Table 1. Comparison of characteristics of the participants who reported/did not report their TTP

		TTP		Total	p value
		Not reported	Reported		
Number		118	180	298	
Age (years)					
Subject		31.9	30.6	31.4	0.14
Partner		33.7	31.9	32.9	0.05
BMI (kg/m^2)		21.2	20.6	20.8	0.08
Number of births (times)		1.9	1.5	1.7	<0.001
Hair mercury level (μg/g)		2.02	2.01	2.01	0.94
Frequency of intercourse (%)					
<1/week		51	41	44	0.19
1/week		30	40	37	
>2/week		19	19	19	
Smoking (%)					
Subject		25	16	19	0.05
Partner		62	54	57	0.20
Milk (glass/week)					
Subject		4.4	5.3	4.9	0.09
Partner		2.6	4.4	3.7	<0.001
Caffeine (mg/week)					
Subject		1114	1332	1247	0.04
Partner		1188	1337	1280	0.17
Alcohol (g/week)					
Subject		75.7	69.7	72.0	0.75
Partner		183	170	175	0.77
Fish consumption (%)					
Subject	<1/week	2.9	1.7	2.1	0.65
	1–2/week	41	39	40	
	3–5/week	42	49	47	
	>5/week	14	11	12	
Partner	<1/week	2.9	1.7	2.1	0.34
	1–2/week	21	17	19	
	3–5/week	57	62	60	
	>5/week	19	19	19	
Meat consumption (%)					
Subject	<1/week	3.9	4.0	3.9	0.98
	1–2/week	38	36	37	
	3–5/week	44	46	46	
	>5/week	14	14	14	
Partner	<1/week	1.0	0.0	0.4	0.40
	1–2/week	20	15	17	
	3–5/week	54	55	55	
	>5/week	25	30	28	

similar to those reported previously (e.g., Curtis et al., 1999) as well as to the distribution obtained in our previous study on Japanese subjects (Arakawa et al., 2003). The present result again shows that the TTP questionnaire is applicable to Japanese women in spite of possible differences in their sexual practices compared to those of Western women, e.g., in their preferred methods of contraception.

The geometric mean of the total mercury concentrations (2.01 μg/g) was close to those obtained in a recent

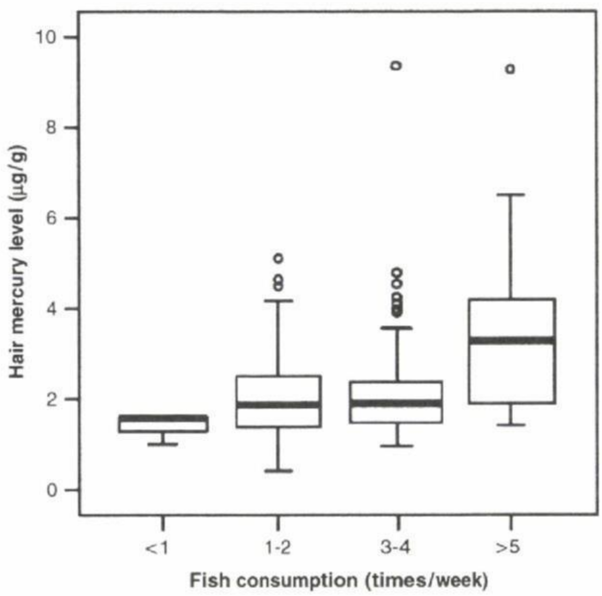

Fig. 2. Relationship between frequency of fish consumption (meals per week) and hair mercury level.

study in which geometric mean values of 1.23–2.50 µg/g were reported for mercury concentrations in the hair of persons from 5 cities in Japan (total number of samples 1666) (Yasutake et al., 2003). This result indicated that fish consumption of our subjects was similar to that of the average Japanese.

There were significant differences in some parameters between the subjects who reported TTP and those who did not (Table 1). Subjects who did not report TTP were older and had a higher number of previous births. The difference in parity could be explained by the age difference: there was a positive correlation between age and parity for the subjects ($r = 0.33$, $p < 0.001$), and the slope of the regression line indicated that the differences in parity were consistent with age difference. Older women who already have children probably do not memorize TTP because they are likely to pay less attention to getting pregnant. In other words, women who were more anxious about and paid more attention to getting pregnant were the subjects of our analysis. Whether this bias has a bearing on our research objectives should be carefully considered.

In our multivariate analyses, parity and age were significant variables that contributed to prolonged TTP when partners' effects were taken into consideration (Table 4). This result was consistent with those of other studies that reported associations between parity and TTP (e.g., Howe et al., 1985; Olsen, 1991), age of female subject and/or her partner and TTP (e.g., Baird and Wilcox, 1985, Howe et al., 1985; Olsen, 1991; Spinelli et al., 1997). Inconsistent results for the logistic regression analysis were obtained when age and life-style parameters for the subject only were included although the reason for the inconsistency was not clear. The reason why alcohol intake was a significant variable for the classification of our subjects into the two groups (Table 3) was also not clear. The results of logistic regression analysis were virtually the same when Cox regression analysis was employed, though statistical significance was not obtained ($p = 0.06$). However, the important finding was that hair mercury concentration was not selected as significant in either of logistic regression analysis and Cox regression analysis.

It is well known that some OCs, such as dioxins and PCBs, can disrupt normal human endocrine functions (Longnecker et al., 1997; Watanabe et al., 1999), and that these compounds concentrate along food chains (IPCS, 1992; Rolff et al., 1993) and thus fish can contain OCs at high concentrations. Therefore, fish consumption is the predominant route of the intake of OCs for the general population with fish-eating habits (Svensson et al., 1991; Tsutsumi et al., 2001). In addition to this, the total mercury concentration in hair has been considered a quantitative indicator of fish consumption (Yamaguchi et al., 1971; Iwasaki et al., 2003). Thus, we employed hair mercury level as a marker of fish consumption, and of the exposure level to OCs, to relate to TTP, based on the assumption that there is a strong correlation between the concentrations of OCs and mercury in fishes, and consequently, in the tissues and body fluids of humans who consume fish.

The result of this study, however, was not consistent with our previous finding that frequent fish eaters showed prolonged TTP (Arakawa et al., 2003). The reasons for this inconsistency may partly be attributable to the fact that we employed total mercury concentration in the hair segment 0–3 cm from the scalp. The hair mercury level represents fish consumption in the last trimester of the subjects' gestation: clearly this does not correspond to the period of TTP. It is possible that the amount of fish consumption changed after pregnancy. This may be the reason for the low correlation between fish-eating frequency and hair mercury level (Fig. 2), which, though statistically significant, was weaker than those recorded in previous studies (Bjornberg et al., 2005). We should, perhaps, have employed samples of hair of > 10 cm from the scalp.

Another possible reason for the negative result of the present study was that hair mercury levels are not correlated with the intake of the chemicals in fish that can affect fecundity. We assumed that the concentrations of OCs and mercury in marine foods would correlate with each other because of their co-biomagnification. However, Bjerregaard and Hansen (2000) reported that the concentrations of OCs and mercury did not correlate in the blood of women who consumed marine food. Therefore, it is clear that we should employ direct indicators that reflect the level of exposure to OCs

Table 2. Comparison of characteristics of the participants between group 1 (TTP: 0–12 months) and group 2 (TTP: >12 months)

		Time to pregnancy		Total	p value
		0–12	>12		
Number		160	20	180	
Age (years)					
Subject		30.6	31.0	30.6	0.70
Partner		31.7	33.7	31.9	0.10
BMI (kg/m^2)		20.7	20.5	20.6	0.83
Number of births (times)		1.5	1.4	1.5	0.23
Hair mercury level (μg/g)		2.01	1.97	2.01	0.82
Frequency of intercourse (%)					
<1/week		40	50	41	0.24
1/week		40	45	40	
>2/week		21	5.0	19	
Smoking (%)					
Subject		15	18	16	0.41
Partner		51	64	54	0.10
Milk (glass/week)					
Subject		5.5	3.4	5.3	0.05
Partner		4.5	3.9	4.4	0.62
Caffeine (mg/week)					
Subject		1314	1469	1332	0.48
Partner		1313	1469	1337	0.30
Alcohol (g/week)					
Subject		59.1	155	69.7	0.12
Partner		169	182	170	0.79
Fish consumption (%)					
Subject	<1/week	1.9	0.0	1.7	0.94
	1–2/week	39	40	39	
	3–5/week	49	50	49	
	>5/week	11	10	11	
Partner	<1/week	1.9	0.0	1.7	0.29
	1–2/week	16	30	17	
	3–5/week	61	65	62	
	>5/week	21	5.0	19	
Meat consumption (%)					
Subject	<1/week	4.5	0.0	4.0	0.68
	1–2/week	36	35	36	
	3–5/week	45	55	46	
	>5/week	15	10	14	
Partner	<1/week	0.0	0.0	0.0	0.03
	1–2/week	14	25	15	
	3–5/week	53	70	55	
	>5/week	33	5.0	30	

to relate to TTP. To date, we know of only one such study: Axmon et al. (2004) investigated the relationship between serum/plasma 2,2′4,4′,5,5′-hexachlorobiphenyl (CB-153) concentrations, a biomarker of total PCB exposure, and TTP. They did not find any association between blood CB-153 concentrations and TTP. They estimated the blood concentrations of CB-153 for the subjects when the index pregnancies took place, namely, 1–36 years previously (median: 20 years), from the CB-153 concentrations in blood that had been drawn from

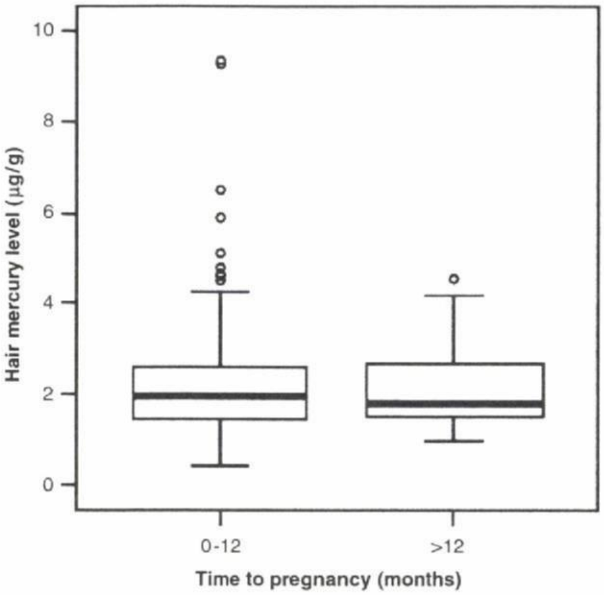

Fig. 3. Comparison of hair mercury level between group 1 (TTP: 0–12 months) and group 2 (TTP > 12 months).

Table 3. Odds ratio of risk factors

	Odds ratio	95% CI	*p* value
Alcohol	1.61	1.05–2.49	0.03

The variable "age" "milk" "alcohol" "caffeine" "meat" "smoking" of the female subjects only were used.

Table 4. Odds ratio of risk factors

	Odds ratio	95% CI	*p* value
Parity	0.44	0.20–0.97	0.04
Age	3.10	1.60–6.04	<0.001

The variables "age" "milk" "alcohol" "caffeine" "meat" "smoking" were generated by summing data for the female subject and her partner because there was a positive correlation between them for these variables.

the subjects in 2000. It is very probable that the blood CB-153 concentrations estimated after so many years would have been subject to gross errors, and this was possibly a major reason why these investigators failed to find any association between blood CB-153 concentrations and TTP.

It may be worth pointing out that the present result indicates a lack of association between TTP and methylmercury exposure in Japanese. In other words, methylmercury exposure at the present level does not affect human fecundity. This may be valuable information in the hazard identification of methylmercury.

In summary, we could not obtain reproducible results showing a positive relationship between fish consumption and TTP in this cohort study using hair mercury levels as an indicator of fish consumption, although such a relationship had been indicated in our earlier study. We will extend our survey by including direct indicators that can reflect exposure level to OCs during the duration of the TTP to characterize the health risk posed by fish consumption, which is important for Japanese and other fish-eating populations.

Acknowledgments

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CONCENTRATIONS OF DIOXINS AND PCBS IN CORD BLOOD IN JAPANESE CHILDREN FROM THE TOHOKU STUDY OF CHILD DEVELOPMENT

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Introduction

From several cohort researches, it has been reported that the perinatal exposure to the methylmercury and persistent organic pollutants (POPs), such as polychlorinated biphenyls (PCBs), has been associated with neurobehavioral development on postnatal growth[1]. We have started a prospective cohort study to examine the effects of perinatal exposures to POPs on neurobehavioral development in Japanese children (The Tohoku Study of Child Development, TSCD)[2]. We registered 599 mother-infant pairs in this cohort study.

Measurement of PCBs in previous epidemiological researches was performed by homologue or limited isomer, but congener-specific analysis was not done. Furthermore, dioxins measurement in cord blood was not reported in many papers since this measurement required high sensitivity and a large amount of samples.

In the present study, we established a measurement procedure to simultaneously determine dioxins and all congener-specific PCBs by high resolution gas chromatograph mass spectrometer (HRGS/HRMS) using isotopic dilution method from the same sample. Then, we determined the concentration of dioxins and PCBs in whole cord blood in Japanese children.

Materials and Methods

PCDDs, PCDFs, dioxin-like PCBs and total PCBs in whole cord blood were analyzed by HRGC/HRMS. After a whole cord blood (about 20 ml) was weighed and added with the clean-up spike containing ^{13}C-labeled standard mixture of PCDDs, PCDFs, dioxin-like PCBs and PCBs, and then crude lipid in sample was extracted. This extract dissolved in *n*-hexane was purified on a multi-layer silica gel column. The purified solution was divided into two aliquots of 80 % for determination of PCDDs, PCDFs and dioxin-like PCBs (aliquot A) and 20 % for determination of congener-specific PCBs (aliquot B). The aliquot B was concentrated without further purification and measured by HRGC/HRMS. The aliquot A was fractionated on active carbon-dispersed silica gel column to PCDDs, PCDFs, *non-ortho* PCBs fraction and *mono-ortho* PCBs fraction, and measured for each fraction by

HRGC/HRMS. HRGC/HRMS analysis was conducted on a 6890 series GC (Agilent Technology, USA) equipped with Autospec-Ultima (Micromass, UK). Measurements of PCDDs , PCDFs and *non-ortho* PCBs were used with SCLV injection system (SGE, Australia).

Results and Discussion

The distribution of total PCBs concentration in whole cord blood was shown in Figure 1. The mean total PCBs concentration was 65.3ng/g-fat (SD 43.4, median 55.6, minimum 12.1, maximum 238.1, n = 163). The mean total TEQ was 13.1pg-TEQ/g-fat (SD 7.7, median 10.9, minimum 3.5, maximum 44.5, n = 84). The correlation between total PCBs and TEQ was shown in Figure 2. This correlation coefficient was 0.93 ($p < 0.01$, n = 84).

Although there are few reports on TEQ in whole cord blood in Japan, a governmental report prepared by Morita[3] is very suggestive. In that report, the TEQ values that were based on the sum of PCDDs and PCDFs were similar or slightly lower than that of our study. Fukata et al.[4] reported the PCBs concentration in cord serum in Japanese, in which the median was 63.0ng/g-fat (n=32). Our results of total PCB concentration were same level as compared with their results.

It was shown that the correlation between TEQ and total PCBs in cord blood was absolutely high in Fig 2. This suggests that it would be possible for measurement of TEQ or total PCBs to evaluate each other value. In addition, using both TEQ and total PCBs should be avoided in a multiple liner regression analysis because of multicollinearity. Consequently, measurement of both TEQ and PCBs in the same sample should be reconsidered.

The sources of dioxins and PCBs were different. In Japan, dioxins were released unintentionally into environment as an impurity of the agricultural chemicals. On the other hand, PCBs is the chemical substance which had been used intentionally for insulation oil, thermal catalyst and coating compound. As the reasons why high correlation was observed between dioxins and total PCBs in the cord blood, it would be suggested that the behavior of both chemicals in the ecosystem and the mechanism which pollutes the human body through the bioaccumulation by the food chain is similar.

Acknowledgments

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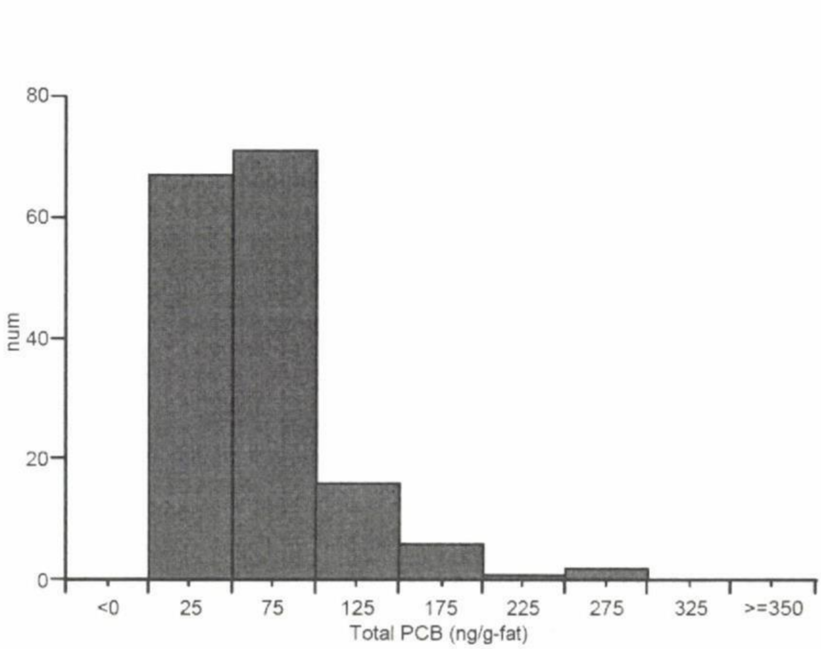

Fig 1. The distribution of total PCBs in whole cord blood

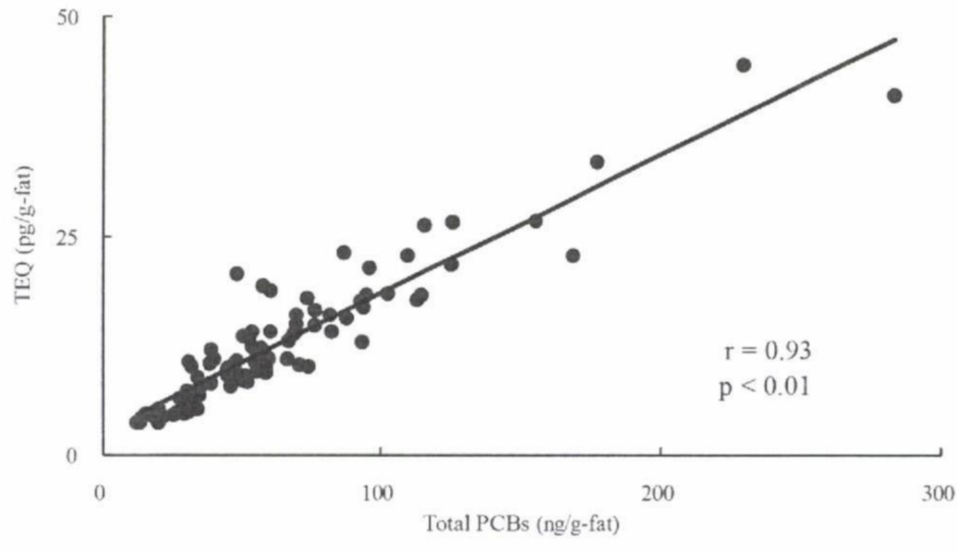

Fig 2. The correlation between total PCBs and TEQ

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EFFECT OF PERINATAL EXPOSURE TO ENVIRONMENTALLY PERSISTENT ORGANIC POLLUTANTS AND HEAVY METALS ON NEUROBEHAVIORAL DEVELOPMENT IN JAPANESE CHILDREN: PCBS EXPOSURE AND NEONATAL NEUROBEHAVIORAL STATUS

Suzuki K[1 2 6], Nakai K[1], Nakamura T[1], Hosokawa T[2], Okamura K[3], Sakai T[4], Kurokawa N[1], Kameo S[1], Murata K[5], and Satoh H[1]

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Introduction

The neurobehavioral effects of perinatal exposures to methylmercury (MeHg) and environmentally persistent organic pollutants (POPs) including polychlorinated biphenyls (PCBs), dioxins and pesticides are of great concern world wide. Several epidemiological studies have reported some associations of perinatal exposure to MeHg or PCBs with neurobehavioral deficit, such as postnatal growth delay and poorer cognitive functions[1]. These chemicals accumulate in human body mostly through the consumption of food, especially fish and shellfish. On the other hand, since fish is rich in nutrients such as polyunsaturated fatty acid (PUFA) essential for normal brain development of fetus, fish has been usually recommended for pregnant women. Therefore, from perspective of risk assessment, these health hazard issues are important for fish eating populations.

We have been performing a prospective cohort study, the Tohoku Study of Child Development (TSCD), to examine the effects of perinatal exposure to MeHg, PCBs, and dioxins on neurobehavioral development in Japanese children[2]. We registered 599 mother-infant pairs from January 2001 to September, 2003 in an urban area of Tohoku district, Japan. Samples such as maternal peripheral blood, cord blood, breast milk and maternal hair were collected for chemical determination. For the assessment of neurobehavioral development, Brazelton Neonatal Behavioral Assessment Scale (NBAS) was administered when children were three days old, and other tests including Bayley scales of infant development second edition and Kaufman assessment battery for children were performed with growth of children.

In the present study, we report the preliminary results on the association of NBAS with total PCB concentration in cord blood and maternal fish intake.

Materials and Methods

Participants were 163 mother-infant pairs whose variables including PCBs concentration in cord blood, NBAS, and other covariates were available. Mean maternal age at the delivery was 31.9 (SD4.2). Infants consisted of 87 boys and 76 girls, and they were all singleton and full-term (36-42 weeks) gestation without congenital anomalies or diseases. Birth weight was 2400g or more. Information was obtained about pregnancy, delivery conditions and infant characteristics from medical record.

PCBs concentration was measured from whole of cord blood collected immediately after delivery. All 209 congeners were analyzed using HR-GC/MS (Metocean environmental Inc, Shizuoka, Japan). Total PCB concentration represented the sum of the all measured congeners, expressed as ng/g-fat.

Maternal fish intake was estimated using the semi-quantitative food frequency questionnaire (FFQ) for 122 individual foods and recipes[3] and 13 additional items regarding fish and shellfish. The FFQ was administered at four days after delivery. Trained investigators showed mothers a real size photograph of each food, then, mothers answered the frequency and the amount of intake per meal.

For other variables of chemical analysis, hair mercury (hair Hg) concentration was analyzed from maternal hair samples taken at two days after delivery. Total hair Hg concentration was measured by cold vapor atomic absorption[4] at National Institutes of Minamata Disease (Minamata, Japan). Thyroid hormones including thyroid-stimulation hormone (TSH), total thyroxine (T4), triiodothyromine (T3), free T4 and free T3, were measured from plasma of cord blood by SRL, Inc. (Tokyo, Japan).

NBAS was administered on three days after delivery. Examiners of the NBAS were trained and certified at the training center for NBAS in Nagasaki University School of Medicine, Japan. Reliability check was conducted throughout the data collection to maintain a 90% level of agreement.

In the statistical analysis, a stepwise multiple regression analyses were performed for adjustment of covariates. The potential covariates were as follows; maternal age at delivery, maternal alcohol drinking during pregnancy, maternal smoking habit, maternal total energy intake, delivery type, parity, gestational age, gender, birth weight, apgar score, TSH and T3 concentration in cord blood, maternal hair Hg concentration, and NBAS examiners. The significance level was set at 5%.

Results and Discussion

The mean total PCB concentration in cord blood was 65.3 ng/g-fat (SD 43.4), and the mean maternal total fish intake was 2.6 kg/year (SD 1.6). Table 1 shows results of multiple regression analyses. A positive association was found between total PCB concentration and the score of the regulation of state cluster. A negative association was found between total fish intake and the score of the habituation cluster.

Early studies reported the negative associations of prenatal PCBs exposure with neurobehavioral development. We hypothesized the negative associations between total PCBs in cord blood and the NBAS. On the other hand, because fish is rich in nutrients such as PUFA essential for brain development, we also hypothesized the beneficial effects of maternal fish intake on the neonatal neurobehavioral status. Our findings are not in line with our hypotheses and the findings from early studies. We do not know clearly the reason for these findings, there are several possibilities. First, it is possible that the level of PCB exposure in our study was too low to detect the effects of PCB exposure on neonatal neurobehavioral status. We supposed that the level of PCB exposure in our study might be lower than those of early studies[5, 6, 7], although it is difficult to compare PCB levels among studies for different analytical methods. Second, since this report is based on the preliminary results, the sample size too small to detect the effects of low level exposure to PCB. The entire participant of our cohort study is 599 mother-infant pairs, and we collected not only samples of cord blood, but also maternal peripheral blood and breast milk. The measurements of chemicals including PCBs are now on going, and the further study will reveal the associations of neurobehavioral development with perinatal PCBs exposure and fish intake.

Table 1. The results of multiple regression analyses

	Habituation	Orientation	Motor	Range of state	Regulation of state	Autonomic stability	Reflex
Total PCBs (ng/g-fat)[1]	0.47	0.34	0.28	-0.04	0.81*	0.16	-1.39
Total fish intake (kg/year)[1]	-0.87*	-0.00	0.20	-0.34	0.73	-0.23	-0.28

(Beta value) * p < .05

[1]Log translations, $Log_{10}X$, were used on the value of total PCB concentration and total fish intake.

Acknowledgements

We thank all families who participated in the study. The study protocol was approved by the Medical Ethics Committee of the Tohoku University Graduate School of Medicine. This research was funded by Japan Ministry of Health, Labour and Welfare, Research on Risk of Chemical Substances.

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ASSOCIATIONS OF NEONATAL NEUROBEHAVIORAL STATUS WITH CORD BLOOD PCB, MATERNAL HAIR MERCURY, AND MATERNAL FISH INTAKE IN THE TOHOKU STUDY OF CHILD DEVELOPMENT

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Abstract

We have been performing a prospective cohort study, the Tohoku Study of Child Development (TSCD), to examine the effects of perinatal exposures to environmentally persistent organic pollutants and heavy metals on neurobehavioral development of offspring. In the present study, we examined the associations of the Neonatal Behavioral Assessment Scale (NBAS) with the total PCB concentrations in cord blood, maternal hair mercury (hair Hg), and maternal fish intake. Multiple regression analyses indicated some significant associations of the NBAS clusters with hair Hg, but there were no significant associations between total PCBs and any cluster of NBAS. These findings suggest that prenatal methylmercury exposure adversely affects neonatal neurobehavioral status.

Introduction

Several epidemiological studies have indicated some associations of perinatal exposure to polychlorinated biphenyls (PCBs) and methylmercury (MeHg) with developmental deficits such as postnatal growth delay and poor cognitive functions. A common form of perinatal exposure is maternal fish intake; however, fish also contain some nutritive factors such as n-3 polyunsaturated fatty acids (n-3 PUFA) essential for normal brain development in the fetus and infant. From the perspective of risk assessment, these health hazard issues are important for fish-eating populations.

We have been performing a prospective cohort study, the Tohoku Study of Child Development (TSCD), to examine the effects of perinatal exposure to PCBs and MeHg on neurobehavioral development in Japanese children[1]. Previously[2], we reported some preliminary data about the associations of neonatal neurobehavioral status with total PCBs in cord blood and maternal fish intake. Since additional data on PCBs in cord blood have recently become available, in the present study we reexamined the associations of neonatal neurobehavioral status with total PCBs in cord blood, maternal hair mercury (hair Hg), and maternal fish intake.

Materials and Methods

The subjects were 392 mother-infant pairs whose variables including the PCB concentration in cord blood, the NBAS, and other covariates were available. The mean maternal age at delivery was 31.9 (SD4.2) years. The infants consisted of 203 boys and 189 girls, and they were all singletons from full-term (36-42 weeks) gestation without congenital anomalies or diseases. Birth weight was 2400g or more. Information was obtained about pregnancy, delivery and infant characteristics from medical records.

The PCB concentration was measured from whole cord blood collected immediately after delivery. All 209 congeners were analyzed using HR-GC/MS (IDEA Consultants, Inc, Tokyo, Japan). The total PCB concentration represented the sum of the all measured congeners, expressed as ng/g-fat.

The hair Hg concentration was analyzed from maternal hair samples taken two days after delivery. The total hair Hg concentration was measured by cold vapor atomic absorption[4] at the National Institute for Minamata Disease (Minamata, Japan).

Maternal fish intake was estimated using a semiquantitative food frequency questionnaire (FFQ) for 122 individual foods and recipes[3] and 13 additional items regarding fish and shellfish. The FFQ was administered four days after delivery. Trained investigators showed life-size photographs of each food to the mothers, after which they were asked to answer questions about the frequency and the amount of intake per meal.

Thyroid hormones, including thyroid-stimulating hormone (TSH), total thyroxine (T4), triiodothyromine (T3), free T4 and free T3, were measured from plasma of cord blood by SRL, Inc. (Tokyo, Japan).

The NBAS was administered three days after delivery. Examiners of the NBAS were trained and certified at the training center for NBAS in Nagasaki University School of Medicine, Japan. Reliability checks were conducted throughout the data collection to maintain a 90% level of agreement.

In the statistical analysis, multiple regression analyses were performed for adjustment of covariates. The potential covariates were as follows: maternal age at delivery, maternal alcohol drinking during pregnancy, maternal smoking habit, maternal total energy intake, delivery type, parity, gestational age, sex, birth weight, Apgar score 1 min after delivery, TSH, T4 and T3 concentrations in cord blood, and the NBAS examiners. The significance level was set at 5%.

Results and Discussion

The mean total PCB concentration in cord blood was 55.9 ng/g-fat (SD 35.3) (median 48.4), the mean total hair Hg level was 2.2 μg/g (SD 1.1) (median 2.0), and total fish intake was 25.9 kg/year (SD 17.8) (median 22.7). Table 1 shows the results of multiple regression analyses. The total PCBs in cord blood and the total fish intake were not significantly associated with any seven clusters of the NBAS. The total hair Hg was negatively associated with the motor score, and positively associated with the range of state and the reflex scores. For the motor and the range of state, higher scores mean more optimal behavioral status, and for the reflex, lower scores mean more optimal status because the reflex score indicates the number of unusual reflexes. Thus, the results suggested that prenatal MeHg exposure adversely affected neonatal neurobehavioral status. In early studies, an adverse effect of prenatal MeHg exposure on neurodevelopment was found in the Faroe Islands[5] and Boston[6], but not in the Seychelles[7]. Our findings are in line with the former two, although the types of examination were different.

Regarding the effects of PCB exposure, early studies demonstrated adverse effects of prenatal PCB exposure on neurodevelopment[8,9]. However, our findings do not agree with those studies. Several possibilities may account for this discrepancy. First, the levels of PCB exposure in Japanese pregnant women have decreased during the past several decades[10]. It is plausible that the level of PCB exposure in our cohort was too low to induce adverse effects on neonatal neurobehavioral status. Second, we used the value of the total PCB concentration as the exposure value. In the Oswego study, highly chlorinated PCBs (Cl7-9) were strongly associated with lower scores of the NBAS, but the total PCB level was not[7]. Therefore, we examined the associations of the NBAS with highly chlorinated PCBs, but found no significance (data not shown). Third, levels of toxicants such as PCB and MeHg and nutritive factors, including n-3 PUFA, vary among different fish types. The Japanese diet relies heavily on steamed rice, fish and vegetables. Indeed, the Japanese eat great amounts of fish, and they also eat many kinds of fish. This food lifestyle may contribute to the differences in the consequences of cohort studies. Further studies will require consideration of the potential risks of fish intake in the context of potential benefits. Since the TSCD study is a prospective cohort study, we will readdress these health issues when the children become older.

Acknowledgments

We thank all the families who participated in the cohort study. The study protocol was approved by the Medical Ethics Committee of the Tohoku University Graduate School of Medicine. This research was funded by the Japan Ministry of Health, Labour, and Welfare, Research on Risks of Chemical Substances.

Table 1. Results of multiple regression analyses

	Total PCBs (ng/g fat)[1]		Total hair Hg (µg/g)[1]		Total fish intake (kg/year)[1]		R^2 of the model
	Standardized beta	F	Standardized beta	F	Standardized beta	F	
Habituation	0.10	0.15	0.37	3.48	-0.31	1.14	0.12
Orientation	0.08	0.21	0.06	0.10	0.17	0.65	0.26
Motor	0.01	0.01	-0.28	5.60*	0.18	2.10	0.16
Range of state	-0.02	0.02	0.38	6.85**	-0.08	0.28	0.11
Regulation of state	0.22	1.27	-0.29	1.70	0.27	1.39	0.08
Autonomic stability	0.02	0.02	0.19	1.07	-0.23	1.38	0.12
Reflex	-0.26	0.72	0.74	4.52*	-0.11	0.09	0.17

* $p < 0.05$ ** $p < 0.01$

[1]Log translations, $Log_{10}X$, were used on values of total PCBs, hair Hg, and total fish intake.

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THE BIOLOGICAL MONITORING PROGRAM OF PERSISTENT ORGANIC POLLUTANTS IN JAPAN: 1. CONCENTRATIONS OF ORGANOCHLORINE PESTICIDES IN BREAST MILK, CORD BLOOD AND MATERNAL BLOOD

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Abstract
Persistent organic pollutants (POPs) are ubiquitous environmental contaminants that accumulate in lipid-rich body tissues. Although POPs are thought to be hazardous to health, the overall epidemiological data are as yet insufficient to draw any conclusions. Thus, exposure monitoring and epidemiological examination of the Japanese population are of importance to determine the health risks due to POPs exposure. The Ministry of the Environment of Japan (MOE) has been conducting systematic monitoring of POPs according to the Stockholm Convention. We provided some biological samples for the POPs biological monitoring project, and reanalyzed the report of the MOE. In this presentation, we summarize the data of organochlorine pesticides in human pair samples of breast milk, cord blood and maternal blood. We also analyzed the associations of pesticide concentrations with TSH and thyroid hormones in maternal and cord blood, since disruption of the hypothalamus-pituitary-thyroid axis is a hypothetical mechanism for POPs-induced adverse effects.

Introduction

Persistent organic pollutants (POPs) are ubiquitous environmental contaminants that accumulate in lipid-rich body tissues. Their lipophilicity and intrinsic resistance to biological degradation processes are responsible for bioaccumulation and biomagnification in the food chain, and consequently they can be found in humans at considerable concentrations. Although these concentrations are usually decreasing and almost at the background level, longer term exposure may cause potential risks to human health.

In humans, some POPs have been claimed to possess endocrine-disrupting potency. DDE exposure is related to TSH and estradiol levels among middle-aged and elderly men.[1] There is a significant negative association between the serum HCB concentration and total T4 in cord blood.[2] These findings suggest that exposure to POPs may affect the hypothalamus-pituitary-thyroid and the hypothalamus-pituitary-gonadal axes. Reproductive defects may be associated in part with exposure to hormonally active environmental chemicals during fetal and childhood development.[3] A growing number of reports have demonstrated the association between adverse effects in children and exposure to POPs at low doses over a longer period. Human perinatal exposure to PCBs has been also shown to be associated with several adverse effects.[4] However, little information is available regarding the delayed neurobehavioral development in infants following exposure to DDE.[5,6] Perinatal exposure to HCB was also shown to be associated with poor social competence in children.[7] Although the overall epidemiological data are not yet sufficient to allow us to draw firm conclusions, exposure monitoring and epidemiological examination of the Japanese population are important for risk assessment.

In Japan, the Ministry of the Environment (MOE) has been conducting systematic monitoring of chemicals over a 30-year period. The MOE initiated refined environmental monitoring including POPs in FY2002 according to the Stockholm Convention.[8,9] Recently, the MOE also added biological monitoring of human samples. Since information on blood levels of POPs in Japan is very limited, this monitoring of all the POPs covered by the convention will contribute to the future effectiveness evaluation. We have been collaborating with the MOE's POPs monitoring project by providing biological samples from our prospective birth cohort study, The Tohoku Study of Child Development (TSCD). We reanalyzed the results from the MOE's monitoring project, and summarize the data of organochlorine pesticides in human pair samples of breast milk, cord blood and maternal blood in this presentation.[10] We also analyzed the associations of pesticide concentrations with TSH and thyroid hormones in maternal and cord blood, since the disruption of the hypothalamus-pituitary-thyroid axis is a hypothetic mechanism for POPs-induced adverse effects.

Materials and Methods

Biological samples analyzed were randomly selected from the participants in the TSCD, and provided anonymously to the MOE. The TSCD study protocol was previously reported.[3] Briefly, maternal peripheral blood was collected using heparin as the anticoagulant agent in the morning when the pregnancy was at 28 weeks. The cord blood was collected immediately after delivery. These whole blood samples were frozen at –80°C until the chemical analysis. Breast milk was collected one month after delivery, and then frozen similarly.

Chemical determination was performed with high resolution gas chromatography-high resolution mass spectrometry (HRGC-HRMS) by IDEA Consultants, Inc. (Tokyo, Japan) as part of the MOE project as described in another report in this book. TSH, total T4 and total T3 were measured from the plasma of cord and maternal blood by SRL, Inc. (Tokyo, Japan). The statistical analyses were performed using JMP ver. 5.1.2. When the levels of data were not normally distributed, the data were log-transformed for statistical analysis.

The TSCD was approved by the Medical Ethics Committee of the Tohoku University Graduate School of Medicine, and all mothers provided signed informed consent.

Table 1 Pesticide concentrations if breast milk, cord blood and maternal blood (pg/g-fat)

Chemicals	Breast milk Median (Min-Max)	Cord blood Median (Min-Max)	Maternal blood Median (Min-Max)
Aldrin	nd (nd)	nd (nd)	nd (nd)
cis-Chlordane	460 (200-3100)	440 (210-1460)	620 (220-2060)
trans-Chlordane	180 (80-1400)	330 (120-770)	190 (130-490)
Oxychlordane	11700 (2700-46800)	4940 (1280-17530)	5520 (1540-17270)
cis -Nonachlor	3400 (860-10570)	960 (280-2780)	1680 (470-4860)
trans -Nonachlor	22480 (6620-100950)	6690 (1690-26260)	12830 (3620-52370)
o,p'-DDD	nd (nd-510)	nd (nd-100)	nd (nd-100)
p,p'-DDD	300 (100-14510)	120 (nd-590)	240 (60-430)
o,p -DDE	380 (180-950)	250 (90-600)	340 (170-730)
p,p'-DDE	143300 (31700-331500)	68180 (12330-385690)	93270 (17280-271390)
o,p -DDT	1220 (550-4170)	450 (190-1420)	700 (200-2130)
p,p'-DDT	7620 (2310-19390)	2450 (560-7330)	3950 (1080-10070)
Dieldrin	4290 (2100-17480)	3140 (1370-13580)	3280 (1440-9810)
Endrin	nd (nd-490)	nd (nd)	nd (nd)
Heptachlor	nd (nd-370)	nd (nd-170)	nd (nd)
trans-Heptachlorepoxide	nd (nd)	nd (nd)	nd (nd)
cis-Heptachlorepoxide	4480 (1800-24140)	2490 (670-12720)	2680 (730-12520)
HCB	16380 (6870-37260)	16700 (6440-39980)	13810 (5560-39580)
α-HCH	290 (150-1570)	310 (130-1910)	220 (120-580)
β-HCH	49010 (11500-213990)	29030 (4860-90490)	29350 (4750-196100)
γ-HCH	220 (50-2340)	340 (150-5080)	220 (100-2180)
δ-HCH	nd (nd-310)	nd (nd-140)	nd (nd)
Mirex	740 (170-1880)	410 (120-1380)	1110 (280-2890)
Parlar-26	1880 (760-7040)	660 (230-3020)	960 (300-2550)
Parlar-40	20 (nd-100)	nd (nd-180)	nd (nd-70)
Parlar-41	230 (nd-560)	nd (nd-240)	110 (nd-220)
Parlar-44	230 (60-640)	nd (nd-380)	70 (nd-200)
Parlar-50	3150 (1280-12490)	850 (280-4140)	1440 (480-4220)
Parlar-62	230 (nd-820)	nd (nd-510)	40 (nd-360)

n=68 for breast milk and cord blood, n=49 for maternal blood.

Table 2. Correlation of pesticide concentrations among breast milk, cord blood and maternal blood

	Breast milk						
	c-CHL	*t*-CHL	OxyCHL	*c*-Nonachlor	*t*-Nonachlor	*p,p'*-DDE	*p,p'*-DDT
Cord blood	0.543**	0.191	0.831**	0.836**	0.871**	0.837**	0.796**
Maternal blood	0.729**	0.291	0.943**	0.954**	0.959**	0.920**	0.878**
	Breast milk						
	Dieldrin	*c*-HCE	HCB	β-HCH	Mirex	Parlar-26	Parlar-50
Cord blood	0.821**	0.800**	0.879**	0.800**	0.673**	0.789**	0.778**
Maternal blood	0.819**	0.928**	0.921**	0.844**	0.894**	0.904**	0.917**

Pearson's r after log-transformed. n=68 for breast milk and cord blood, and n=49 for breast milk and maternal blood.

** $p<0.001$

Results and Discussion

Concentrations of pesticides in breast milk, cord blood and maternal blood are shown in Table 1. The highest values were observed for *p,p'*-DDE in all three materials. Since the use of DDT was prohibited in 1971 in Japan, this finding indicates the nature of intrinsic resistance to biological degradation of DDT/DDE. Mirex and toxaphene were measurable in most samples. Since neither chemical has ever been used in Japan, the route and the source of contamination are not fully understood.

High correlations of most chemicals among the three materials were observed as shown in Table 2. These findings indicated the usefulness of breast milk as a monitoring material for human exposure. Breast milk is rich in fat and therefore lipophilic chemicals such as POPs are accumulated, and it can be easily obtained from lactating women. In breast milk, most chemicals also correlate with each other (data not shown).

Since the working hypothesis on POPs-induced adverse effects is the disruption of the hypothalamus-pituitary-thyroid axis, associations of pesticides with TSH, T4 and T3 in maternal blood and cord blood were analyzed as shown in Table 3. It was confirmed that dioxins, PCBs and DDT/DDE were associated with TSH and thyroid hormones. In addition, other minor pesticides such as HCB, nonachlor, and toxaphenes were also associated with TSH, T4 and T3. Since there were clear multicollinearities among the chemicals, the causal relationships between the pesticide exposures and the levels of TSH and thyroid hormone remain to be clarified.

POPs exhibit bioaccumulation and biomagnification in the food chain, and therefore human exposure is thought to be mainly through the consumption of fish. However, maternal fish intake did not correlate with the concentrations of any of the chemicals (data not shown).

Further monitoring assessments and epidemiological examinations will make it possible to understand the exposure characteristics and biological effects of POPs on humans.

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