living in Osaka Prefecture. Before the start of research, the original sample donor agreement and the approval of the laboratory ethics committee were obtained. To minimize maternal factors, milk specimens were obtained from women who were primiparous and whose age ranged from 25 to 29 years old. The milk specimens were collected between 1973 and 2000 and stored at -20°C . Equivalent amounts of milk fat were mixed from 20 to 39 milk specimens and prepared for each year's sample. PCB congeners were analyzed by HRGC/HRMS using a capillary column for the selected ion monitoring (SIM) of PCBs. The number of samples for each year is shown in Table 1.

All of the reagents used for the analyses were special analytical grade tested for residual PCBs, pesticide and dioxin (Wako Pure Chemical Industries). PCBs and dioxin standards used were from Wellington Laboratories.

Milk samples for each year for HRGC/HRMS were prepared by mixing the obtained milk specimens. Milk fat (about 2.0 g) for each year was decomposed in 100 ml of 0.5 N KOH/ethanol solution for two hours, with stirring at room temperature, after the addition of ^{13}C -2,3,7,8-substituted polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and ^{13}C coplanar-PCBs (co-PCBs) as surrogates, PCDDs, PCDFs and PCBs were then extracted with 80 ml of hexane three times. The hexane extract was purified with a multistage column of silica gel coated with AgNO_3 , H_2SO_4 and KOH, and an aluminum oxide column. The effluent was dried and PCBs were separated from co-PCBs on an activated carbon column. The effluent was dried and reconstituted to *n*-nonane.

PCB congeners analysis: HRGC (HP5890 II, Hewlett Packard, USA)/HRMS (JMS700, JEOL, Japan) using a capillary column equipped with a splitless injector was carried out for SIM of PCBs. *Non-ortho* PCBs (mixture of four) and *mono-ortho* PCBs (mixture of eight) were measured separately. The chromatographic parameters were as follows: 5% phenyl-95% dimethylsiloxane-fused silica capillary column (DB-5MS; J&W; film thickness, 0.25 μm , 30 m $\times$ 0.25 mm ID, USA), programmed from 130 $^{\circ}\text{C}$ (2 min) $\rightarrow$ 15 $^{\circ}\text{C}/\text{min}$ $\rightarrow$ 230 $^{\circ}\text{C}$ $\rightarrow$ 4 $^{\circ}\text{C}/\text{min}$ $\rightarrow$ 300 $^{\circ}\text{C}$ (*non-ortho* PCBs) and 130 $^{\circ}\text{C}$ (2 min) $\rightarrow$ 15 $^{\circ}\text{C}/\text{min}$ $\rightarrow$ 180 $^{\circ}\text{C}$ $\rightarrow$ 3 $^{\circ}\text{C}/\text{min}$ $\rightarrow$ 300 $^{\circ}\text{C}$ (*mono-ortho* PCBs); injection temperature, 290 $^{\circ}\text{C}$; sample size 2 ml. The mass

spectrometry operating conditions were as follows: ionization, EI, 270 $^{\circ}\text{C}$, 38 eV; emission current, 600 $\text{\AA}$; SEM voltage, 1.8 kV; and mass resolution, 10,000. The detection limit of *non-ortho* PCB was 1 pg/g fat and *mono-ortho* PCB was 0.01 ng/g fat. The TEQs of dioxins and co-PCBs were calculated according to World Health Organization (WHO) methods.

PCB analysis with packed column: Using KC-500 (chlorine content=about 54.6%) as a standard, the total amount of PCBs was measured by GC-ECD using the 2% OV-1 packed column (5). The calculation method is based on the total height of seven major peaks for which the gas chromatographic retention times are greater than *p,p'*-DDE, as recommended by one of the official calculation methods for PCBs (6).

Results

The recovery ratios for a standard solution for PCBs in human breast milk were about 100%. The detection limits were 1 pg/g fat for *non-ortho* PCBs, and 0.1 ng/g fat for *mono-ortho* PCBs. Among the 154 PCB congeners occurring 51 PCB congeners ranging from TeCBs to HpCBs were detected in the milk fat samples collected in 1973. However, only 48 PCB congeners were detected in the samples from 2000 (Table 1). Twelve types of major PCB congener were detected in human breast milk. In terms of composition ratio, the congeners ranked from high to low were as follows: #138, #153, #118, #74, #66, #99, #180, #105, #187, #60, #170, and #52. The concentrations of all PCBs in breast milk were found to steadily yearly decrease, and their changes over time differed greatly among congeners. There might be a much higher concentration in breast milk for a PCB congener with chlorine in the position of 2,4,5. TeCB concentrations in breast milk decreased to about 5.14% of their highest concentrations (0.176 $\mu\text{g}/\text{g}$ fat in 1973, 0.009 in 2000). PeCB concentrations had declined to about 11.7% of their highest levels (0.205 $\mu\text{g}/\text{g}$ fat in 1973, 0.024 in 2000), HxCBs to about 23.1% (0.307 $\mu\text{g}/\text{g}$ fat in 1974, 0.071 in 1998), and HpCBs to about 33.8% (0.006 $\mu\text{g}/\text{g}$ fat in 1978, 0.039 in 2000) (Fig. 1). These findings suggest that the half-lives of congeners in the body become longer for PCB congeners with high chlorine contents.

PCB congener concentrations decreased from those in the 1970s (when the levels of contamination in human breast milk were the highest): congener #138 decreased to about 21.3% of

Fig. 1 Trends of PCB congeners on human breast milk fat basis. (25 to 29 year-old mothers, no data for 1987)

Table 1 Concentrations (ng/g fat) of PCB congeners in human breast milk from 1973 to 2000

Year	Number of samples	Tetra-PCB										Penta-PCB										Hexa-PCB				
		#52	#49	#44	#74	#66	#60	#81 ^a	#77 ^a	#95	#92	#101	#99	#119	#87	#85	#110	#108/107	#118 ^b	#114 ^b	#105 ^b	#126 ^a	#151	#135	#147	#149
1973	30	16.39	8.04	4.52	81.92	45.48	19.62	0.077	0.277	5.44	5.06	12.63	44.21	0.07	7.78	1.56	2.51	4.91	84.77	3.98	31.46	0.310	5.69	2.40	1.27	3.64
1974	22	7.71	4.57	1.88	79.06	34.19	16.03	0.038	0.203	2.78	2.82	6.04	43.21	0.11	6.53	1.04	1.48	3.73	80.12	3.92	28.34	0.310	4.72	1.70	0.89	2.50
1975	33	7.55	3.95	1.55	86.93	28.58	14.96	0.027	0.193	2.55	2.93	5.80	44.54	0.01	7.13	0.97	1.25	3.55	78.40	4.30	28.24	0.275	3.28	1.56	0.91	1.70
1976	21	7.48	2.97	1.49	62.98	21.40	11.28	0.030	0.140	2.38	3.14	6.26	37.26	0.00	5.77	0.71	1.11	3.15	64.35	3.65	24.12	0.226	3.95	1.30	1.01	2.11
1977	32	4.81	2.57	1.16	58.14	17.11	9.18	0.022	0.128	2.04	2.27	4.93	33.63	0.06	4.98	0.69	0.89	2.80	60.76	3.19	21.31	0.218	3.04	1.47	0.89	1.61
1978	31	4.35	2.58	1.15	65.70	16.30	8.50	0.016	0.149	2.21	2.34	5.56	41.50	0.08	5.97	0.72	1.01	3.80	70.54	3.92	24.30	0.248	2.60	1.47	1.06	1.56
1979	32	4.14	2.01	0.98	51.35	11.90	5.77	0.017	0.099	1.63	1.91	3.92	31.87	0.06	4.74	0.61	0.86	2.33	51.97	2.89	18.44	0.197	2.76	1.21	0.87	1.71
1980	31	2.79	1.95	0.90	47.05	8.41	4.43	0.012	0.084	1.25	1.45	3.09	27.05	0.03	3.81	0.44	0.70	2.00	44.14	2.83	14.69	0.161	2.41	0.82	0.75	1.20
1981	31	2.57	1.85	0.77	34.30	5.84	3.30	0.012	0.079	1.58	1.72	3.99	30.87	0.07	4.54	0.58	0.74	1.98	45.42	3.03	15.79	0.152	2.05	0.91	0.77	1.57
1982	25	2.13	1.73	0.69	43.55	6.82	3.38	0.011	0.070	1.14	1.34	3.00	27.84	0.07	3.69	0.45	0.57	1.69	39.32	2.51	13.09	0.146	2.07	0.82	0.86	1.03
1983	20	1.62	1.32	0.59	39.80	6.35	3.04	0.011	0.075	0.94	1.11	2.70	23.42	0.05	3.22	0.44	0.52	1.80	39.25	2.65	12.44	0.159	1.79	0.74	0.82	1.27
1984	25	1.25	1.15	0.64	32.13	4.54	2.30	0.008	0.057	0.84	0.85	1.86	20.92	0.03	2.53	0.31	0.49	1.28	31.28	2.33	10.81	0.122	1.73	0.63	0.47	0.97
1985	33	1.24	1.23	0.60	29.85	4.17	1.75	0.010	0.045	0.89	0.88	2.27	21.31	0.06	2.64	0.37	0.54	1.17	27.30	3.95	8.09	0.107	1.26	0.61	0.57	1.07
1986	35	1.82	1.20	0.47	10.65	2.04	1.00	0.009	0.053	1.04	0.82	2.22	17.21	0.04	2.32	0.25	0.37	0.93	21.06	1.70	7.38	0.106	0.94	0.45	0.46	0.71
1988	32	2.39	1.84	0.68	8.85	1.51	0.80	0.010	0.056	1.26	1.34	3.13	25.09	0.01	3.23	0.31	0.50	0.94	23.96	1.71	9.03	0.123	1.01	0.51	0.39	0.76
1989	31	0.77	0.70	0.40	17.04	2.76	1.15	0.009	0.052	0.64	0.64	1.49	13.40	0.04	1.70	0.25	0.37	0.95	20.04	1.62	6.31	0.111	0.76	0.28	0.35	0.55
1990	31	1.01	0.61	0.32	17.14	2.75	1.14	0.008	0.035	0.55	0.61	1.51	12.54	0.05	1.78	0.28	0.36	0.96	18.66	1.51	5.88	0.103	0.83	0.37	0.35	0.53
1991	39	0.86	0.91	0.39	16.36	2.89	1.01	0.007	0.034	0.73	0.76	1.84	14.17	0.04	1.76	0.29	0.43	1.14	18.67	1.43	5.70	0.102	0.88	0.40	0.42	0.93
1992	29	0.51	0.51	0.25	11.85	1.91	0.76	0.005	0.021	0.59	0.53	1.22	10.62	0.03	1.22	0.20	0.33	0.76	14.13	1.08	4.43	0.083	0.63	0.36	0.35	0.56
1993	33	0.70	0.71	0.31	14.22	2.20	0.84	0.004	0.022	0.52	0.61	1.64	11.15	0.03	1.38	0.21	0.31	0.81	15.15	1.17	4.80	0.085	0.65	0.35	0.35	0.52
1994	28	0.72	0.67	0.32	11.25	1.79	0.68	0.005	0.022	0.60	0.54	1.41	10.02	0.01	1.38	0.22	0.25	0.80	13.60	1.08	4.20	0.079	0.81	0.46	0.42	0.53
1995	27	0.56	0.49	0.28	10.15	1.93	0.69	0.017	0.017	0.48	0.56	1.48	8.83	0.04	1.16	0.17	0.28	0.74	12.94	0.95	3.86	0.075	0.68	0.37	0.24	0.57
1996	32	0.42	0.59	0.26	9.03	1.76	0.67	0.004	0.018	0.45	0.45	1.27	9.27	0.05	1.04	0.18	0.27	0.72	12.32	0.91	3.23	0.075	0.58	0.25	0.13	0.46
1997	27	0.74	0.62	0.36	8.18	1.83	0.71	0.004	0.019	0.47	0.39	1.00	7.76	0.03	0.86	0.15	0.26	0.67	10.93	0.79	3.30	0.051	0.53	0.23	0.25	0.40
1998	35	0.20	0.28	0.10	5.18	1.06	0.34	0.003	0.012	0.24	0.31	0.78	7.00	0.04	0.87	0.16	0.17	0.60	10.57	0.77	2.66	0.045	0.25	0.21	0.23	0.23
1999	30	0.51	0.52	0.24	8.04	1.82	0.62	0.004	0.007	0.43	0.46	1.12	8.55	0.08	0.96	0.23	0.23	0.72	10.37	0.64	3.48	0.045	0.40	0.21	0.30	0.33
2000	27	0.45	0.43	0.16	6.15	1.38	0.48	0.003	0.007	0.37	0.44	1.04	7.00	0.03	0.82	0.19	0.20	0.67	9.85	0.60	2.60	0.053	0.46	0.27	0.24	0.42

^a *Non-ortho* co-PCB for which WHO has determined TEF.

^b *Mono-ortho* co-PCB for which WHO has determined TEF.

No data for 1987.

Table 1 Continued

Year	Number of samples	Hexa-PCB												Hepta-PCB												Total-PCBs ^c	Packed column ^d
		#134/133	#146	#153	#137/130	#163/164	#138	#128	#167 ^b	#156 ^b	#157 ^b	#169 ^a	#178	#187	#183	#181	#177	#171	#192/172	#180	#191	#170	#189 ^b				
1973	30	1.25	14.19	106.93	7.60	2.87	113.75	3.77	3.89	12.62	2.31	0.035	3.71	22.42	8.79	2.07	8.19	2.69	2.20	35.46	0.47	18.61	0.43	120.0	1434.6		
1974	22	1.47	15.76	125.90	8.52	2.29	119.33	3.17	5.09	13.53	2.51	0.037	4.12	21.44	7.27	1.17	7.47	2.35	2.05	29.15	0.45	17.88	0.42	109.8	1577.4		
1975	33	1.34	13.57	102.65	6.44	2.98	104.29	2.65	3.91	13.28	2.46	0.042	4.00	20.96	7.89	1.14	8.28	2.67	2.40	37.77	0.67	20.01	0.59	122.2	1244.1		
1976	21	1.33	13.34	103.64	8.82	0.00	98.19	1.75	3.96	12.25	2.25	0.034	3.59	21.59	8.23	1.05	8.04	2.95	2.01	28.08	0.48	19.38	0.38	110.3	1128.6		
1977	32	1.50	13.78	106.53	5.70	3.02	96.06	2.15	3.78	11.83	2.10	0.037	3.81	21.01	7.59	0.93	7.01	2.40	1.96	34.70	0.42	18.97	0.38	113.2	1147.6		
1978	31	1.57	14.46	117.10	6.14	4.04	110.84	2.09	3.97	12.87	2.45	0.041	4.20	23.59	7.69	1.03	9.60	3.31	2.49	38.06	0.61	24.73	0.44	131.1	1341.1		
1979	32	1.62	16.17	120.91	7.40	2.05	109.30	2.08	4.20	13.33	2.41	0.041	4.29	22.82	7.27	0.47	4.69	2.65	2.01	40.66	0.60	22.77	0.54	124.5	1109.7		
1980	31	1.48	12.79	94.16	6.32	1.62	89.23	1.34	3.35	10.50	1.74	0.041	5.31	27.11	10.13	0.46	8.39	2.58	2.06	36.87	0.28	20.09	0.58	126.2	1001.0		
1981	31	1.37	12.74	95.71	4.55	2.69	80.80	1.39	3.22	10.66	2.08	0.040	3.86	21.17	6.63	0.73	6.38	2.25	2.18	33.47	0.40	21.93	0.54	112.3	1036.0		
1982	25	1.54	14.12	111.97	5.03	3.31	98.89	1.53	3.69	11.70	2.07	0.041	4.47	20.86	9.19	0.50	7.92	2.39	2.34	37.93	0.71	23.73	0.47	124.3	1037.1		
1983	20	1.71	14.79	113.04	5.17	3.41	94.66	1.08	3.42	12.27	2.33	0.047	5.46	24.27	7.48	0.71	7.47	3.08	2.03	36.52	0.48	23.28	0.63	126.1	1172.2		
1984	25	1.56	13.89	107.02	4.94	2.55	89.41	1.06	3.28	10.99	2.27	0.043	4.27	20.22	6.21	0.39	5.51	1.82	2.10	29.76	0.48	21.18	0.42	105.6	886.8		
1985	33	1.13	10.29	82.91	3.95	2.53	65.90	0.89	2.11	7.79	1.43	0.038	3.41	17.91	6.13	0.48	4.42	2.34	2.04	36.11	0.45	18.15	0.37	101.1	736.6		
1986	35	1.29	8.20	62.03	2.79	1.35	47.24	0.56	1.64	6.78	1.16	0.036	2.19	13.96	3.84	0.36	3.84	1.23	1.34	23.87	0.31	12.27	0.22	71.4	667.8		
1988	32	1.33	9.67	65.66	3.45	1.33	57.14	0.53	1.96	6.55	1.32	0.040	3.04	15.98	4.95	0.33	4.39	1.92	1.67	28.31	0.32	12.72	0.42	82.0	533.9		
1989	31	0.86	7.52	60.58	3.09	0.73	47.09	0.62	1.69	6.93	1.22	0.039	2.38	10.82	3.13	0.22	3.75	1.53	1.42	21.66	0.30	10.34	0.37	64.1	534.2		
1990	31	1.05	8.61	58.46	2.73	1.34	46.30	0.49	1.71	6.19	1.06	0.040	3.50	13.36	4.09	0.15	3.85	1.71	1.51	23.44	0.36	13.01	0.31	72.6	479.8		
1991	39	1.11	9.02	68.88	3.23	1.29	53.55	0.59	2.11	6.32	1.14	0.041	2.78	13.82	3.62	0.50	4.37	1.41	1.25	23.87	0.20	12.21	0.36	71.9	361.8		
1992	29	0.90	7.01	52.59	2.16	1.22	43.91	0.57	1.32	5.84	1.03	0.036	2.46	11.91	3.24	0.24	3.74	1.48	1.43	18.11	0.27	10.50	0.32	60.6	305.7		
1993	33	0.93	7.89	53.66	2.31	0.96	39.60	0.54	1.46	5.42	0.98	0.038	2.26	12.46	3.51	0.00	2.62	1.23	1.11	17.86	0.20	11.65	0.46	59.8	278.9		
1994	28	1.16	9.01	64.11	2.60	1.44	46.25	0.58	1.61	6.00	1.37	0.040	3.59	15.24	4.12	0.00	2.97	1.39	1.42	21.22	0.34	12.87	0.26	70.8	323.4		
1995	27	1.06	6.77	46.69	2.40	0.00	36.79	0.41	1.54	4.60	0.89	0.034	2.48	10.25	2.46	0.00	2.47	0.92	1.40	19.57	0.00	9.99	0.18	55.2	276.7		
1996	32	0.85	5.92	40.91	1.44	0.84	30.43	0.42	1.19	3.60	0.71	0.036	2.34	10.14	3.12	0.00	2.88	1.03	1.04	18.26	0.25	8.17	0.22	51.8	330.5		
1997	27	0.55	4.97	35.80	1.12	0.74	25.89	0.34	0.92	2.70	0.53	0.031	1.79	8.41	2.33	0.00	1.95	0.69	0.77	13.70	0.10	7.10	0.16	40.2	230.7		
1998	35	0.58	4.94	32.15	1.30	0.85	25.42	0.30	0.98	2.97	0.64	0.034	1.73	9.67	1.74	0.00	1.90	0.71	0.92	16.02	0.10	8.80	0.18	45.4	209.8		
1999	30	0.71	5.96	38.97	2.00	0.00	28.29	0.40	1.02	3.06	0.59	0.034	3.14	15.70	3.94	0.00	3.28	1.05	0.88	20.22	0.30	11.90	0.33	64.4	259.3		
2000	27	0.88	5.62	41.07	2.08	0.00	31.19	0.38	0.97	2.98	0.62	0.028	2.41	10.15	2.49	0.00	2.32	0.79	1.02	12.85	0.17	6.65	0.31	42.8	207.6		

^a Non-ortho co-PCB for which WHO has determined TEF.

^b Mono-ortho co-PCB for which WHO has determined TEF.

^c Total concentration of TeCB to HpCB of each congeners.

^d Using KC-500 as standard.

No data for 1987.

the concentrations in the 1970s (119.3 ng/g fat in 1974, 25.4 in 1998), #153 to about 25.5% (125.9 ng/g fat in 1974, 32.2 in 1998), #118 to about 11.6% (84.8 ng/g fat in 1973, 9.9 in 2000), #74 to about 6.3% (81.9 ng/g fat in 1973, 5.2 in 1998), #66 to about 2.3% (45.5 ng/g fat in 1973, 1.1 in 1998), #99 to about 15.7% (44.5 ng/g fat in 1975, 7.0 in 1998), #180 to about 34.0% (37.8 ng/g fat in 1975, 12.8 in 2000), #105 to about 8.3% (23.6 ng/g fat in 1978, 2.6 in 2000), #187 to about 35.7% (23.6 ng/g fat in 1978, and 8.4 in 1997), #60 to about 2.47% (19.6 ng/g fat in 1973, 0.48 in 2000), #170 to about 26.9% (24.7 ng/g fat in 1978, 6.6 in 2000) and #52 to about 1.24% (16.4 ng/g fat in 1973, 0.20 in 1998).

With #153 as a reference, as it had the highest congener composition ratio of concentrations in breast milk, HpCBs and HxCBs composition ratios exhibited almost no changes between 1973 and 2000 (Figs. 2 and 3), but PeCBs and TeCBs congener composition ratios decreased. In particular, the composition ratio of TeCBs declined markedly (Figs. 4 and 5).

We reported on congeners and congeners of PCBs in total diet samples (fishery food, meat, egg, milk and their products) (7). PCB contamination of the Japanese population is largely caused by seafood intake, but it is thought that the PCB congener composition ratio in fish and that in breast milk differ greatly (7). The absorption or metabolism in the human body differs greatly depending on the congener, resulting in different internal storage concentrations among the PCB congeners. The chromatogram pattern of PCBs in breast milk was similar to the pattern of KC-500. Therefore, KC-500 was used as the standard of PCBs in 70's, when the measurement of PCBs was started in human breast milk.

A nearly parallel correlation has been recognized between the packed column GC-ECD pattern method (the analytical method used in the past) using KC-500 as the standard and the analytical values for each congener (total from TeCBs to HpCBs) by HRGC/HRMS, although the former method gave values that were from 1.2 to 2.5 times higher than those obtained by the latter method for each congener (Fig. 6). This disparity was shown to decrease from yearly. The KC-500 PCB congener composition ratio is shown in Figure 7. It is puzzling that there is a marked difference between the PCB congener composition of breast milk and KC-500. These findings suggest that the half-lives of PCBs in breast milk are longer than those that were reported previously (4), and that the PCBs are highly persistent in the body. In Japan, KC-300 of PCBs is mainly used. As for KC-300, there are many PCBs of low chlorine concentration and the pattern of packed column is patterning and quite different from that of breast milk. The pattern method was used previously because there was no microanalysis equipment similar to that used today. However, the organohalogen compounds that was not originally PCB in the analysis, the value differed when each congener was measured by the microanalyses method, that is, HRGC/HRMS in recent years; the packed column method has been used for PCBs, and PCB concentration in actual breast milk was different. Naturally, the half-life was also different, and it was actually longer than that determined by the pattern method.

Dioxins with chemical structures similar to PCBs are known to be highly persistent. We previously reported on

Fig. 2 HpCB congener proportions in breast milk.

Fig. 3 HxCB congener proportions in breast milk.

Fig. 4 PeCB congener proportions in breast milk.

Fig. 5 TeCB congener proportions in breast milk.

Fig. 6 Concentrations of determined by PCB congener analysis and packed column analysis on human breast milk fat basis. (25 to 29 year-old mothers, no data for 1987)

Fig. 7 PCB congener proportions in KC-500.

Fig. 8 Time-courses of *non-ortho* co-PCBs in human breast milk (fat basis).

changes in dioxin concentration in breast milk from 1973 to 1996 (8). The present study included a detailed analysis of co-PCBs, which are one type of dioxin, and found that the concentration of #77, which is a TeCB, decreased to 2.4% of earlier concentrations (277 pg/g fat in 1973, 7 in 1999) whereas #169, an HxCB, showed no significant decrease (35 pg/g fat in 1973, 34 in 1999) (Fig. 8). One possible reason for this finding may be that these concentrations are related not only to contamination from PCBs products, but also to contamination caused by the incineration of chlorinated products.

Discussion

There are 209 congeners of PCBs, depending on the

number and substitution sites of chlorine atoms. Because PCBs are stable in acids and alkalis and do not easily decompose under high temperatures, they were used in Japan mainly as insulating materials in condensers and other equipment, and large amounts are said to remain in Japan. The worldwide cumulative total of PCBs produced is about 1.2 million tons (9), of which about 60,000 tons were produced in Japan (10). These PCBs pollute the environment and accumulate in the human body (11–14). The impacts of PCBs on humans differ greatly depending on the congener. In particular, co-PCBs, which have a flat structure, are similar to dioxins (15). In 1998, the WHO determined the tolerable daily intake of dioxins for humans to be 1–4 pg/kg/day, and established dioxin toxic equivalency factor (TEF) for four types of *non-ortho* PCB and eight types of *mono-ortho* PCB (16). The TEFs of co-PCBs (0.0001–0.1) are relatively small, but because PCBs tend to concentrate in the body, the TEQs of PCBs in breast milk and blood may be comparable to those of PCDDs and PCDFs. There have also been reports of toxicity from hormone effects and neurotoxicity related to PCBs for which their TEFs have not been reported (15). The TEFs of PCBs differs greatly with the type of accumulating congener (17). For these reasons, it is important to measure the concentrations of each type of PCB congener that accumulates in the body.

Since 1972, we have continued our research into the organochlorine compound contamination of the breast milk of lactating women residing in Osaka Prefecture. Our previous reports included the findings of research from 1972 to 1998 into the contamination of breast milk by PCBs, hexachlorobenzene, *beta*-HCH, *p,p'*-DDE, *p,p'*-DDT, heptachlor epoxide (HCE) and chlordane. The reports of PCB concentrations were based on the packed column GC-ECD pattern method (4). To accurately ascertain pollution from PCBs, it is necessary to conduct analysis of each congener by HRGC/HRMS. Comparisons HRGC/HRMS results with those of the packed column GC-ECD pattern method are also required. In addition, a method for analyzing individual PCB congeners that is simpler than HRGC/HRMS is needed.

Our results indicated that PCB concentrations in breast milk and their changes over time differ greatly depending on the congener. The toxicity of PCBs also differs greatly among congeners. For this reason, it is not possible to accurately evaluate PCB toxicity on the basis of overall PCB concentrations. Thus, the analysis of congeners is also essential for toxicity assessment. Choi et al. reported on co-PCBs in the human adipose tissue collected in 1970, 1971 and 2000 (18). We have also found, in agreement with the report of Choi et al. (18), that the concentrations of tetra- and penta-chlorinated PCBs decreased, but those of #169 (*non-ortho* HxCB) and #189 (*mono-ortho* HpCB) increased. These findings suggest changes in the environmental pollution by PCBs from year to year.

In 1974, the Ministry of International Trade and Industry and the Ministry of Health and Welfare of Japan designated PCBs as specified chemical substances that cause environmental pollution. Almost thirty years have passed since their use was prohibited. However, PCBs are persistent compounds. The source of organochlorine compounds in the human body is mostly from food (19). The contamination of fish and shellfish

has been a serious problem for the Japanese, who consume a large amount of seafood in their diet (20). PCBs still exist in the environment and remain a serious problem. PCBs of low chloride content metabolize more quickly than PCBs of high chloride content (21). This is thought to be one reason for the rapid decrease in the concentration of low-chloride PCBs in breast milk. Another factor that must be considered is pollution from sources other than PCB products (22).

Astebro reported that 20% PCBs were present as sealants in building materials (23) and were used from 1968 to 1972 in Japan (24). There is a possibility that PCB contaminants derived from the sealants become a problem in the future. Further research needs to be carried out for each congener of

PCBs in the environment and in the body, including breast milk.

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