

# PCB Contamination Assessment of Yusho Patients by Using Preserved Human Umbilical Cord

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**Abstract** PCB concentrations in umbilical cord preserved from the time when Yusho patients and healthy subjects gave birth were examined. The total concentration of the 12 DL-PCB isomers ranged from 130 to 12,000 pg/g in the umbilical samples, was about 700 pg/g around 1950 but began to increase in the mid 1960s, reached about 12,000 pg/g between 1968 and 1970 immediately after the Yusho incident. However, the DL-PCB concentration was high between 1968 and 1970 in not only the designated Yusho patients but also healthy subjects, and the maximum DL-PCB concentration was close between the two groups

**Keywords** Umbilical cord · Yusho · PCB · Contamination level

The Kanemi Yusho (oil poisoning) incident, which occurred in Japan in 1968, is a typical example of human contamination due to polychlorinated biphenyl (PCB) (Furue et al. 2005; Ryan et al. 1990). The damage caused to the health of Yusho victims has not been clarified even now, more than 30 years after the incident. The PCB contamination level of Yusho patients has been evaluated using various biological samples including blood, mother's milk, hair, sebum, and adipose tissue (Hirakawa et al.

2007; Ryan et al. 1993; Matsueda et al. 1993). However, no study using the preserved umbilical cord of Yusho victims has been conducted. It is a Japanese custom to cut off part of the umbilical cord of a baby at its birth and preserve it in, usually, a small wooden box at home. Since chemicals contained in these preserved umbilical cords are thought to reflect their concentrations in the mothers at childbirth, their measurement is considered to allow the assessment of human contamination level at that time.

In this study, we examined the PCB concentrations in umbilical cord preserved from the time when Kanemi Yusho patients gave birth to evaluate their contamination level in the period around the incident.

## Materials and Methods

Human umbilical cord preserved from childbirth by 6 Yusho victims (Mothers A–F) and 9 healthy women (Mothers G–O) were examined. The preserved umbilical cords of the Yusho victims were provided by the KANEMI Yusho Patients Support Center (Table 1). Of the 6 Yusho victims, 2 were designated patients (Mothers D, E), and 4 were suspected of having the disease (Mothers A, B, C and F). Three samples of preserved umbilical cords were obtained from the 2 designated patients, and 8 from the 4 suspected victims (Total, 11 samples; Samples A-1 to F-1). The mothers who provided the samples gave birth between 1968, immediately after the Yusho incident, and 2000, giving birth to 5 male and 6 female infants. From the 9 healthy subjects, 15 samples of preserved umbilical cord were obtained (Samples G-1 to O-1 in Table 1). The period of these subjects' childbirths was from 1950 to 1986, and 5 samples were obtained from childbirths near the time of the incident (1966–1970).

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**Table 1** Preserved umbilical cord samples

Donor (mother)	Sampels <sup>a</sup>	Neonate's sex	Year of childbirth
<i>Yusho victims (Designated and suspected Yusho patients)</i>			
A	A-1	Male	1970
B	B-1	Male	1981
	B-2	Female	1982
C	C-1	Female	1984
	C-1	Female	1986
	C-2	Female	1988
D (Designated)	D-3	Male	1968
E (Designated)	E-1	Female	1968
F	E-2	Male	1970
	F-1	Male	1995
	F-2	Female	2000
<i>Healthy subjects</i>			
G	G-1	Male	1962
	G-2	Female	1965
	G-3	Male	1968
H	H-1	Female	1966
	H-2	Female	1968
I	I-1	Male	1950
J	J-1	Male	1979
	J-2	Female	1982
	J-3	Female	1986
K	K-1	Male	1975
	K-2	Male	1980
L	L-1	Male	1970
M	M-1	Female	1963
N	N-1	Male	1966
O	O-1	Male	1964

<sup>a</sup> Preserved human umbilical cord

Figure 1 shows Method for the measurement of the DL-PCB concentration in the preserved umbilical samples. The surface of the samples was lightly washed with acetone, about 0.1 g of each sample was cut off as strips using stainless steel scissors, and they was pulverized with anhydrous sodium sulfate (1.0 g) in a mortar. A clean-up spike (consisting of 200 pg each of 12 types of <sup>13</sup>C-DL-PCB) was added to these pulverized samples, and PCB was extracted by circulating toluene (100 mL) for 5 h. After hot filtration, the toluene extract was concentrated, and PCB was purified by silver nitrate silica gel column chromatography. Silver nitrate silica gel column chromatography was performed using a column packed with 1.0 g of 10% silver nitrate silica gel and eluting it with 20 mL of hexane. Then, the eluate was concentrated again, dissolved with 10 µL of nonane, and supplemented with a syringe spike (consisting of 200 pg each of <sup>13</sup>C-TeCB #70, <sup>13</sup>C-PeCB #111, <sup>13</sup>C-HxCB #138, and <sup>13</sup>C-HpCB #178) to a total

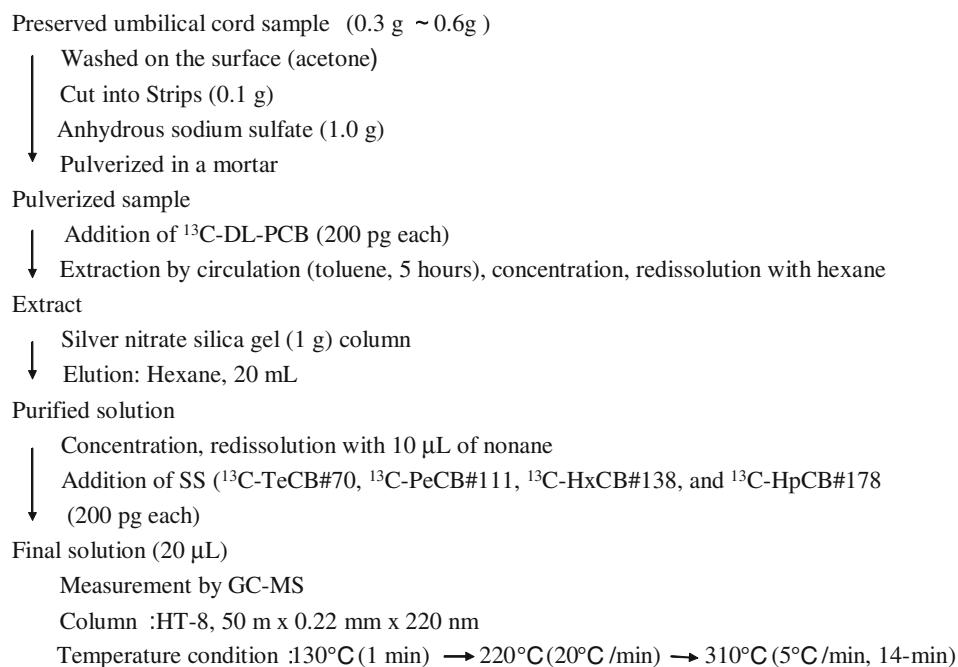
volume of 20 µL. This final solution was measured for 12 isomers of DL-PCB by high-resolution gas chromatography (GC)/mass spectrometry (MS). The capillary column attached to the gas chromatograph was HT-8 (50 m × 0.22 mm × 220 nm), and its temperature was increased from 130°C (1 min) → 220°C (20°C/min) → 310°C (5°C/min, 14 min).

## Results and Discussion

Table 2 shows the concentrations of 12 DL-PCB isomers contained in the preserved umbilical cord provided by designated and suspected Yusho patients. The recovery of the clean up spike (12 types of <sup>13</sup>C-DL-PCB) were 53%–77% for 2 <sup>13</sup>C-TeCBs, 50%–85% for 5 <sup>13</sup>C-PeCBs, 56%–93% for 4 <sup>13</sup>C-HxCBs and 52%–102% for <sup>13</sup>C-HpCB in the 11 umbilical samples (Samples A-1 to E-4). The total concentration of the 12 DL-PCB isomers ranged from 130–11,000 pg/g. It was highest at 11,000 pg/g in Sample E-2 provided by Mother E, who was a designated Yusho patient. It was relatively high at 2,100 pg/g in Sample E-1 provided by the same mother. Moreover, it was high at 6,600 pg/g in Sample D-1 provided by Mother D, who was another designated Yusho patient. In the 8 samples provided by the 4 mothers (Mother A, B, C and F) suspected to have Yusho, it was 130–1,400 pg/g, being lower than the levels in the designated patients. The highest concentration (11,000 pg/g) observed in this study was about 100 times higher than the lowest concentration (130 pg/g). Also, the DL-PCB concentrations in the designated patients (Mother D and E) were higher than those in the suspected Yusho patients (Mother A, B, C and F). When the concentrations of the 12 DL-PCB isomers were compared, the concentration of PeCB #118 was the highest, followed by PeCB #105, HxCB #156, and TeCB #77 in Sample E-2, which showed the highest total DL-PCB concentration. The same concentration order (#118 → #105 → #156 → #77) was also observed in all the other samples. Therefore, the composition of DL-PCB isomers is considered to be similar in all umbilical samples despite 10- to 100-fold differences in the total DL-PCB concentration.

Table 3 shows the percentages of various isomers relative to the total DL-PCB concentration in preserved umbilical samples. The sum of the concentrations of 4 isomers, i.e., #77, #118, #105, and #156, accounted for about 90% or more of the total concentration. This tendency was observed in all samples regardless of the total DL-PCB concentration, neonate's sex, or year of childbirth.

Table 4 compares the total DL-PCB concentrations in the samples from the same mothers. In Mother C, the concentration at the birth of her first child was 680 pg/g, but those at the birth of her 2nd and 3rd children were 450

**Fig. 1** Analytical method for PCB in umbilical samples**Table 2** DL-PCB isomer concentrations (pg/g) in preserved umbilical samples from designated and suspected Yusho patients

	Suspected						Designated			Suspected	
	A-1	B-1	B-2	C-1	C-2	C-3	D-1	E-1	E-2	F-1	F-2
TeCB (#81)	–	–	–	–	–	–	–	–	93	–	–
TeCB (#77)	81	40	30	45	73	33	800	130	460	30	14
PeCB (#123)	–	–	–	–	–	–	–	–	–	–	–
PeCB (#118)	710	280	400	360	240	180	3,000	1,200	6,600	140	80
PeCB (#114)	48	16	23	19	17	19	200	–	–	–	–
PeCB (#105)	330	120	190	130	100	77	2,100	520	2,800	50	40
PeCB (#126)	–	–	–	–	–	–	32	–	–	–	–
HxCB (#167)	48	36	22	34	–	10	130	40	310	–	–
HxCB (#156)	170	84	87	71	22	21	320	110	900	–	–
HxCB (#157)	43	30	24	22	–	–	130	32	270	–	–
HxCB (#169)	–	–	–	–	–	–	–	–	–	–	–
HpCB (#189)	–	–	–	–	–	–	–	20	–	–	–
Total	1,400	590	780	680	450	350	6,600	2,100	11,000	220	130

–: N.D., <5 pg/g for TeCBs, PeCBs and <10 pg/g for HxCBs, HpCB

and 350 pg/g, respectively, showing progressive decreases in the PCB concentration. Such decreases were also observed in Mother F, suggesting that the PCB concentrations in the bodies of these subjects decreased with successive childbirths. Unlike Mothers C and F, the umbilical PCB concentration was higher at the birth of the 2nd child than at the birth of the 1st child in Mothers B and E. Therefore, the DL-PCB concentration in the umbilical cord did not necessarily decrease with childbirths. However, in Mother B, the umbilical PCB concentration was 590 pg/g at the birth of the 1st child and 780 pg/g at the

birth of the 2nd child, showing small changes, as in the other mothers. In this study, the umbilical DL-PCB concentrations in the 2 samples from Mother E were 2,100 and 11,000 pg/g, being higher than the values in the other mothers. Therefore, the PCB concentration in the body of Mother E is considered to have been higher than in Mothers B, C, and F.

Table 5 shows the PCB concentrations in preserved umbilical cord of Yusho victims and healthy individuals. The concentrations of DL-PCB isomers and total DL-PCB concentrations were calculated separately between the

**Table 3** Percentages (%) of various DL-PCB isomers relative to the total DL-PCB concentration in preserved umbilical samples from designated and suspected Yusho patients

	A-1	B-1	B-2	C-1	C-2	C-3	D-1	E-1	E-2	F-1	F-2
TeCB (#81)	–	–	–	–	–	–	–	–	0.8	–	–
TeCB (#77)	5.7	6.3	4.2	6.5	16	9.5	12	6.1	4.0	14	11
PeCB (#123)	–	–	–	–	–	–	–	–	–	–	–
PeCB (#118)	49	46	52	53	52	53	45	59	58	64	60
PeCB (#114)	3.4	2.6	3.0	2.8	3.7	5.4	3.0	–	–	–	–
PeCB (#105)	23	20	24	20	23	22	31	25	24	22	29
PeCB (#126)	–	–	–	–	–	–	0.5	–	–	–	–
HxCB (#167)	3.4	6.1	2.8	4.9	–	2.9	1.9	1.9	2.7	–	–
HxCB (#156)	12	14	11	10	4.8	6.0	4.7	5.3	7.8	–	–
HxCB (#157)	3.0	5.0	3.0	3.2	–	–	2.0	1.4	2.3	–	–
HxCB (#169)	–	–	–	–	–	–	–	–	–	–	–
HpCB (#189)	–	–	–	–	–	–	–	1.0	–	–	–
HpCB (#77) + (#118) + (#105) + (#156)	90	87	92	90	96	91	93	95	94	100	100

Total concentration of DL-PCB isomers = 100%, –: 0%

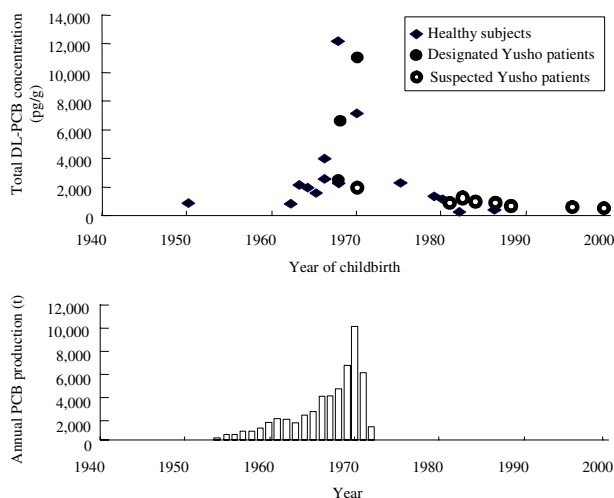
**Table 4** Comparison of the total DL-PCB concentration (pg/g) in umbilical samples among siblings

Donor (mother)	Preserved umbilical sample		Neonate's sex	Year of childbirth	Total DL-PCB concentration (pg/g)
B	B-1	Brother	Male	1981	590
	B-2	Sister	Female	1982	780
C	C-1	1st daughter	Female	1982	680
	C-2	2nd daughter	Female	1986	450
	C-3	3rd daughter	Female	1988	350
E (Designated)	E-1	Sister	Female	1068	2,100
	E-2	Brother	Male	1970	11,000
F	F-1	Brother	Male	1995	220
	F-2	Sister	Female	2000	130

**Table 5** DL-PCB concentrations in preserved umbilical cord of Yusho victims and healthy subjects (pg/g dry weight, mean (maximum–minimum))

	Yusho victims (n = 11)		Healthy subjects (n = 15)
	Designated patients (n = 3)	Suspected patients (n = 8)	
TeCB (#81)	93 (N.D. ~93)	N.D (N.D. ~N.D.)	45 (N.D. ~200)
TeCB (#77)	460 (130–800)	43 (14–81)	340 (3.0–2,000)
PeCB (#123)	N.D. (N.D. ~N.D.)	N.D. (N.D. ~N.D.)	68 (N.D. ~530)
PeCB (#118)	3,600 (120–6,600)	300 (80–710)	1,400 (150–5,300)
PeCB (#114)	200 (N.D. ~200)	24 (N.D. ~48)	110 (N.D. ~260)
PeCB (#105)	1,800 (520–2,800)	130 (40–330)	600 (58–2,500)
PeCB (#126)	32 (N.D. ~32)	N.D. (N.D. ~N.D.)	20 (N.D. ~30)
HxCB (#167)	160 (40–310)	32 (N.D. ~43)	110 (N.D. ~300)
HxCB (#156)	440 (110–900)	76 (N.D ~170)	160 (N.D. ~580)
HxCB (#157)	140 (32–270)	30 (N.D. ~43)	59 (N.D. ~160)
HxCB (#169)	N.D. (N.D. ~N.D.)	N.D. (N.D. ~N.D.)	N.D. (N.D. ~N.D.)
HpCB (#189)	20 (N.D. ~20)	N.D. (N.D. ~N.D.)	N.D. (N.D. ~N.D.)
Total	6,500 (2,000–11,000)	580 (130–1,400)	2,700 (250–12,000)

N.D.: not detected



**Fig. 2** Comparison of the total DL-PCB concentration (pg/g) in preserved umbilical cord of Yusho patients and healthy subjects by the year of childbirth

designated ( $n = 3$ ) and suspected ( $n = 8$ ) Yusho patients. The values are shown as the mean and range (minimum–maximum). The mean DL-PCB concentration in the preserved umbilical cord at the childbirths of the designated patients was 6,500 pg/g, which was more than 10 times higher than the level in the suspected Yusho patients (580 pg/g). It was also clearly higher compared with the mean of the healthy subjects (2,700 pg/g). However, the maximum total concentration in the suspected Yusho patients was 1,400 pg/g, which was clearly lower than 11,000 pg/g in the designated patients, but the value in the healthy subjects (12,000 pg/g) was close to that in the designated patients. The mean concentrations of various DL-PCB isomers tended to be higher in the designated than in the suspected patients or healthy subjects, but the maximum concentrations were similar between the designated patients and healthy subjects. These results suggest that PCB may be detected at high concentrations in preserved umbilical cord of healthy individuals as well as designated Yusho patients.

Figure 2 plots the total DL-PCB concentrations in preserved umbilical cord of the Yusho victims ( $n = 11$ ) and healthy subjects ( $n = 15$ ) according to the year of birth. The plotted total concentrations ( $n = 26$ ) was about 700 pg/g around 1950 but began to increase in the mid 1960s, reached about 12,000 pg/g between 1968 and 1970 immediately after the Yusho incident, and decreased thereafter to about 300 pg/g around 1990.

Otake et al. compared the changes in the PCB concentrations in preserved human umbilical cord of Japanese subjects with those in the annual production of PCB

products in Japan, and reported a possible association between the PCB concentration in the preserved umbilical cord and human contamination by PCB products (Otake et al. 2004). They also clarified that major components of PCB isomers detected from preserved umbilical cord were in agreement with those contained in PCB products. In our study, also, the PCB concentration in the preserved umbilical cords increased with the annual production of PCB products and began to decrease after 1972, when the production of PCB was discontinued. However, the PCB concentration in the preserved umbilical cord was high between 1968 and 1970 in not only the designated Yusho patients but also healthy subjects, and the maximum DL-PCB concentration was close between the two groups. Therefore, both designated Yusho patients and healthy subjects are considered to have been contaminated to a similar level in the period immediately after the Yusho incident. However, as PCB in biological samples from designated Yusho patients shows a characteristic isomer composition compared with that in samples from healthy individuals, comparison of the concentrations of isomers that are known to be high in designated Yusho patients may lead to a conclusion different from that of this study, which was based on the total PCB concentration.

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