into four groups. Each bottle contained 50-100 g tissue. The representative samples were finally prepared by homogenization (Iyengar and Rapp 2001). The entire cord was stored in a clean glass tube without any preparation.

The mothers were finally asked to provide a sample of breast milk (more than 50 ml) one month after the delivery. A clean glass bottle was used for the shipping of breast milk.

Questionnaire. Several types of questionnaire were administered after the delivery. To assess the fish-intake and the general nutrition status of the mothers a food-intake frequent questionnaire (FFQ) for 122 individual foods and recipes (Date et al. 1996) and some additional items regarding seafood was administered. This is a standardized FFQ that enables the assessment of the intake of not only major nutrients but also several essential nutrients including retinol and folic acid in the Japanese population.

Other questionnaires were administered with the following items: educational background, occupation, income, smoking habit including passive smoking, alcohol consumption during pregnancy, hair treatments including bleaching, permanent wave and coloring, and dental amalgam treatment.

Neurodevelopment assessment. All testers who performed neurodevelopment assessments were not informed of exposure information including alcohol consumption/smoking habit, FFQ data, and feeding method.

The Brazelton Neonatal Behavioral Assessment Scale (NBAS) was administered when the infants were 3 days old. The testers had been trained in the training center at Nagasaki University School of Medicine, Japan.

Cognitive functions of the infants at 7 months old were evaluated using the Bayley Scale of Infant Development (BSID), second edition, the Kyoto Scale of Psychological Development (KSPD), and the Fagan Test of Infant Intelligence (FTII). BSID, an established psychodevelopmental test tool, consists of three major scales: the Mental Scale, the Psychomotor Scale, and the Behavior Rating Scale; only the first two scales are used. The mental scale assesses the infant's level of cognitive function (memory, learning, and problem solving), language development (expressive/receptive language, and vocalization), and personal/social development. The motor scale assesses fine and gross motor functions. Since there is no Japanese version of the standardized protocol of BSID, we translated the original manual into Japanese. To examine its reliability, the evaluation of testers were examined on the basis of the Gold Standard developed at the University of Rochester School of Medicine (Davidson et al. 1995). In addition, raw scores were used in the analysis because of the lack of Japanese age norms. KSPD is a Japanese standard developmental test (Maehara et al. 2002); therefore, the developmental performance of the infants is expressed as the developmental age (DA) for each behavior area and for all areas. The developmental quotient (DQ) is obtained by dividing the estimated DA by the chronological age and then multiplying the quotient by 100. FTII is a noninvasive test of information processing that may be applied to infants up to one year of age (Fagan and Detterman 1992).

BSID and KSPD were also used for the assessment of neurobehavioral development when the children were 18 months old. The Japanese version of Kaufman Assessment Battery for Children (K-ABC) was employed to assess the development and intelligence of children when they are 42 months old. The growth and development of the children will be followed up until they are 6-7 years old, but the battery of neurobehavioral tests is as yet undetermined.

Chemical determinations. Total mercury analysis was carried out by cold vapor atomic absorption spectrometry (Akagi and Nishimura 1991) with minor modifications. Briefly, without washing the hair samples, each sample, weighing approximately 20 mg, was acid digested with 0.5

ml of HNO_3, 0.5 ml of $HClO_4$ and 2 ml of H_2SO_4 at 200°C for 30 minutes. The resultant ionic mercury was then reduced to mercury vapor by adding 0.5 ml of 10% tin chloride to a flameless atomic absorption monitor (HG-201, Sanso Co., Ltd., Tokyo). Analytical accuracy was ensured by analyzing the Human Hair Reference Material NIES CRM No. 13 from the National Institute of Environmental Studies (Lot #650, Tsukuba). In fish-eating populations, total mercury in hair consists mostly of MeHg. Indeed, a few samples were analyzed to know the exact MeHg concentration by the method of Akagi and Nishimura (1991). MeHg in hair first extracted with hydrochloric acid and then with benzene. The organic layer was subjected to electron-capture detection gas chromatography (ECD-GC) at the National Institute for Minamata Diseases. The concentration of MeHg was confirmed to be more than 95% of the total mercury content. Total mercury analysis was also applied to other samples similarly.

Assessment of PCB exposure was performed by determining PCB levels in cord blood, placenta, breast milk, and maternal blood. All 209 PCB congeners were analyzed by high-resolution gas chromatography/high-resolution mass spectrometry (HRGC/HRMS) using the isotope dilution method. The analytical method was as follows: after biological samples were spiked with the ^{13}C-labeled standard mixture of PCBs, lipids in a sample were extracted and weighed. The extract redissolved by an organic solvent was purified in a multi-layer silica gel column. The purified solution was concentrated and analyzed for PCB after the addition of ^{13}C-labeled syringe spike. Four nonplanar PCB congeners (International Union for Pure and Applied Chemistry (IUPAC) nos. 118, 138, 153, and 180) are the predominant congeners found in human tissues and typically account for approximately 50-60% of total PCB (data not shown). Some earlier epidemiological studies attempted to assess PCB exposure using the sum of the above four major PCB congeners. For comparison with those earlier studies, the sum is also calculated in the present study.

A reporter gene assay of the toxic potency of dioxins and related chemicals was used for the assessment of dioxins. The Chemically Activated LUciferase gene eXpression (CALUX) assay was developed by Xenobiotic Detection Systems (XDS, Durham NC, USA) using a patented recombinant mouse cell line that contains the luciferase reporter gene under the control of dioxin-responsive elements (Denison et al. 1998). This analytical process consisted of the first extraction process as in PCB analysis and then column purification using sulfuric acid-impregnated silica gel and activated carbon column. The last purified extracts were given to the cells to produce luciferase, and the amount of light generated by the luciferase was directly related to dioxin toxic equivalent (TEQ) value. This assay has several advantages including its high sensitivity, easy pretreatment, and rapid determination, in comparison with HRGC/HRMS. This assay also requires only a smaller sample volume, which is another important advantage for epidemiological studies.

Cadmium and lead were determined by graphite furnace atomic absorption spectrometry and inductively coupled plasma mass spectrometry, respectively, after samples were digested in a microwave oven with ultrapure nitric acid. The standard reference material for analysis was NIST 1577b (bovine liver). Other major biochemical analyses of maternal and cord blood samples included those of plasma selenium and thyroid hormones including TSH, and total/free T4 and T3. Selenium was determined fluorometrically (Watkinson 1966). The assay of thyroid hormones were performed using a radioimmunoassay technique.

Potential confounders/covariates. The quality of the home environment was assessed using a questionnaire, the Evaluation of Environmental Stimulation (EES) (Anme et al. 1998), which has been established in Japan modified after the Home Observation for Measurement of the Environment (HOME) score (Caldwell and Bradley 2001).

HOME is a validated instrument for the assessment of the home environment, but there is no Japanese version that matches the Japanese cultural context. The EES is a questionnaire that directly evaluates the interaction between the child and the caregiver. It was shown that the results of EES highly correlated with those of HOME (Anme et al. 1998).

The parental socioeconomic status (SES) was rated using the Hollingshead Four Factor Index of Social Status (Hollingshead 1975) with several modifications to make the category and prestige of occupation match the Japanese economical context.

Maternal intelligence quotient was measured using the Raven standard progressive matrices. Only the Raven colored progressive matrices have already been introduced in Japan only for people older than 40 years old. We therefore used the original Raven standard version and analyzed results using the raw data.

Other major potential confounders included were as follows: age at examination (days), gestational age (weeks), and alcohol consumption/smoking habits during pregnancy for the mothers, and the Apgar score, neonatal illness/jaundice, spontaneous delivery, parity, chronic diseases, and duration of breastfeeding (months) for the infants.

TABLE 2. *Variables measured at the Tohoku study*

Measurement	Description
Exposure assessment	
PCBs	Cord blood, placenta, breast milk, and maternal blood
Dioxins	Cord blood, placenta, and breast milk, expressed by CALUX-TEQ
Pesticides	Breast milk and placenta, but the exact assay method has not been decided.
MeHg	Maternal hair at delivery, maternal blood, cord blood, and placenta
Heavy metals	Other heavy metals including Pb and Cd, in cord blood, maternal blood, and placenta
Other biochemical measurements	
Selenium	Cord blood and maternal blood
TSH, T4/T3	Cord blood and maternal blood
Neurodevelopment assessments	
NBAS	Infants at 3 days old
BSID	Infants at 7 and 18 months old
KSPD	Infants at 7 and 18 months old
FTII	Infants at 7 months old
K-ABC	Children at 42 months old
Confounders/covariates	
EES	A questionnaire regarding the home environment
SES	Hollingshead four factor index with modifications for application in Japan
Maternal IQ	Raven standard progressive matrices
FFQ	An interview method, with 122 single foods and recipes, and some additional seafood items
Questionnaires	Alcohol consumption/smoking during pregnancy, educational background, hair cosmetic treatments, dental amalgam, and duration of breastfeeding (months),
Other factors	**Mother**: age at delivery, spontaneous delivery/cesarean section, and chronic diseases **Infant**: Apgar score, body weight, body height, head circumference at birth, gestational age (weeks), neonatal illness/jaundice, parity, and age at examination (days)

Results and Discussion

The present report describes the study design and protocol for the prospective cohort study on the effects of perinatal exposures to MeHg and other environmentally POPs on neurobehavioral development in Japanese children. All variables measured are summarized in Table 2. To our knowledge, this is the first cohort study that examines these hazardous risks to children in Japan.

Recruitment. We recruited 687 healthy pregnant women between January 2001 and September 2003 at the obstetrical wards of two hospitals in Sendai, but the final number of babies registered in this study is not yet determined because the delivery of pregnant women registered in this study is ongoing. The percentage of babies fulfilling the criteria for inclusion with the mothers' consent to participate in the assessment using NBAS was 85%. The percentage of babies participating in the next assessment at 7 months old was 86% of those participating in the assessment using NBAS. This reduction was mainly due to family relocation from Sendai to other places. Sample size is essential for the statistical power, and this is especially important to test whether exposures to low levels of chemicals have the hazardous effects. In addition to the theoretical approach to decide the appropriate sample size, recent epidemiological studies that assessed neurobehavioral consequences of perinatal exposure to PCBs are useful in considering this issue. The Dutch cohort study was started with 418 healthy infants and 395 children were examined at 42 months of age (94% of the original cohort) (Patandin et al. 1999). The German cohort study consisted of 171 mother-infant pairs; 126 mothers provided milk samples and 91 mothers remained in the final examination of children at 42 months of age (approximately 70% of the mothers participating in the postnatal follow-up cohort) (Winneke et al. 1998). In the Faroe cohort study, PCBs could be analyzed in cord tissues from 435 of 1022 children who underwent neurodevelopment examination at 7 years old (Grandjean et al. 2001). These cohort studies showed a negative correlation between prenatal/postnatal PCB exposure and neurobehavioral development in children. Considering that the exposure level of Japanese women was similar to that of European women, and that the potential risk is almost identical, our sample size is probably sufficient.

Neurodevelopment assessment. There are six sets of cohort studies on health hazardous effects of perinatal PCB exposure in children, and all these studies approached this issue by the method of neurodevelopment assessment. Four sets of studies employed BSID to measure the development of infants, and three of them found a significant correlation between the outcomes of BSID and PCB exposure (Schantz et al. 2003). Based on these findings, BSID is expected to be a useful tool for evaluating the risks and the results can be easily compared among the studies. This was the reason why we employed BSID as one of the major components in our tests. On the other hand, BSID is a developmental test based on the developmental milestone concept, and there are no standardized data in Japan. Thus, BSID does not provide us information on MPI and PDI, the two standard indexes of the relative status of development in a population. We therefore used KSPD, the most commonly used neurodevelopmental test in Japan, to calculate DQ. Both BSID and KSPD were originally developed based on the work of Gessell (Ikuzawa et al. 1985; Black and Matula 1999). We also applied FTII and K-ABC to assess children at the ages of 7 and 42 months, respectively. The present study was the first trial to use FTII in Japan. FTII is a novelty preference task designed to predict the later development and intelligence of children (Fagan and Detterman 1992). These two intelligence tests were shown to be sensitive in detecting the adverse effects of low levels of perinatal PCB exposure (Jacobson et al. 1985; Patandin et al. 1999; Darvill et al. 2000; Walkowiak et al. 2001).

Chemical determinations. In a review (Schantz et al. 2003) of epidemiological studies on the possible adverse effects of perinatal expo-

sure to PCBs, it was concluded that a more complete information regarding the neurotoxicity of individual congeners or congener groups may be helpful for risk assessment. There are 209 PCB congeners, and a large number of these congeners were indeed found to be present in human tissues. Since their relative potency to produce nerve system effects is entirely unknown, a congener-specific analytical technique is essential for risk assessment. Despite the fact that several recent studies have used sophisticated congener-specific analytical techniques, there have been no attempts to analyze individual PCB congeners probably present in cord blood, mainly due to the lack of assay sensitivity. The delay of cognitive development may be more related to prenatal PCB exposure, as measured by the sum of concentrations of three or four major PCB congeners in either cord or maternal blood, but not with the postnatal PCB exposure, as measured by the sum of concentrations of PCBs in breast milk samples (Schantz et al. 2003). These findings suggest the importance of PCB congener-specific analysis in cord blood. In the present study, the detailed assessment of individual PCB congeners in cord blood and other samples was designed using a very sensitive HRGC/HRMS.

Only the Dutch cohort study (Patandin et al. 1999) examined the adverse effects of dioxin exposure on neurobehavioral development in children, in which the perinatal exposure, as measured by GC/MS in breast milk samples collected at 2 weeks postpartum, showed no noticeable correlation with cognitive functions measured later. However, the interpretation of these findings is complicated by the results that total PCB in breast milk samples showed no correlation with cognition functions, even though the same study showed negative correlation when total PCB in cord blood was used for analysis. These findings suggest that the characterization of prenatal exposure is more important to clarify the adverse effects of dioxins; the effects of prenatal dioxins exposure should be examined by analyzing levels of dioxins in cord blood. Because dioxins could not be measured by HRGC/HRMS in small volume of cord blood and maternal blood samples, the CALUX assay, a reporter gene assay to determine the all dioxin-like substances, is useful for this purpose. Previously, we already confirmed that data obtained by CALUX assay showed an extremely good correlation with TEQs obtained by HRGC/HRMS in environmental materials (Nakamura et al. 2002).

However, in practice, several problems in exposure assessment remain. First, the metabolites of PCBs are likely included in the adverse effects of PCB exposure. The main hypotheses are that PCB effects on neurodevelopment include the disruption of thyroid hormone homeostasis (Porterfield and Source 2000), and that candidate PCB congeners that may disturb the homeostasis may include several minor congeners and their OH-metabolites (Cheek et al. 1999; Chauhan et al. 2000). The measurement of all possible metabolites of PCBs is not realistic. Second, although there are limited available data describing the neurotoxicity of pesticides in humans, these chemicals may indeed affect the neurodevelopment of children (Schettler 2001). In the present study, however, the assay methods for pesticides including organochlorine and organophosphorus chemicals are not yet determined because the number of chemicals is too large. Third, the measurement of all chemicals including PCBs, dioxins, heavy metals and pesticides from cord blood is difficult because of the shortage of sample volume and the insufficient detection limit. Other biological samples such as placenta are promising for identifying the surrogate marker for exposure assessment. A recent report suggested a good correlation of total PCB in placenta with that in cord blood, maternal blood, and breast milk samples (Wang et al. 2004). Further studies are necessary in order to examine the importance and usefulness of placenta and cord tissues in the assessment of prenatal exposure effects.

Confounders. Despite the major source of MeHg and POPs is via fish intake, fish consumption itself is though to have several beneficial

aspects. Selenium is considered to play an essential role in protection against MeHg toxicity (Watanabe 2002). Fish is usually rich in selenium, and almost 70% of the daily total selenium is through the fish intake in Japan (Miyazaki et al. 2002). However, the bioavailability of fish-derived selenium is still controversial. Fish is also rich in PUFA which may be essential for the normal development of an infant brain (Horwood and Fergusson 1998). However, the beneficial effects of increased amount of PUFA in cord blood on the later developmental period are also still controversial (Bakker et al. 2003). In the present study, these confounding factors including selenium and PUFA were considered from nutritional perspectives in the risk assessment of eating fish.

The results of this cohort study will allow us to evaluate associations between the neurobehavioral development of children and perinatal exposures to MeHg and environmentally POPs in Japan. A recent report from the cohort at Faroe Islands (Murata et al. 2004) indicated that the adverse effects of prenatal exposure to MeHg were still observed in the children at age 14 years by neurophysiological tests, suggesting that some neurotoxic effects from prenatal exposures are irreversible. To clarify this issue, the subjects should be followed until their adolescent ages. The present report describes the study design for children aged 0 to 42 months. When any significant associations between child development and chemical exposures are observed in this study, the further follow-up is essential to know the persistency of adverse effects.

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Maternal and Fetal Mercury and *n*-3 Polyunsaturated Fatty Acids as a Risk and Benefit of Fish Consumption to Fetus

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Maternal fish consumption brings both risks and benefits to the fetus from the standpoint of methylmercury (MeHg) and *n*-3 PUFA (polyunsaturated fatty acids). MeHg is one of the most risky substances to come through fish consumption, and mercury concentrations in red blood cells (RBC-Hg) are the best biomarker of MeHg exposure. Docosahexaenoic acid (DHA, C22:6*n*-3), which is one of the most important fatty acids for normal brain development and function, is also derived from fish consumption. Our objective in this study was to examine the relationships between RBC-Hg and plasma fatty acid composition in mother and fetus at parturition. Venous blood samples were collected from 63 pairs of mothers and fetuses (umbilical cord blood) at delivery. In all cases, fetal RBC-Hg levels were higher than maternal RBC-Hg levels. The geometric mean of fetal RBC-Hg was 13.4 ng/g, which was significantly ($p < 0.01$) higher than that of maternal RBC-Hg (8.41 ng/g). While the average fetal/maternal RBC-Hg ratio was 1.6, the individual ratios varied from 1.08 to 2.19, suggesting considerable individual differences in MeHg concentrations between maternal and fetal circulations at delivery. A significant correlation was observed between maternal and fetal DHA concentrations ($r = 0.37$, $p < 0.01$). Further, a significant correlation was observed between RBC-Hg and plasma DHA in fetus ($r = 0.35$, $p < 0.01$). These results confirm that both MeHg and DHA which originated from fish consumption transferred from maternal to fetal circulation and existed in the fetal circulation with a positive correlation. Pregnant women in particular need not give up eating fish to obtain such benefits. However, they would do well to at least consume smaller fish, which contains less MeHg, thereby balancing the risks and benefits from fish consumption.

Introduction

Methylmercury (MeHg) is a well-known and widespread environmental neurotoxicant. In the natural course of events, most human exposure to MeHg is through fish and sea mammal consumption. Generally, the larger fish and sea mammals at the top of the food chain, such as shark, tuna, and whale, contain higher levels of MeHg than the smaller ones. Fetuses are known to be a high-risk group for MeHg exposure (*1*–*3*) since the susceptibility of the developing brain itself is high (*3*–*5*) and higher MeHg accumulates in cord blood than in maternal blood (*6*–*10*). Therefore, the effect of MeHg exposure on pregnant women remains an important issue for elucidation, especially in populations which consume much fish and sea mammals (*3*, *11*–*14*). Serum or plasma is known as a good biomarker of elementary or mercuric Hg exposure (*15*). On the other hand, mercury concentration in red blood cells (RBC-Hg) is the best biomarker of MeHg exposure (*3*, *16*–*18*). Additionally, more than 90% of that in RBC is known to be in the methyl form in high-fish-consuming populations (*19*). Further, hematocrit (Htc) values are quite different between mother and fetus at parturition (Table 1). Therefore, we used total Hg concentrations not in whole blood but in RBCs to reveal the MeHg levels in mothers and fetuses in the present study.

On the other hand, human intake of the *n*-3 longer chain of polyunsaturated fatty acids (PUFAs), such as eicosapentaenoic acid (EPA, C20:5*n*-3) and docosahexaenoic acid (DHA, C22:6*n*-3), is also known to occur through marine products, mainly from fish consumption. Both of these fatty acids are very beneficial for human health (*20*, *21*). Especially, DHA is known to be an important *n*-3 PUFA for normal brain development and function (*22*–*25*). Rapid brain growth occurs primarily during the third trimester in humans (*26*, *27*), and the amount of these fatty acids increases dramatically during the period (*25*). This period corresponds to when the human brain is most susceptible to MeHg (*27*), and also a high accumulation of MeHg in the brain may occur during the period (*5*).

We conducted a study to determine the relationship between RBC-Hg and plasma fatty acid concentrations in fetus to evaluate the risks and benefits of maternal fish consumption by comparing 63 maternal–fetal pairs of blood samples.

Materials and Methods

Sixty-three healthy Japanese pregnant women, ranging in age from 21 to 41 yr (average 29.6 ± 4.4 yr), planning to deliver in Munakata Suikokai General Hospital, Munakata City, Fukuoka, Japan, gave informed consent to take part in the present trial. Blood samples were collected from the mothers and umbilical cord. The samples included 13 mL of venous umbilical cord blood at birth and 10 mL of venous maternal blood 1 day after parturition before breakfast. Both blood samples were obtained by venipuncture with a small amount of heparin–Na and centrifuged at 3000 rpm for 10 min to separate into RBCs and plasma. Samples were stored at −80 °C until analysis. This study was approved by the Ethics Committee of the National Institute for Minamata Disease (NIMD).

Total Hg in 0.5 g of RBC was determined by cold vapor atomic absorption spectrophotometry (CVAAS) according to the method of Akagi and Nishimura (*29*). The method

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TABLE 1. Subject Characteristics (n = 63) and Values, Including Maternal and Fetal Mercury Concentrations in Red Blood Cells and Hematocrit Values

category	mean (geomean)	SD	min	max
maternal age (yr)	29.6	4.37	21	41
maternal RBC-Hg level (ng/g)	9.12 (8.41)	3.63	3.76	19.1
maternal Htc value	31.5	3.18	23.9	38.4
fetal RBC-Hg level (ng/g)	14.7 (13.4)	6.37	4.92	35.4
fetal Htc value	45.2	3.64	38.1	53.7
fetus/mother RBC-Hg ratio	1.6	0.27	1.08	2.19

involves sample digestion with HNO_3, $HClO_4$, and H_2SO_4 followed by reduction to Hg^0 by $SnCl_2$. The detection limit was 0.1 ng/g. Accuracy was ensured by using certified reference material (DORM-2, dogfish muscle prepared by the National Research Council of Canada) as the quality control material; the Hg concentration found averaged 4.53 μg/g, as compared to the assigned value of 4.64 ± 0.26 μg/g. The total analytical precision of this analysis was estimated to be 3.9%. Fatty acid composition analysis in plasma was performed by SRL Inc. (Tokyo, Japan). Lipid was extracted from the sample according to the method of Folch et al. (*30*), and tricosanoic acid (C23:0) was added as an internal standard and then hydrolyzed with 0.5 M HCl. Free fatty acids were extracted with chloroform, and methylated with 0.4 M potassium methoxide–methanol solution and 14 wt % boron trifluoride–methanol. Fatty acid methyl esters were separated by capillary gas chromatography (GC17A, Shimadzu Co., Japan) and identified by comparison with standards (Sigma Chemical Co., Poole, U.K.). Fatty acid compositions were expressed as concentration (μg/mL of plasma) and percentage by weight of total fatty acids.

Statistics. The differences in RBC-Hg concentrations between paired samples were determined by paired t-tests. The associations between RBC-Hg and plasma fatty acid concentrations were studied by Pearson and Spearman correlation analysis. Each fetal/mother ratio of fatty acid was analyzed by a one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test. Significant differences were compared with the sum of the saturated and monounsaturated fatty acids assumed as a reference value. A p value less than or equal to 0.05 was considered to demonstrate statistical significance.

Results

Table 1 presents the subject characteristics and values, including maternal and fetal mercury concentrations in red blood cells and Htc values. The geometric mean of fetal RBC-Hg at 13.4 ng/g was 1.6 times higher (p < 0.001) than that of maternal RBC-Hg (8.41 ng/g). There was a considerable difference in Htc values between maternal and fetal blood. The mean fetal Htc value (45.2 ± 3.6) was about 1.4 times higher than the maternal level (31.5 ± 3.2). In all 63 cases fetal RBC-Hg levels were higher than maternal RBC-Hg levels. A strong correlation was observed in RBC-Hg between mothers and fetuses (r=0.92, p < 0.001; Figure 1), the average fetal/maternal RBC ratio was 1.6, and the individual ratios varied from 1.08 to 2.19 (Figure 2).

All of the fatty acid concentrations were lower in the fetuses' plasma than in their mothers' plasma (Table 2). However, the fetal/maternal ratio varied with each fatty acid. The ratio for the sum of saturated and monounsaturated fatty acid concentrations was 0.27. The ratios of n-3 and n-6 fatty acid concentrations were compared with the value for the sum of saturated and monounsaturated fatty acids

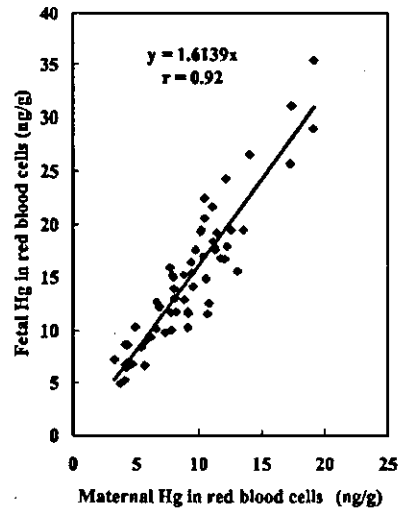

FIGURE 1. Correlation between maternal and fetal mercury concentrations in red blood cells in 63 maternal–fetal pairs. In all 63 cases fetal RBC-Hg levels were higher than maternal RBC-Hg levels. A strong correlation was observed in RBC-Hg between mothers and fetuses (r = 0.92, p < 0.001).

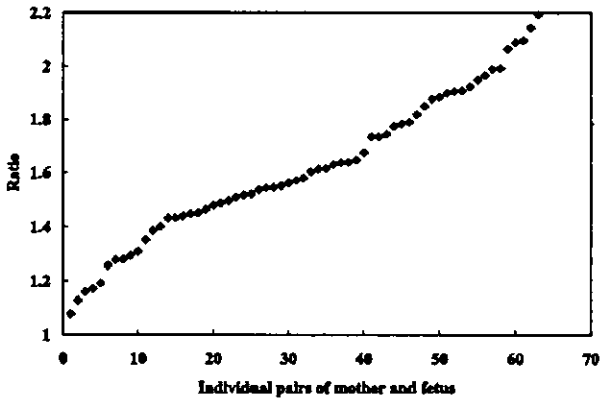

FIGURE 2. Individual fetus/mother ratios of Hg concentrations in red blood cells in 63 maternal–fetal pairs. The average fetal/maternal RBC ratio was 1.6, and the individual ratios varied from 1.08 to 2.19.

assumed as a reference value. The ratios for linoleic acid (LN, C18:2n-6)) and linolenic acid (LnN, C18:3n-3) were significantly (p < 0.01) lower than the value for the sum of saturated and monounsaturated fatty acids. On the other hand, those for arachidonic acid (AA, C20:4n-6), DHA, and dihomo-γ-linolenic acid (DGLA, C20:3n-6) were significantly (p < 0.01) higher than the reference value. Further, there were significant correlations in LN (r = 0.31, p < 0.05), DGLA (r = 0.34, p < 0.01), AA (r = 0.39, p < 0.01), EPA (r = 0.39, p < 0.01), and DHA (r = 0.37, p < 0.01) concentrations between maternal and fetal plasma (Table 2).

Maternal RBC-Hg concentrations showed significant correlation coefficients with maternal plasma EPA (r = 0.36, p < 0.01) and DHA (r = 0.33, p < 0.05) concentrations (Table 3). Further, fetal RBC-Hg concentrations showed a significant positive correlation with fetal plasma EPA (r = 0.32, p < 0.05) and DHA (r = 0.35, p < 0.01) (Table 3 and Figure 3).

Discussion

MeHg is one of the most risky substances to fetus brain, and most of the human exposure to MeHg is through maternal fish consumption. On the other hand, DHA, which is important for the fetus brain and its growth, is derived also from maternal fish consumption. If human exposure to MeHg were independent of nutrition from fish, we would aim at

TABLE 2. Comparison of Maternal and Plasma Fatty Acid Concentrations and Correlation Coefficient between Maternal and Fetal Plasma Fatty Acid Concentrations in 63 Maternal–Fetal Pairs[a]

fatty acid	mother ($n = 63$) μg/mL	mother ($n = 63$) %	fetus ($n = 63$) μg/mL	fetus ($n = 63$) %	fetus/mother	correlation coeff
saturated and monounsaturated	2361 (476)	61.6	619 (156)	65.0	0.273 (0.08)	
linoleic (C18:2*n*-6)	1013 (177)	26.4	118 (34)	12.4	0.118 (0.03)[b]	0.31[c]
linolenic (C18:3*n*-3)	30 (9)	0.8	1.1 (1.3)	0.1	0.070 (0.04)[b]	0.23
dihomo-γ-linolenic (C20:3*n*-6)	55 (17)	1.4	33 (9.2)	3.5	0.628 (0.20)[b]	0.34[b]
arachidonic (C20:4*n*-6)	187 (45)	4.9	115 (29)	12.0	0.637 (0.16)[b]	0.39[b]
eicosapentaenoic (C20:5*n*-3)	35 (19)	0.9	7.1 (3.7)	0.7	0.308 (0.13)	0.39[b]
docosahexaenoic (C22:6*n*-3)	149 (41)	3.9	59 (16)	6.2	0.417 (0.12)[b]	0.37[b]

[a] Saturated = palmitic (C16:0) and stearic (C18:0); monounsaturated = palmitoleic (C16:1*n*-7) and oleic (C18:1*n*-9). Values are mean (SD). Fetal/mother plasma fatty acid concentration ratio for *n*-3 and *n*-6 series against the sum of saturated and monounsaturated fatty acids as a reference value. Data were analyzed by a one-way ANOVA followed by Dunnett's multiple comparison test. The associations between maternal and fetal fatty acids were studied by correlation analysis. [b] $p < 0.01$. [c] $p < 0.05$.

TABLE 3. Correlation Coefficient between Mercury Concentrations in Red Blood Cells and Fatty Acids in Both Mothers and Fetuses[a]

		maternal mercury	fetal mercury
maternal ($n = 63$)	linoleic (C18:2*n*-6)	−0.09	−0.10
	linolenic (C18:3*n*-3)	0.23	0.19
	dihomo-γ-linolenic (C20:3*n*-6)	−0.20	−0.01
	arachidonic (C20:4*n*-6)	−0.05	−0.14
	eicosapentaenoic (C20:5*n*-3)	0.24	0.17
	docosahexaenoic (C22:6*n*-3)	0.27[c]	0.21
fetal ($n = 63$)	linoleic (C18:2*n*-6)	−0.06	−0.04
	linolenic (C18:3*n*-3)	0.06	0.10
	dihomo-γ-linolenic (C20:3*n*-6)	−0.08	−0.08
	arachidonic (C20:4*n*-6)	−0.23	−0.20
	eicosapentaenoic (C20:5*n*-3)	0.36[b]	0.32[c]
	docosahexaenoic (C22:6*n*-3)	0.33[b]	0.35[b]

[a] The associations between maternal and fetal fatty acids were studied by correlation analysis. [b] $p < 0.01$. [c] $p < 0.05$.

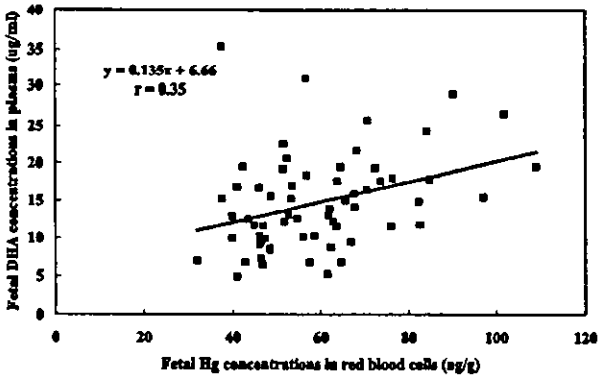

FIGURE 3. Correlations between RBC-Hg concentrations and plasma DHA concentrations in 63 fetuses.

zero exposure. However, the exposure is through fish, which is an important source of protein especially for Japanese and other Asian people. Fish also contain *n*-3 PUFA and other nutrients (*31*). Therefore, this study was designed mainly to determine the relationship between MeHg exposure and *n*-3 PUFA concentrations in fetus to consider the risks and benefits of maternal fish consumption during the gestation period.

The RBC-Hg level in umbilical cord blood was about 1.6 times higher than those in the mothers, and there was a significant correlation between them. This suggests that MeHg actively transfers to the fetus across the placenta via a neutral amino acid carrier, as demonstrated by previous studies (*9, 10*). This higher Hg accumulation in the fetuses than in mothers is widely acknowledged from human and animal studies (*1–3, 8–10*). However, the individual fetal/maternal RBC-Hg ratio varied from 1.08 to 2.19, indicating the individual differences in MeHg concentrations between maternal and fetal circulations at late gestation. This will be partly explained by the individual differences in MeHg transfer from mother to fetus through the placenta. The maternal MeHg level tends to be influenced by the latest meal. On the other hand, blood/organ ratios of MeHg concentration will be settled at parturition in fetal circulation. The results suggest that not the maternal side biomarker but the fetal side biomarker is much more advantageous to evaluate the subtle effects of MeHg exposure on the fetus during gestation.

DHA and AA are abundant in the brain (*22–25, 28*), and the DGLA concentration is higher than those of LN, EPA, and LnN (*22*). During rapid brain growth, large amounts of DHA and AA from maternal circulation must reach the fetus to meet its needs for development (*23, 25, 33*). The rapid quantitative accretion of both DHA and AA during the third trimester of pregnancy was noticed in human brain (*25, 28, 33*). Breast milk also contains these fatty acids (*22, 33*). The result of the high fetal/maternal ratio of DHA, AA, and DGLA (Table 2) also may indicate that the fatty acids which are important for the brain and its growth were selectively transferred from maternal circulation to fetal circulation, as was demonstrated in the previous study by Sakamoto and Kubota (*34*).

There were significant correlations in the EPA ($r = 0.39$, $p < 0.01$) and DHA ($r = 0.37$, $p < 0.01$) concentrations between maternal and fetal plasma (Table 2), indicating that EPA and DHA in fetal circulation which originated from fish consumption reflected the existence of these fatty acids in maternal circulation. Maternal RBC-Hg concentrations had significant correlation coefficients with both fetal plasma ($r = 0.36$, $p < 0.01$) and DHA ($r = 0.33$, $p < 0.01$) levels; further, fetal RBC-Hg concentrations had significant correlation coefficients with both fetal plasma EPA ($r = 0.32$, $p < 0.05$) and DHA ($r = 0.35$, $p < 0.01$) levels, indicating that both the MeHg and these *n*-3 PUFAs existing in fetal circulation showed a positive correlation (Table 3 and Figure 3). This is, to our knowledge, the first report to indicate significant correlation coefficients between the MeHg level and these fatty acids originating from fish consumption. These two results indicate that both MeHg and DHA, which act contrary to the normal growth and function of the developing brain, were taken into maternal circulation through maternal fish consumption and transfer to fetal circulation, and that they showed positive correlations. Therefore, if the ordinary fish consumed are low in MeHg but rich in DHA, children's health will especially benefit from fish consumption. However, if the fish MeHg concentration is high enough to ruin the effect

of DHA, fish consumption will retard children's development. Pregnant women in particular would do well to consume at least smaller fish, thereby reducing the risk from large fish but allowing them to continue to eat them to confer the benefits. The different outcomes of the two main cohort studies in the Faroe Islands (*11*) and Seychelles Islands (*13*) regarding the effect of fetal MeHg exposure on children's development may be partly explained by the difference in the amount of DHA. However, the average MeHg exposure level was slightly higher in the Seychelles Islands than in the Faroe Islands. The Seychelles study (*13*) concluded there was no adverse effect from MeHg exposure through fish consumption, whereas the Faroe Islands study (*11*) demonstrated a negative developmental effect due to MeHg exposure. In any event, DHA concentrations in the fetal biomarker should be measured as a confounding factor to examine the subtle effects of MeHg exposure from fish consumption.

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A Cohort Study of Effects of Perinatal Exposures to Methylmercury and Environmentally Persistent Organic Pollutants on Neurobehavioral Development in Japanese Children: Study design and status report

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Abstract: Adverse effects of perinatal exposures to methylmercury (MeHg) and environmentally persistent organic pollutants (POPs) have been apparent from several birth cohort studies, but little is known about the hazardous effects in Japanese, whose fish consumption is high. The present study was designed to examine the effects of perinatal exposures to MeHg, polychlorinated biphenyls (PCB), dioxins, pesticides, and other chemicals in Japanese children. Six hundred eighty-seven pregnant women were participated in this study with their written informed consent. Maternal peripheral blood, cord blood, cord tissue, placenta, and breast milk samples were collected for chemical analysis. Maternal hair was also taken for MeHg analysis. Infants born at full term were assessed by a battery of neurobehavioral tests. The children will be continuously followed up to ages 6-7. The results of this cohort study will allow us to evaluate associations between the neurobehavioral development of children and perinatal exposures to MeHg and environmentally POPs in Japan.

Key words: epidemiology, methylmercury, pregnant women

INTRODUCTION

The neurobehavioral effects of prenatal exposures to methylmercury (MeHg) and environmentally persistent organic pollutants (POPs) including polychlorinated biphenyls (PCBs), dioxins, and pesticides are of great concern worldwide (NAKAI, 2002). It was shown that prenatal MeHg exposure causes the delay of development of cognitive functions in Faroe Islands (GRANDJEAN, 1997), although studies conducted in the Seychelles showed the absence of toxic effects of prenatal exposures to MeHg (DAVIDSON, 1998). Several epidemiological studies have also shown the evidence of the adverse effects of perinatal PCB exposure on neurodevelopment.

In this report we present a protocol of our cohort study, the Tohoku Study of Child Development, of the effects of perinatal exposures to MeHg and POPs on neurobehavioral development among Japanese children (NAKAI, 2004). We hypothesize that the prenatal/postnatal exposures to the above chemicals delay or disturb the normal growth and neurobehavioral development of children.

STUDY DESIGN

Healthy pregnant women were recruited with their informed consent at obstetrical wards of two hospitals in Tohoku, Japan. To establish an optimal study population, only infants born at term (36 to 42 weeks of gestation) without congenital anomalies or diseases are included. Pregnancy and delivery should have been completed without overt signs of serious illness or complications. The study protocol was approved by the Medical Ethics Committee of the Tohoku University Graduate School of Medicine.

The hair samples were collected from the mothers after delivery. Maternal peripheral blood samples were collected at 28 weeks of pregnancy. They were centrifuged within 4 hours for 20 minutes at 3000 rpm; plasma and whole blood were stored at –80 °C until analysis. A blood sample from the umbilical cord was collected into a bottle using heparin as the anticoagulant after the delivery. Placenta and cord tissues were also collected after the delivery. The mothers were finally asked to provide a sample of breast milk one month after the delivery.

Questionnaire. Several types of questionnaire were administered after the delivery. To assess the fish-intake and the general nutrition status of the mothers a food-intake frequent questionnaire (FFQ) for 122 individual foods and recipes, and some additional items regarding seafood was administered. This is a standardized FFQ that enables the assessment of the intake of not only major nutrients but also several essential nutrients including retinol and folic acid in the Japanese population. Other questionnaires were administered with the following items: educational background, occupation, income, smoking habit including passive smoking, alcohol consumption during pregnancy, hair treatments including bleaching, permanent wave and coloring, and dental amalgam treatment.

Neurodevelopment assessment. All testers who performed neurodevelopment assessments were not informed of exposure information including alcohol consumption/ smoking habit, FFQ data, and feeding method. The Brazelton Neonatal Behavioral Assessment Scale (NBAS) was administered when the infants were 3 days old. Cognitive functions of the infants at 7 months old were evaluated using the Bayley Scale of Infant Development, second edition (BSID), the Kyoto Scale of Psychological Development (KSPD), and the Fagan Test of Infant Intelligence (FTII). BSID and KSPD were also used for the assessment of neurobehavioral development when the children were 18 months old. The Japanese version of Kaufman Assessment Battery for Children (K-ABC) was employed to assess the development and intelligence of children when they are 42 months old. The growth and development of the children will be followed up until they are 6-7 years old.

Chemical determinations. Total mercury analysis was carried out by cold vapor atomic absorption spectrometry. Briefly, without washing the hair samples, each sample was acid digested with HNO_3, $HClO_4$ and H_2SO_4 at 200 °C for 30 minutes. The resultant ionic mercury was then reduced to mercury vapor by tin chloride to a flameless atomic absorption monitor (HG-201, Sanso Co., Ltd., Tokyo). Analytical accuracy was ensured by analyzing the Human Hair Reference Material NIES CRM No. 13 from the National Institute of Environmental Studies (Lot #650, Tsukuba). In fish-eating populations, total mercury in hair consists mostly of MeHg (more than 95 %).

Assessment of PCB exposure was performed by determining PCB levels in cord blood, placenta, breast milk, and maternal blood. All 209 PCB congeners were analyzed by high-resolution gas chromatography/high-resolution mass spectrometry (HRGC/HRMS) using the isotope dilution method.

A reporter gene assay of the toxic potency of dioxins and related chemicals was used for the assessment of dioxins. The CALUX (Chemically Activated LUciferase gene eXpression) assay was developed by Xenobiotic Detection Systems (XDS, NC, USA) using a patented recombinant mouse cell line that contains the luciferase reporter gene under the control of dioxin-responsive elements. This assay has several advantages including its high sensitivity, easy pretreatment, and rapid determination, in comparison with HRGC/HRMS.

Cadmium and lead were determined by graphite furnace atomic absorption spectrometry and inductively coupled plasma mass spectrometry, respectively, after samples were digested in a microwave oven with ultrapure nitric acid. Other major biochemical analyses of maternal and cord blood samples included those of plasma selenium and thyroid hormones. Selenium was determined fluorometrically. The assay of TSH, and total/free T4 and T3 were performed using a radioimmunoassay technique.

Potential confounders/covariates. The quality of the home environment was assessed using a questionnaire, the Evaluation of Environmental Stimulation (EES), which has been established in Japan modified after the Home Observation for Measurement of the Environment (HOME) score. The parental socioeconomic status (SES) was rated using the Hollingshead Four Factor Index of Social. Other major potential confounders included were as follows: intelligence quotient by the Raven standard progressive matrices, age at examination (days), gestational age (weeks), and alcohol consumption/smoking habits during pregnancy for the mothers, and the Apgar score, neonatal illness/jaundice, delivery type, parity, chronic diseases, and duration of breastfeeding (months) for the infants.

RESULTS AND DISCUSSION

The present report describes the study design and protocol for the prospective cohort study of the effects of prenatal exposures to MeHg and other environmentally POPs on neurobehavioral development in Japanese children. To our knowledge, this is the first cohort study that examines these hazardous risks to children in Japan.

We recruited 687 healthy pregnant women between January 2000 and September 2003. Although the final number of babies registered in this study is not yet determined because the delivery of pregnant women registered in this study is ongoing, the percentage of babies fulfilling the criteria for inclusion with the mothers' consent to participate in the assessment using NBAS was 85 %. The percentage of babies participating in the next assessment at 7 months old was 86 % of those participating in the assessment using NBAS. This reduction was mainly due to family relocation to other places.

The results of this cohort study will allow us to evaluate associations between the neurobehavioral development of children and prenatal exposures to MeHg and environmentally POPs in Japan. A recent report from the cohort at Faroe Islands (MURATA ET AL., 2004) indicated that the adverse effects of prenatal exposure to MeHg were still observed in the children at age 14 years by neurophysiological tests, suggesting that some neurotoxic effects from prenatal exposures are irreversible. To clarify this issue, the subjects should be followed until their adolescent ages. The present report describes the study design for children aged 0 to 42 months. When any significant associations between child development and chemical exposures is observed in this study, the further follow-up is essential to know the persistency of adverse effects.

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Organochlorine Pesticide Residues in Human Breast Milk and Placenta in Tohoku, Japan

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Introduction

Organochlorine pesticides are compounds widespread in the environment due to their persistence and highly lipophilic nature, and they accumulate in biological systems. Newborns are exposed to these organochlorine compounds across the placenta and through breastfeeding. Perinatal exposure to these compounds may induce several adverse effects such as lower birth weight [1], neurodevelopmental delay [2], and disturbance of thyroid hormone status [3]. DDT, especially, has been suggested to be a neuroendocrine disruptor as well as a functional teratogen in humans [4 5]. Other pesticides such as dieldrin and endosulfan were also recognized to have estrogenic hormonal activity in animal studies.

Recently, we have started a birth cohort study to examine the effects of exposure to persistent organochemical pollutants and heavy metals on neurodevelopment in Japanese children, The Tohoku Study of Child Development [6]. In this cohort study, biological samples, including maternal peripheral blood, cord blood, placenta, cord tissue, and breast milk have been collected from more than six hundred mother-infant pairs for chemical determinations. The growth of infants has been monitored using neurodevelopmental tests, including the Brazelton Neonatal Behavioral Assessment Scale, the Bayley Scale of Infant Development, the Kyoto Scale of Psychological Development, and others. Exposures to dioxin and related compounds, polychlorinated biphenyls, methylmercury, and several heavy metals were assessed. Additionally, since perinatal exposure to organochlorine pesticides may affect the neurodevelopment of children, we examined the effects of those pesticides in the cohort study.

In the present study, several organochlorine pesticides were analyzed in human breast milk and placenta from 20 mothers to identify the major pesticide compounds found in the cohort subjects. The relationship between pesticides in breast milk and the placenta was analyzed to examine the utilization of the placenta as the material for exposure assessment. Some information regarding the factors affecting the contamination of breast milk and the placenta with organochlorine pesticides

are also discussed.

Methods and Materials

This study was performed as part of our prospective cohort study [6]. Healthy pregnant women were recruited with their informed consent at obstetrical wards of two hospitals in Tohoku between January 2001 and September 2003. Twenty subjects were randomly selected from the registered subjects of the cohort study, and pairs of breast milk samples and placenta samples were used. The ages of mothers ranged from 21 to 39. The placenta was taken immediately after the delivery, and divided into 20-30 pieces that were randomly separated into 4 groups. Each bottle contained 50-100 g of tissue. The representative samples were finally prepared by homogenization. The mothers were asked to provide breast milk one month after the delivery. The breast milk sample was taken directly into a clean glass bottle. These samples were frozen at –80°C until analysis. Each mother completed a questionnaire to provide personal information such as the number of births, smoking, alcohol consumption during pregnancy, occupation, educational background, food intake, and place of residence. The study protocol was approved by the Medical Ethics Committee of the Tohoku University Graduate School of Medicine.

The pesticides examined were hexachlorobenzene (HCB), α-hexachlorocyclohexane (HCH), β-HCH, γ-HCH, δ-HCH, cis-chlordane, trans-chlordane, oxy-chlordane, cis-nonachlor, trans-nonachlor, p,p'-DDT, o,p'-DDT, p,p'-DDE, o,p'-DDE, p,p'-DDD, o,p'-DDD, aldrin, endrin, dieldrin, α-endosulfun, β-endosulfun, heptachlor, heptachlorepoxide, and methoxychlor. Gas chromatographic determination of these organochlorine pesticides was performed with the collaboration of SRL, Inc. (Tokyo, Japan) for sample extraction and Toray Research Center (Tokyo, Japan) for gas chromatography. Briefly, after the samples were spiked with $^{13}C_6$-HCB, $^{13}C_6$-β-HCH, $^{13}C_{12}$-p,p'-DDT, $^{13}C_{12}$-endosulfun, and $^{13}C_{10}$-chlordane, they were extracted with ethanol/hexane. The organic extracts were finally purified with the use of a Florisil column, and the eluates were concentrated and spiked with $^{13}C_{12}$-pentaPCB(#118). A mass spectrometer (AutoSpec, Micromass) coupled to a Hewlett-Packard model HP6800 capillary gas chromatograph equipped with a capillary column (BPX-35, 0.25 mm ID x 25 m, film thickness 0.33 μm, SGE) was used for determination of pesticides. Residue levels were expressed as ng/g extracted fat.

Results and Discussion

HCB, β-HCH, oxy-chlordane, cis-nonachlor, trans-nonachlor, p,p'-DDT, p,p'-DDE, dieldrin, and heptachlorepoxide were found from all breast milk samples and placenta samples as shown in Table 1, whereas levels of α-HCH, γ-HCH, δ-HCH, cis-chlordane, trans-chlordane, o,p'-DDT, o,p'-DDE, p,p'-DDD, o,p'-DDD, aldrin, endrin, α-endosulfun, β-endosulfun, heptachlor, and methoxychlor were very low or below the detection limit (data not shown). Since using of these organochlorine compounds had been prohibited in the field in the 1970-1980s in Japan, these results reconfirmed their environmentally persistent nature. In Japan, the concentrations of PCBs, β-HCH, and DDTs in breast milk declined gradually from the peak levels observed at the mid-1970s and almost reached equilibrium states [7]. However, it remains to be elucidated whether the current low levels of organochlorine pesticides affect the neurodevelopment of children.

The concentration of organochlorine pesticides in breast milk mainly depends on their accumulation in the maternal fatty tissue and their subsequent mobilization. Indeed, numerous studies around the world have used human breast milk samples to determine maternal body burden and lactational transfer of pesticides to infants. Since there were excellent correlations of all major pesticides between breast milk samples and placenta samples (Table 1, and the two typical relationships in Fig. 1), placenta is also suggested to be the useful material to estimate the maternal body burden. In addition, the concentrations of some organochlorine pesticides such as HCB, oxy-chlordane, and trans-nonachlor, in the placenta samples had significant negative correlations with parity (Table 2). This finding clearly shows that the mothers eliminate these pesticides during pregnancy and by breastfeeding them into their children. Considering that the concentration of pesticides in breast milk samples had no significant correlation with parity, monitoring of the placental pesticide concentration may contribute to determining the prenatal exposure of infants to organochlorine pesticides. The placenta is a relatively large organ, and is usually discarded after delivery. Utilization of the placenta is possibly suggested for the purpose of assessment of exposure to chemicals.

Table 1: Organochlorine pesticide concentrations in the human milk samples and placenta samples, and the relationship between the 2 samples.

Pesticide	Milk (ng/g-fat)	Placenta (ng/g-fat)	Correlation Coefficient Milk x Placenta
Hexachlorobenzene	17.1±10.1	9.9±4.1	0.693**
β-HCH	83.4±55.1	21.5±12.6	0.919**
oxy-Chlordane	7.2±3.4	2.3±0.9	0.644**
cis-Nonachlor	3.7±1.7	0.8±0.4	0.589**
trans-Nonachlor	18.8±8.6	3.8±2.2	0.679**
p,p'-DDT	6.2±3.5	1.4±0.6	0.746**
p,p'-DDE	142.3±73.5	46.0±34.6	0.569**
Dieldrin	5.0±3.6	1.7±1.1	0.808**
Heptachlorepoxide	3.7±1.4	1.4±0.3	0.881**

Spearman's correlation analysis, $^{**}p<0.01$, $^{*}p<0.05$

Table 2: Correlation coefficient values of organochlorine pesticides with fish intake, maternal age at delivery, and parity.

Pesticide	Fish consumption		Maternal age		Parity	
	Milk	Placenta	Milk	Placenta	Milk	Placenta
Hexachlorobenzene	0.023	-0.127	-0.421	-0.223	-0.429	-0.625**
β-HCH	0.064	0.034	0.244	0.375	0.025	-0.064
oxy-Chlordane	0.609**	0.515*	-0.208	0.033	-0.428	-0.521*
cis-Nonachlor	0.486*	0.356	-0.085	0.234	-0.093	-0.354
trans-Nonachlor	0.701**	0.475*	-0.133	0.155	-0.282	-0.471*
p.p'-DDT	0.341	0.267	-0.179	0.06	-0.053	0.089
p.p'-DDE	0.054	0.412	-0.165	0.169	-0.174	-0.131
Dieldrin	0.463*	0.518*	-0.109	0.004	0.12	0.033
Heptachlorepoxide	0.566**	0.711**	-0.185	-0.169	-0.054	-0.235

Spearman's correlation analysis, $^{**}p<0.01$, $^{*}p<0.05$

Some organochlorine pesticides have been thought to be introduced to humans partly through the consumption of fish and related products [8]. The concentrations of oxy-chlordane, nonachlors, dieldrin, and heptachlorepoxide in breast milk samples and placenta samples were indeed correlated with fish consumption; however, HCB, HCH, and DDE had no association. These results indicated that the contribution of fish consumption to the intake of pesticides was dependent on the kind of pesticide. More information regarding risk analysis of pesticide intake is needed for risk management. Maternal age at the time of delivery and parity have been shown to be important factors affecting the concentration of pesticides in breast milk samples [8]. Although parity was a potent factor in our data (Table 2), maternal age had no significant relationship with the concentrations of pesticides in breast milk samples and placenta samples. However, since parity correlated significantly with maternal age (data not shown), multiple regression analysis should be performed to control for the effects of covariates. These issues, and identification of the factors affecting the contamination levels of organochlorine pesticides in breast milk and placenta will be readdressed when we increase the sample size.