Hirokazu Kodama and Hideo Ota*: Studies on the Transfer of PCB from Mothers to Infants (1) [PCB ni Bishiiko ni Kansuru Kenkyu (1)]

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STUDIES ON THE TRANSFER OF PCB FROM MOTHERS TO INFANTS (1)

Summary

Epidemiological study on the transfer of PCB to infants from their mothers was investigated from 1974 to 1976. The following samples were collected and analyzed for PCB: maternal blood and cord blood at delivery, maternal blood, infant blood and breast milk at 3 months after birth, and infant blood at 1 year and 2 years after birth.

The level of PCB in the breast milk was nearly middle level in Japan. The levels of PCB in the cord blood and the maternal blood at 3 months after birth were lower than that in the maternal blood at delivery, significantly. When the cord blood was considered as the infant blood at birth, the level of PCB in the blood of breast fed infants rose gradually with ingestion of breast milk, exceeded the level in the blood of their mothers after 3 months, and tended to increase until 1 year and then significantly decreased at 2 years after birth. However, the level of PCB in the blood of artificially fed infants remained at the low concentration level during the same period.

These results suggested that the amount of PCB from lactation was higher than that from placenta on the transfer of PCB to infants from their mothers.

1. Introduction

The transfer of polychlorinated biphenyls (PCB) from mother to infant in experimental animals was reported by Kitamura et al. [1], Masuda et al. [2], Mizutani et al. [3], and Takagi et al. [4]. Since an adverse effect of PCB on the next generation was suspected, various surveys have been conducted on the status of contamination of breast milk and blood of lactating females. A few cases of Kanemi oil disease found in newborn babies and in breast-fed babies are the only available sources of information on the transfer of PCB to newborn babies at present [5]. On the other hand, studies on the status of contamination of newborn babies from normal healthy females are currently lacking.

The authors have investigated the transfer of PCB from mother to infants epidemiologically. The transfer of PCB by breast feeding was confirmed by comparing the status of contamination in breast-fed infants with the status of contamination in those who were not breast fed. In order to investigate the status of infants after weaning, data was collected for a period of three years from 1974 to 1976. The results obtained are reported in this study.

II. Procedures

1) Subjects

Mothers who had eutocia at a city hospital in Aichi Prefecture, and who agreed to cooperate in this survey, and their infants were used as subjects. Mothers who were lactating at three months after child birth were considered as lactating mothers, and their infants were breast-fed babies. Mothers who were not lactating at that time were considered as nonlactating mothers, and their infants were bottle-fed babies. The same subjects were followed over time.

2) Specimens examined and number of specimens

The specimens which were collected for the determination of PCB concentration were maternal blood at birth, cord blood, maternal blood at three months after birth (called maternal blood in this text) and breast milk. The specimens collected from the babies were blood at three months after birth (blood of three month old babies), blood at one year after birth (blood of one year old babies) and blood at two years after birth (blood of two year old babies).

The number of specimens examined during a three year period is presented in Table I.

3) Time of collection of specimens and amount of specimens

The specimens were collected in each period from August to March. The specimens were collected in a fasting state before meals so that the effect of foods was assumed to be minimal. Specimens from newborn babies were collected in 1974, blood specimens from one year old babies were collected in 1975, and blood specimens from two year old babies were collected in 1976. Blood specimens were also collected from newborn babies in 1975 and blood specimens from one year old babies were collected in 1976.

The amount of specimens collected were as follows: maternal blood and cord blood: 20-30 g; infant blood: 5-10 g; breast milk: 100-150 ml.

4) Analytical method

PCB in the blood and milk was analyzed by the continuous steam distillation method using a modified oil testing type quantitative analysis apparatus [6]. The PCB concentration was calculated from the sum of the major peak heights based on KC-500 as a standard and using an absolute calibration method.

The milk fat content was determined by the method of Gerber [7].

The frequency distribution of the results obtained showed an almost log-normal distribution. Thus, the data obtained was statistically analyzed after logarithmic conversion. The arithmetic mean was examined by Smirnov's rejection test and was calculated with a rejection level at a probability of less than 5%. A difference between the means was examined by the Student t-test.

III. Results

1) PCB in breast milk

The milk fat content and the PCB concentration in breast milk are given in Table II.

The mean concentration of milk fat in each year period are similar, while the mean concentration of PCB in breast milk fluctuated. According to the results of an annual survey on the PCB concentration in breast milk on a whole milk basis in various areas in Aichi Prefecture [8], PCB concentrations were as follows: 27 $\pm$ 17 (mean $\pm$ S.D.) ppb in 1973; 28 $\pm$ 23 ppb in 1974; 24 $\pm$ 14 ppb in 1975. The value is not available in 1976, since surveys in the area aside from the present survey were not conducted. According to the results of a national survey on the PCB concentration in breast milk, conducted by the Ministry of Public Health and Welfare [9], the PCB concentration in breast milk in this Prefecture seems to be almost close to the value of the national average. As in other Prefectures, the PCB concentration in breast milk showed a slight trend to decline over the years.

When the average PCB concentration in whole milk, 19.1 ppb, which was calculated as an average of three years as given in Table II, was multiplied by the average daily milk intake for three month old babies, 878 ml, which was reported by Suzuki [10], an average daily intake of PCB in breast-fed three month old babies was estimated to be approximately 16.8 μg. This estimated value was much greater than the average daily PCB intake of mothers, 4.55 μg, which was reported by Yoshida et al. [11].

2) PCB concentrations in the maternal blood and the cord blood at birth

The PCB concentrations in maternal blood and cord blood at birth, which were calculated as an average in the respective years, are presented in Table III.

The average PCB concentration in maternal blood at birth in 1974 was similar to that in 1975. The value in 1976 was slightly lower than in previous years. However, the difference between the values in each year was not statistically significant according to the *t*-test. From our experience, the PCB concentration in the blood of normal adults ranges around 2-3 ppb. Thus, the PCB concentration found in this study in maternal blood at birth was somewhat higher than the normal concentration.

The PCB concentration in cord blood in 1974 was not significantly different from that in 1975. On the other hand, the value in 1976 was significantly lower than those values in 1974 and 1975, at a level of $P < 1\%$ and $P < 5\%$, respectively.

Comparing the PCB concentration in the maternal blood at birth with that in cord blood at birth, the PCB concentration in the cord blood was significantly higher in each year period at a level of $P < 1\%$. The correlation between the PCB concentration in maternal blood at birth and that in cord blood at birth was examined with respect to the results of 74 cases during the three year period. A coefficient of correlation was calculated to be 0.445. The correlation between these values was confirmed to be significant at a level of $P < 1\%$.

3) PCB concentrations in maternal blood and infant blood

The PCB concentrations in the maternal blood at three months after birth, the blood of three month old babies and the blood of one year old babies, which are calculated as an average of the respective years, are presented in Table IV.

Only 9 cases were available for examination of the PCB concentration in the maternal blood of lactating mothers. Thus, the number of samples was not sufficient to determine a significant difference between the level in 1975 and the level in 1976.

The PCB concentration in maternal blood of lactating mothers showed a trend to decline slightly with years. However, these difference are not statistically significant.

In a comparison of the PCB concentration in the maternal blood of lactating mothers with that in the maternal blood of nonlactating mothers, the level in lactating mothers was found to be slightly lower than that in nonlactating mothers in each year period. According to the results throughout the three year period, no significant difference was observed.

When the PCB concentration in maternal blood, shown in Table IV, was compared with the PCB concentration in maternal blood at birth, the level in mothers at three months after birth was significantly lower than that in mothers at birth at a level of $P < 1\%$.

No significant difference was observed in the PCB concentration in the blood of three month old breast-fed and bottle-fed babies. Regarding the PCB concentrations in the blood of one year old babies, no significant difference was observed between the years although the number of specimens examined was limited. When bottle-fed babies were compared with breast-fed babies, the PCB concentration, either in the blood of three month old babies or in the blood of one year old babies, was significantly higher in the breast-fed babies than in the bottle-fed babies at a level of $P < 1\%$.

Regarding the data through the three year period, when the PCB concentration in the blood of three month old babies was compared with that in the blood of one year old babies, no significant difference was observed between breast-fed babies and the bottle-fed

babies. However, the following phenomenon was observed. In bottle-fed babies, the PCB concentration in the blood of three month old babies blood was higher than the level in the blood of one year old babies. On the other hand, in breast-fed babies, the blood PCB concentration increased with time.

When the PCB concentration in the blood of three month old babies shown in Table IV was compared with the PCB concentration in the cord blood shown in Table III, the PCB concentration in the blood of bottle-fed babies was not significantly different from that in the cord blood, while the PCB concentration in the blood of breast-fed babies was significantly higher in each year period than that in the cord blood at a level of $P < 1\%$.

When the PCB concentration in the blood of three month old babies was compared with the PCB concentration in maternal blood, the PCB concentration in the blood of bottle-fed babies was significantly lower than that in the blood of nonlactating mothers at a level of $P < 5\%$ throughout the three year period. On the other hand, the PCB concentration in the blood of breast-fed babies was not significantly different from that in the blood of lactating mothers in 1974, while the PCB concentration in the blood of breast-fed babies either in 1975 or 1976 or during the three year period was significantly higher than that in the blood of lactating mothers at a level of $P < 5\%$.

The average PCB concentration in the blood of two year old babies who were born in 1974 was 0.4 ± 0.5 (mean $\pm$ S.D.) ppb for bottle-fed babies while the average PCB concentration in the 14 cases of breast-fed babies was 2.3 ± 1.5 ppb.

The PCB concentration in the blood of two year old bottle-fed babies was also lower than that in the blood of one year old bottle-fed babies. However, the difference was not significant. The PCB concentration in the blood of two year old breast-fed babies was significantly lower than that in the blood of one year old breast-fed babies at a level of $P < 5\%$. As in the case of babies three months and one year old, the PCB concentration in the blood of two

year old breast-fed babies was significantly higher than that in the blood of two year old bottle-fed babies at a level of $P < 1\%$.

The change in the PCB concentration in the cord blood and the blood of 20 babies, which was collected at three months, one year and two years after birth in 1974 is shown in Figure 1.

The correlations between the PCB levels in the same subjects, but at different times; and between the PCB levels in the different subjects, but at the same time, through the entire three experimental years, are presented in Table IV.

The PCB concentration in the blood of nonlactating mothers showed a significant correlation with that in the maternal blood at birth ($N = 9$, $r = 0.855$) at a level of $P < 1\%$; and with that in the cord blood ($N = 8$, $r = 0.823$) at a level of $P < 5\%$.

The PCB concentration in the blood of lactating mothers showed a significant correlation with that in the maternal blood at birth ($N = 46$, $r = 0.823$), and with that in breast milk (whole milk: $N = 46$, $4 = 0.399$; milk fat: $N = 46$, $r = 0.434$) at a level of $P < 1\%$; and with that in cord blood ($N = 45$, $r = 0.316$) at a level of $P < 5\%$.

The PCB concentration in the blood of three month old bottle-fed babies showed a significant correlation with that in the maternal blood at birth ($N = 15$, $r = 0.541$) at a level of $P < 5\%$. No other significant correlations were observed.

The PCB concentration in the blood of three month old breast-fed babies showed a significant correlation with that in breast milk (whole milk: $N = 42$, $r = 0.510$; milk fat: $N = 42$, $r = 0.549$), with that in maternal blood at birth ($N = 42$, $r = 0.516$), with that in cord blood ($N = 42$, $r = 0.440$), and with that in the blood of lactating mothers ($N = 42$, $r = 0.594$) at a level of $P < 1\%$. A significant correlation was also found at a level of $P < 1\%$ between the PCB concentration in the blood of one year old babies and that in the blood of three month old babies ($N = 24$, $r = 0.792$), and between the PCB concentration in the blood of two year old babies and that in one year old babies ($N = 12$, $r = 0.795$).

IV. Discussion

1) PCB concentration in breast milk

Since there is a likelihood of PCB contamination entering the bodies of mothers and affecting their offspring, the PCB concentration in breast milk, which can be collected relatively easily as a specimen, should be determined to investigate the status of contamination by PCB. Takeshita et al. [12] suggested that the PCB content in breast milk shows qualitatively good agreement with the PCB content in maternal adipose tissue. Therefore, if the PCB concentration in the adipose tissue of subjects is high, the PCB concentration in the breast milk from those subjects is expected to be high. The authors have previously selected 10 lactating mothers; breast milk was collected twice from each subject with a one week interval. The PCB concentration in the respective breast milk was determined. Regarding the PCB level in the whole milk, the values fluctuated even in the same individuals. On the other hand, the PCB level in milk fat was found to be relatively constant [13]. Thus, the PCB concentration in the whole milk is assumed to fluctuate with the changes in the milk fat content when the specimens are collected. Yakushiji et al. [14] have conducted a similar experiment. They suggested that a considerable difference in the PCB concentration in breast milk appears between the specimen collected before nursing and that collected after nursing even in the same individual. Thus, they pointed out that the PCB levels fluctuate with the change in time of collection and conditions prevailing at the time the specimens are collected. It was also reported that the average PCB concentration increases if there is a greater percentage of first pregnancies among the subjects examined. Since the number of subjects used was relatively small in each year in this study, the fluctuation in the PCB concentration in the breast milk can be assumed to be due to the difference in the pregnancy history of the subjects included each year, and due to the differences in the status of food intake in the subjects involved.

Tsuchiya et al. [15] have reported that the absolute amount of PCB taken in by babies can be estimated by multiplying the milk intake of babies with the PCB level in whole breast milk. Thus, the contamination index is obtained assuming the breast milk as a "diet" for the babies. However, this index must be carefully interpreted because of the above-mentioned reasons. Therefore, the average daily PCB intake of three month old breast-fed babies (16.8 µg) estimated from the results of this study should be considered only as a criterion for estimating the status of contamination by PCB in babies.

2) PCB in the maternal blood and cord blood at birth

The PCB concentration in the maternal blood at birth was found to be slightly higher than that generally found in normal adults according to the present data. According to the reports presented by Alvarez et al. [16] and Ueno et al. [17], the lipid content in the blood increases during the course of gestation and it reaches at birth a level 1.5 to 2 times the level found in the nonpregnant female. Since PCB is a fat-soluble substance, the PCB concentration in the maternal blood at birth is assumed to be higher than that in the blood of nonpregnant females. Yakushiji et al. [18] have investigated the change in the PCB concentration during the gestation period in the case of pregnant females who had PCB occupationally before pregnancy. The PCB concentration in the maternal blood was found to be unchanged for 7 months before child birth. Thus, this subject deserves further consideration in the future.

When the PCB concentration in the maternal blood at birth was compared with that in the cord blood, the PCB concentration in the cord was found to be significantly lower than that in the maternal blood at birth. This result agreed with the data reported by Akiyama et al. [19]. A significant correlation was found between the PCB level in the maternal blood and that in the cord blood in this study, whereas Akiyama et al. failed to find a significant correlation from their results.

Nelson et al. [20] have reported that the lipid content in the cord blood is significantly lower than that in the maternal blood at birth and that the actual value in the cord blood is approximately 1/3 of that in the maternal blood. Since PCB is a fat-soluble substance, a correlation between PCB and the lipid content in the biological system must be considered. Regarding the placental transfer of PCB, the PCB concentration in the maternal blood must be compared with that in the cord blood, considering those values in relation to the lipid content of the blood. However, this subject was not investigated in this study. Akiyama et al. [19] have reported that the PCB concentration in the cord blood per unit fat content is significantly lower than that in the maternal blood per unit fat content. Thus, the placental transfer of PCB in mothers is assumed to be controlled by the blood-placental barrier. However, this control is not complete so that the PCB concentration in the cord blood increases as the PCB concentration in the maternal blood increases.

3) PCB concentration in the maternal blood and infant blood

No significant difference was observed between the PCB concentration in the blood of nonlactating mothers and that in the blood of lactating mothers at three months after birth. However, it is known that the large amounts of PCB accumulated in the mother is excreted from the body during the lactation period through the breast milk [4]. Thus, this subject must be investigated in further detail in the future using an increased number of subjects to determine the effect of the status of lactation and the status of the diet during the lactation period.

The extent of PCB contamination in babies via their mothers was investigated assuming the PCB concentration in the blood of the baby at birth. In the case of three month old bottle-fed babies, the PCB concentration in the blood was unchanged from that in the blood of babies at birth. On the other hand, in the case of breast-fed babies at the same age, the PCB concentration remarkably increased due to the transfer of PCB from mothers to babies by breast milk.

In addition, the PCB concentration in the blood of babies exceeded the PCB concentration in the blood of the mothers. Matsuo [21] has reported on the absorption and metabolism of fat in infants. The fat absorption in breast-fed babies is found to be higher than that in bottle-fed babies. On the other hand, the excretion rate of fat in breast-fed babies is slow. Therefore, PCB transferred by the breast-fed milk is accumulated in the body of the infant with the absorption of fat and the above-mentioned result is obtained due to the fact that the biological degradation of PCB is very slow.

An increasing trend in the PCB concentration in the blood of breast-fed babies was found in the same babies at one year after birth. On the other hand, a decreasing trend was found in the PCB concentration in the blood of three month old bottle-fed babies as compared to one year old bottle-fed babies. Thus, PCB does not seem to be taken on after birth by bottle-fed babies. In the case of breast-fed babies, the PCB concentration in the blood of one year old babies does not seem to decrease even though milk is being replaced by other food during the weaning period. This PCB level at one year after birth was slightly higher than that at three months after birth. In spite of the fact that the milk intake of infants decreases around the age of one year after birth, since both breast milk and weaning food are taken in, the amount of PCB transferred from breast milk to the babies is still higher than the amount of PCB which can be excreted. The PCB concentration in the blood of breast-fed babies at two years after birth, decreased significantly compared with that in the blood of one year old breast-fed babies. This phenomenon is due to the fact that these babies are completely weaned and their food intake is close to that of adults, and that the PCB concentration accumulated during the breast-feeding period is gradually excreted from the body. However, a remarkable difference in the PCB concentration in the blood of breast-fed babies and bottle-fed babies was observed even at two years after birth. The authors will follow continuously the changes in the PCB concentration in the blood of these infants examined in the present study.

V. Conclusion

The transfer of PCB from mothers to infants was investigated for three consecutive years beginning with 1974. The following results were obtained:

1) The average PCB concentration in the breast milk of lactating mothers in this study was 19.1 $\pm$ 12.7 ppb. This level seems to be similar to the national average. Breast-fed babies are assumed to be taking PCB in breast milk at a concentration higher than the PCB intake of their lactating mothers.

2) When the PCB concentration in the maternal blood at birth was compared with that in the cord blood, the PCB concentration in the cord blood was found to be significantly lower than that in the maternal blood at birth. A significant correlation was obtained between these two values.

3) The PCB concentration in the maternal blood at three months after birth was not significantly different between lactating mothers and nonlactating mothers. However, both values were significantly lower than the PCB concentration in the maternal blood at birth.

4) Regarding the PCB concentration in the blood of bottle-fed and breast-fed babies, the PCB concentrations in the blood of breast-fed babies, three months, one year, and two years old were significantly higher than those in the blood of bottle-fed babies of the same ages.

5) The PCB concentration in the blood of bottle-fed babies showed a gradually decreasing trend after birth, while the PCB concentration in the blood of breast-fed babies sharply increased during a three month period after birth and tended to increase until one year after birth. The PCB concentration finally tended to decrease around two years after birth, by which time nursing had presumably ceased.

6) The PCB concentration in the blood of lactating mothers at three months after birth showed a significant correlation with the PCB concentration in the blood of three month old breast-fed babies.

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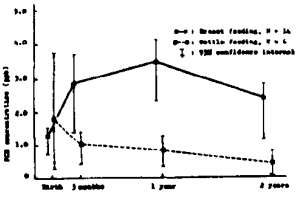


Fig. 1 Time course of PCB concentration in babies blood

Table 1. Number of samples in each period from 1974 to 1976

		1974	1975	1976	Total
Maternal blood at delivery		27	21	28	76
Cord blood		27	20	27	74
Maternal blood at 3 months after birth		19	21	15	55
Infant blood	3 months old	27	19	11	57
	1 year old	19	14	-	33
	2 years old	20			20
Breast milk at 3 months after birth		19	18	9	46

Table 11. Level of PCB in the breast milk at 3 months after birth

		1974		1975		1976		Total	
		No. of samples	Mean ± S.D. (Max.~Min.)	No. of samples	Mean ± S.D. (Max.~Min.)	No. of samples	Mean ± S.D. (Max.~Min.)	No. of samples	Mean ± S.D. (Max.~Min.)
Milk fat (%)		19	3.6 ± 1.2 (5.8~1.9)	18	3.8 ± 1.6 (7.0~1.4)	9	3.3 ± 0.9 (4.3~1.9)	46	3.6 ± 1.3 (7.0~1.4)
PCB conc. (ppb)	whole basis	19	12.3 ± 8.1 (31.2~2.6)	18	26.7 ± 14.4 (64.2~8.0)	9	18.3 ± 7.4 (32.1~7.2)	46	19.1 ± 12.7 (64.2~2.6)
	fat basis	19	350 ± 236 (938~90)	18	990 ± 424 (1,834~362)	9	561 ± 170 (836~363)	46	564 ± 372 (1,834~90)

Values of PCB were calculated as Kanechlor 500

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Table III. Level of PCB in the maternal blood and the cord blood at delivery

	1974		1975		1976		Total	
	No. of samples	Mean ± S.D. (Max. ~ Min.)	No. of samples	Mean ± S.D. (Max. ~ Min.)	No. of samples	Mean ± S.D. (Max. ~ Min.)	No. of samples	Mean ± S.D. (Max. ~ Min.)
Maternal blood (ppb)	27	5.1 ± 3.0 (15.5 ~ 1.4)	21	5.1 ± 3.3 (12.1 ~ 0.8)	28	3.6 ± 2.1 (11.1 ~ 1.2)	76	4.5 ± 2.9 (15.5 ~ 0.8)
Cord blood (ppb)	27	1.5 ± 1.3 (5.6 ~ N.D.*)	20	1.1 ± 0.8 (3.1 ~ N.D.)	27	0.8 ± 0.6 (2.7 ~ N.D.)	74	1.1 ± 1.0 (5.6 ~ N.D.)

*: Not detected
Values of PCB were calculated as Kanechlor 500

Table IV. Level of PCB in the mothers and the babies blood

			1974		1975		1976		Total	
			No. of samples	Mean ± S.D. (Max. ~ Min.)	No. of samples	Mean ± S.D. (Max. ~ Min.)	No. of samples	Mean ± S.D. (Max. ~ Min.)	No. of samples	Mean ± S.D. (Max. ~ Min.)
Mothers blood (ppb)		I	—	—	3	4.1 ± 1.0 (5.4 ~ 3.1)	6	2.0 ± 1.1 (4.0 ~ 0.6)	9	2.7 ± 1.4 (5.4 ~ 0.6)
		II	19	2.9 ± 2.4 (10.8 ~ 0.5)	18	2.6 ± 1.9 (6.2 ~ N.D.*)	9	1.5 ± 0.7 (2.9 ~ 0.4)	46	2.5 ± 2.0 (10.8 ~ N.D.)
Babies blood (ppb)	3 months old	I	8	1.7 ± 3.0 (7.6 ~ N.D.)	3	1.9 ± 0.8 (3.0 ~ 1.0)	4	1.1 ± 0.7 (2.0 ~ N.D.)	15	1.6 ± 2.2 (7.6 ~ N.D.)
		II	19	3.3 ± 2.0 (8.7 ~ 0.2)	16	4.5 ± 2.6 (10.9 ~ 0.7)	7	2.6 ± 1.1 (4.4 ~ 1.1)	42	3.6 ± 2.3 (10.9 ~ 0.2)
	1 year old	I	6	0.9 ± 0.6 (2.1 ~ 0.3)	2	N.D.	—	—	8	0.7 ± 0.5 (2.1 ~ N.D.)
		II	13	4.0 ± 2.4 (10.6 ~ 1.7)	12	5.4 ± 4.7 (17.7 ~ 0.8)	—	—	25	4.7 ± 3.7 (17.7 ~ 0.8)

I : Bottle feeding, II : Breast feeding, *: Not detected
Values of PCB were calculated as Kanechlor 500

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