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Table 3. Relationship of POPs with maternal age, gestational age and concentrations of TSH and thyroid hormones

	Maternal age	Gestational age	TSH and thyroid hormones					
			Maternal blood			Cord blood		
			TSH	T4	T3	TSH	T4	T3
Breast milk (n=68, except for n=67 for maternal TSH/T4/T3)								
TEQ	ns	ns	0.232§	ns	0.225§	ns	-0.224§	ns
PCB	ns	ns	0.205§	0.231§	0.257*	ns	ns	ns
trans-Nonachlor	ns	ns	ns	ns	0.281*	ns	-0.238§	ns
p,p'-DDE	ns	ns	ns	ns	ns	ns	ns	ns
p,p'-DDT	ns	ns	ns	ns	ns	ns	ns	ns
Dieldrin	0.229§	-0.265*	ns	ns	0.235§	ns	ns	ns
cis -Heptachlorepoxide	ns	ns	ns	ns	ns	0.243*	-0.212§	ns
HCB	ns	ns	ns	ns	ns	ns	-0.228§	ns
β-HCH	ns	ns	ns	ns	ns	ns	ns	ns
Mirex	0.422**	-0.232§	0.234§	ns	ns	0.233§	ns	ns
Parlar-26	ns	ns	ns	0.291*	0.395**	ns	ns	ns
Parlar-50	ns	ns	ns	0.287*	0.381**	ns	ns	ns
Maternal blood (n=49, except for n=48 for maternal TSH/T4/T3)								
TEQ	ns	ns	0.264§	0.272§	0.375**	ns	ns	ns
PCB	ns	ns	0.277§	0.287*	0.402**	ns	ns	ns
trans-Nonachlor	ns	ns	0.274§	ns	0.429**	ns	-0.243§	ns
p,p'-DDE	ns	ns	0.242§	ns	0.252§	ns	ns	-0.251§
p,p'-DDT	ns	ns	ns	0.299*	0.426**	ns	-0.279§	ns
Dieldrin	0.351*	ns	ns	ns	0.247§	ns	ns	ns
cis-Heptachlorepoxide	ns	ns	ns	ns	ns	ns	ns	ns
HCB	ns	ns	0.251§	0.310*	0.391**	ns	ns	ns
β-HCH	ns	ns	ns	ns	0.267§	ns	ns	ns
Mirex	0.391**	-0.251§	0.342*	ns	0.324*	0.284*	ns	ns
Parlar-26	ns	ns	ns	0.267§	0.468**	0.261§	ns	ns
Parlar-50	ns	ns	ns	0.296*	0.500**	0.269§	ns	ns
Cord blood (n=68, except for n=67 for maternal TSH/T4/T3)								
TEQ	ns	ns	0.203§	ns	ns	ns	-0.270*	ns
PCB	ns	ns	0.239§	ns	0.240§	ns	ns	ns
trans-Nonachlor	ns	ns	0.238§	ns	0.244*	ns	-0.217§	ns
p,p'-DDE	ns	ns	ns	ns	ns	ns	-0.208§	ns
p,p'-DDT	ns	ns	0.206§	ns	ns	ns	-0.238§	ns
Dieldrin	ns	-0.237§	ns	ns	ns	ns	-0.302*	-0.260*
cis -Heptachlorepoxide	ns	-0.278*	ns	ns	ns	0.262*	-0.335**	-0.208§
HCB	ns	ns	ns	ns	ns	0.202§	-0.293*	ns
β-HCH	0.206§	ns	ns	ns	ns	ns	ns	ns
Mirex	0.294*	-0.239§	0.234§	ns	ns	ns	ns	ns
Parlar-26	ns	-0.237§	ns	ns	0.300*	ns	ns	ns
Parlar-50	ns	-0.220§	ns	ns	0.324**	ns	ns	ns

Pearson's r after log-transformed. § $p<0.1$, * $p<0.05$, ** $p<0.01$

THE BIOLOGICAL MONITORING PROGRAM OF PERSISTENT ORGANIC POLLUTANTS IN JAPAN: 2. CONCENTRATIONS OF DIOXINS AND POLYCHLORINATED BIPHENYLS IN BREAST MILK, CORD BLOOD AND MATERNAL BLOOD

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Abstract

Persistent organic pollutants (POPs) such as dioxins and polychlorinated biphenyls (PCBs) are bioaccumulative chemical toxins that are resistant to degradation. POPs are thought of as hazardous contaminants. The Ministry of the Environment of Japan (MOE) has been conducting environmental monitoring of POPs since FY2002 on the basis of the Stockholm Convention on POPs. Since we provided some biological samples for the POPs biological monitoring project, we reanalyzed the report from the MOE. In this presentation, we summarize the data on dioxins and PCBs in human pair samples of breast milk, cord blood and maternal blood. We also analyze the associations of the concentrations of these compounds with thyroid-stimulating hormone (TSH) and thyroid hormones in maternal and cord blood, since disruption of the hypothalamus-pituitary-thyroid axis is a hypothetical mechanism for dioxin- and PCB-induced adverse effects. Concentrations of dioxins and PCBs in each biological sample were at levels similar to those in previous reports on Japanese, and high correlations among the three biological samples were observed. Furthermore, single regression analysis showed a statistically significant correlation of dioxins and PCBs with TSH and thyroid hormones such as total thyroxine (T4) and triiodothyromine (T3).

Introduction

POPs such as polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and PCBs are bioaccumulative chemical toxins that are resistant to degradation. Generally, the main source of exposure to POPs for the general population is thought to be food because the physicochemical attributes of POPs such as lipophilicity and persistence cause bioaccumulation in the food chain, and consequently they can be found in humans at considerable concentrations. Although these concentrations tend to decrease and to be at the background level in industrialized nations, long-term exposure may cause potential risks to human health.

In humans, POPs have been claimed to possess endocrine-disrupting potency. Dioxins, expressed as toxic equivalent (TEQ) levels, were correlated significantly with lower T3 and T4 levels in maternal blood, and with higher blood concentrations in breast-fed infants[1]. There was a significant negative association between dioxin concentrations in breast milk and total T4 in the blood of breast-fed infants[2]. On the other hand, higher environmental background exposure to dioxins significantly increased the T4 concentration in the blood of infants[3]. These findings suggest that exposure to dioxins may affect the hypothalamus-pituitary-thyroid axis. 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. In particular, human perinatal exposure to PCBs has been shown to be associated with immunological changes[4], neural and developmental changes[5, 6], lower psychomotor development[7, 8], defects of short-term memory and spatial learning ability[9] and lower cognitive development[10]. Therefore, monitoring and epidemiological verification of exposure to POPs are necessary to assess the health risks to the Japanese population.

In Japan, the MOE has been conducting the POPs monitoring project[11] since FY2002 for the monitoring of chemicals in each of the environmental media and to obtain data that can contribute to effective evaluations in the Stockholm Convention on POPs. Recently the biological monitoring of human samples was added. We have been collaborating with the POPs biological monitoring project of the MOE by providing biological samples

from our prospective birth cohort study, The Tohoku Study of Child Development (TSCD)[12]. We reanalyzed the results and summarized the data of dioxins and PCBs in human pair samples of breast milk, cord blood and maternal blood[13]. We also analyzed the associations of the concentrations of these compounds with TSH and thyroid hormones in maternal and cord blood, since the disruption of the hypothalamus-pituitary-thyroid axis is a hypothetical mechanism for dioxin- and PCB-induced adverse effects.

Materials and Methods

The biological samples analyzed were randomly selected from the participants in the TSCD, and provided anonymously to the MOE. These samples were measured by IDEA Consultants, Inc. (Tokyo, Japan) as part of the MOE project. This study protocol was previously reported[12]. Briefly, the 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. The breast milk was collected one month after delivery, and then frozen similarly. The TSCD was approved by the Medical Ethics Committee of the Tohoku University Graduate School of Medicine, and all mothers provided signed informed consent.

Chemical analysis was conducted following the methods in the environmental monitoring report on persistent organic pollutants (POPs) in Japan 2002-2004[14]. Briefly, the biological samples were spiked with ^{13}C-labeled POPs as internal standards before extraction. The samples were extracted with liquid-liquid extraction and then extracts were purified by multilayer silica gel column chromatography. Active carbon dispersed silica gel column chromatography was further used for purification of PCDD/Fs and dioxin-like PCBs (DL-PCBs). For the other POPs, extracts were purified by Florisil column chromatography except for silica gel column chromatography for toxaphene. Congener-specific determination of the compounds was performed by high resolution gas chromatograph-high resolution mass spectrometry (HRGC-HRMS) or negative ion chemical ionization mass spectrometry (GC-NICIMS) for toxaphene by isotope dilution quantification. Although control samples were analyzed for every 9-sample batch, they did not contain significant amounts of the target compound. 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..

Results and Discussion

Concentrations of TEQ and total PCBs in breast milk, cord blood and maternal blood are shown in Table 1. TEQ was calculated by the WHO (1998) toxic equivalency factor[15] (TEF) assuming that the amount of congeners below the determination limit was zero. These data were roughly in agreement with previous studies[16-21]. In these biological samples, TEQ and PCB levels in breast milk were higher than in cord blood and maternal blood. Concentrations of TEQ and total PCBs among the three biological samples showed high correlations (Figure 1). Therefore, to predict the concentrations of dioxins and PCBs for the purpose of biological monitoring, it might be useful to measure the concentrations in breast milk. The homologue pattern of PCBs in breast milk was similar in composition to those of cord blood and maternal blood. The predominant homologues in the biological samples were HxCBs, followed by HpCBs, PeCBs and TeCBs.

The correlations between TEQ and PCBs in breast milk, cord blood and maternal blood were very high (Table 2). It was found that the contribution ratio of DL-PCBs to total TEQ was about 40% and the percentage of the DL-PCB concentration in total PCBs was 10% by the congener-specific analysis of PCBs in these biological samples. Because of the high correlation between TEQ and PCBs, the levels of exposure to dioxins for the population could be estimated from the results of PCB measurements. We may be able to simplify the monitoring method by eliminating the determination of dioxins from the analytical procedures.

The working hypothesis is that dioxins and PCBs cause adverse effects via disruption of thyroid hormone regulation and metabolism. Indeed, as shown in Table 3, there were significant correlations of PCBs with T3, and T4 in maternal blood ($p<0.05$). Similarly, there were correlations of TEQ and PCBs with several thyroid function indicators in breast milk and cord blood ($p<0.05$). Although the exact mechanisms by which dioxins and PCBs affect the levels of TSH and thyroid hormones are not fully understood, these results suggest that exposure to dioxins and PCBs could cause hormonal disturbance of thyroid function.

Table 1. Concentrations of TEQ (pg-TEQ/g-fat) and PCBs (ng/g-fat) in breast milk, cord blood and maternal blood

Compound names	Breast milk Median (Min - Max)	Cord blood Median (Min - Max)	Maternal blood Median (Min - Max)
Dioxins			
PCDD/Fs-TEQ	9.9 (2.0-25)	5.4 (0.28-16)	8.6 (2.8-26)
DL-PCBs-TEQ	6.8 (2.1-21)	2.9 (0.74-7.3)	4.8 (1.4-11)
Total TEQ	17 (4.2-45)	8.3 (1.1-22)	13 (4.8-33)
PCBs			
Total PCBs	102 (31-274)	40 (12-128)	76 (20-163)

n=68 for breast milk and cord blood, n=49 for maternal blood.
TEQ was calculated by the WHO (1998) TEF assuming that the amount of congeners below the determination limit was zero.

Acknowledgments

We thank all the families who participated in the study. This presentation was based on the environmental monitoring report on POPs from the MOE, with support in part from the Japan Ministry of Health, Labour and Welfare (Research on Risk of Chemical Substances) and Japan Ministry of Education, Culture, Sports, Science and Technology (Grant-in-Aid for Scientific Research (B)).

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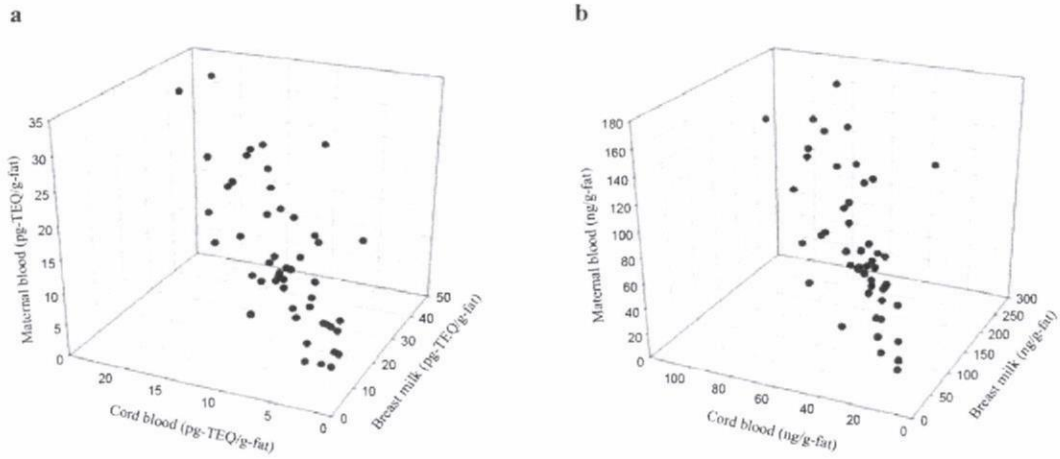

Fig. 1 Relationship of TEQ (a) and PCBs (b) among pair samples of breast milk, cord blood and maternal blood.

Table 2. Pearson correlation coefficients of TEQ and PCBs in breast milk with those of cord blood and maternal blood

	Breast milk		Cord blood		Maternal blood	
	TEQ	PCBs	TEQ	PCBs	TEQ	PCBs
Breast milk						
TEQ	-					
PCBs	0.901	-				
Cord blood						
TEQ	0.763	0.633	-			
PCBs	0.716	0.808	0.826	-		
Maternal blood						
TEQ	0.938	0.854	0.794	0.756	-	
PCBs	0.841	0.927	0.699	0.843	0.892	-

Pearson's r ($p < 0.001$) after log-transformed. n=49 for breast milk, cord blood and maternal blood.

Table 3. Pearson correlation coefficients of TEQ and PCBs with TSH, total T4 and total T3 in breast milk, cord blood and maternal blood

	Maternal blood			Cord blood		
	TSH	T4	T3	TSH	T4	T3
Breast milk (n=68, except for n=67 for maternal TSH/T4/T3)						
TEQ	0.232	0.158	0.225	0.146	-0.224	-0.138
PCBs	0.205	0.231	0.257*	0.137	-0.163	-0.075
Cord blood (n=68, except for n=67 for maternal TSH/T4/T3)						
TEQ	0.203	0.038	0.150	0.071	-0.270*	-0.051
PCBs	0.239	0.131	0.240	0.024	-0.144	0.085
Maternal blood (n=49, except for n=48 for maternal TSH/T4/T3)						
TEQ	0.264	0.271	0.375*	0.145	-0.238	-0.094
PCBs	0.278	0.287*	0.402*	0.185	-0.150	-0.038

n=68 for breast milk and cord blood, n=49 for maternal blood.
Pearson's r after log-transformed. * $p<0.05$

Relationship between child birth weight and concentration of polychlorinated biphenyls (PCBs) of the mother in Japan. –Tohoku Study of Child Development (TSCD)–

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Introduction

Birth weight is considered to be a predictor of a variety of adverse developments in childhood and beyond, including obesity, high blood pressure, cardiovascular disease and depression. There is widespread concern about potentially adverse health effects of environmental chemicals on children. Infants exposed *in utero* and during the early neonatal period are particularly vulnerable because of cell differentiation, the immaturity of metabolic pathways, and development of vital organ systems[1]. Exposure to persistent organochlorine compounds, including pesticides and industrial chemicals, is associated with detrimental effects on childhood neurobehavioral development. Studies in rhesus monkeys and rats showed that prenatal exposure to polychlorinated biphenyls (PCBs) was associated with reduced birth weight. In addition, certain unusual exposures such as those resulting from accidental poisoning by PCBs in Japan and Taiwan, have definitely been associated with low birth weight. Some studies also observed reduced birth weight and shorter gestation in infants with elevated PCB levels whose mothers consumed contaminated fish[2,3]. Thus, there is reason to believe that exposure to PCBs *in utero* may adversely affect the developing infant. The present study examines the association between exposure to PCBs *in utero* and infant birth weight.

Materials and Methods

We have been conducting a prospective cohort study, the Tohoku Study of Child Development (TSCD), to examine the effects of perinatal exposure to PCBs and methylmercury on neurobehavioral development in Japanese children. From January 2001 through September 2003 we recruited healthy pregnant women with their informed consent at obstetrics wards of two urban hospitals in the Tohoku region of Japan. Our cohort study is being conducted in a large city with a population of more than one million in order to assess the effect of the average exposure in pregnant Japanese women. The details of the study protocol were reported previously[4]. The TSCD was approved by the Medical Ethics Committee of the Tohoku University Graduate School of Medicine, and all mothers provided signed informed consent. In this analysis, the subjects were mother-infant pairs whose variables including the PCB concentration in cord blood, birth weight and other covariates were available. The infants were all singletons from full-term (37-42 weeks) gestation without congenital anomalies or diseases.

Birth weight in all infants was 2500g or more since low birth weight was used as an exclusion criterion. Information was obtained about pregnancy, delivery and infant characteristics from medical records. We obtained information about smoking status (nonsmoker/ex-smoker and current smoker) and alcohol drinking (yes/no) during pregnancy from a questionnaire. Umbilical cord blood was collected into a clean bottle immediately after birth. The samples were frozen at -80°C until analysis. All 209 PCB congeners were analyzed using HR-GC/HR-MS. The analytical procedure was described previously[5]. The total PCB concentration represented the sum of all the measured congeners, expressed as ng/g-fat. The total mercury concentration in cord blood was measured by cold vapor atomic absorption. In the statistical analysis, total PCBs and mercury concentrations, birth weight and maternal body mass index (BMI) before pregnancy were logarithmically transformed because of skewed distribution. Parametric methods were applied throughout. Multiple regression analyses were performed for adjustment of covariates. The potential confounders were considered and identified on the basis of previous studies[2,3,6-10]. They were the maternal age at delivery, maternal BMI before pregnancy, mercury concentration in cord blood, maternal alcohol drinking and smoking during pregnancy, parity, gestational age, and the sex and birth weight of the infant. The significance level was set at 5%.

Results and discussion

The number of mother-infant pairs was 438. The characteristics of the mothers and infants are shown in Table 1. The mean maternal age at delivery was 31.3 (SD 4.4, range 20-42) years. BMI before pregnancy ranged between 16.0 and 45.0 kg/m^2, with only 27 (6.1%) being over 25 kg/m^2, which is defined as overweight. The mean weight of all infants was 3100.5 (SD 319.7) g, the median was 3097.0 g, with a range between 2506 and 4176 g. The infants consisted of 225 boys and 213 girls. The mean total PCB concentration in cord blood was 54.0 ng/g-fat (SD 33.1) (median 47.2), and total maternal fish intake was 23.5 kg/year (SD 16.7) (median 20.5). Table 2 shows the results of multiple regression analyses. The BMI before pregnancy and gestational age were positively associated with birth weight. There was no significant difference statistically in birth weight between nonsmokers and smokers (including ex-smokers) during pregnancy. The total PCB and mercury concentration in cord blood were negatively associated with birth weight, whereas the total fish intake was positively associated with birth weight. Thus, the results suggested that prenatal PCB exposure adversely affected fetal growth.

Our study found a significant decrease in birth weight associated with the total PCB concentration in cord blood at delivery. Several studies have investigated the potential association between PCB exposure and birth weight[2,3,6,8-11]. However, one strength of our study is that we used the cord blood PCB concentrations as the indicator of intrauterine exposure, not approximations such as a food frequency questionnaire. Although we have found that PCBs and mercury may be associated with reduced birth weight, the underlying mechanisms remain unknown. In addition, levels of toxicants such as PCB and mercury, as well as nutritive factors, including n-3 PUFA, vary among different fish types. The Japanese diet relies heavily on rice, fish and vegetables. Indeed, the Japanese eat great amounts of many kinds of fish. Regarding fish consumption, there is a report that some polyunsaturated fatty acids ingested from fish, in particular docosapentaenoic acid (DPA), increase birth weight[7].

Another report showed that fish consumption was a major source of mercury exposure for pregnant women, and a relationship between elevated mercury levels and increased risk of very preterm delivery[12]. Although we have found that fish consumption is associated with an increase in birth weight, we have not yet considered the polyunsaturated fatty acids that may be confounders in this analysis. Polyunsaturated fatty acids are provided by seafood and may be beneficial for pregnancy and offspring. Since both polyunsaturated fatty acids and PCBs have the same origin and thus are likely to be correlated, fish and seafood consumption may confound the association between PCBs and birth weight. 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

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Table 1. Characteristics of the study population in this analysis

Variables	Mean±SD	Range
Birth weight (g)	3100.5±319.7	2506-4176
Mother's age at delivery (years)	31.3±4.4	20-42
Fish consumption (g/year)	23460±16716	0-147278
Body mass index before pregnancy (kg/m^2)	21.0±2.85	16.0-45.0
Gestational age (weeks)	39.7±1.13	37.0-41.9
Total PCB concentration (ng/g-fat)	54.0±33.12	6.37-274.21
Total mercury concentration (ng/g)	11.4±6.24	1.77-43.90

Variables	Number of participants by categories	
Infant gender	Boys: n=225	Girls: n=213
Parity	First: n=233	Second or more: n=205
Smoking status during pregnancy	Nonsmoker: n=396	Smoker/ex-smoker: n=42
Alcohol drinking during pregnancy	No: n=349	Yes: n=88

Table 2. Multiple linear regression results for independent predictors of birth weight in this analysis

Variables	β	Standardized β	p value
Mother's age at delivery	0.001	0.062	0.207
Fish consumption (g/year)	0.011	0.110	0.016
Body mass index before pregnancy (kg/m^2)	0.170	0.199	<0.001
Gestational age (weeks)	0.030	0.330	<0.001
Total PCB concentration (ng/g-fat)	-0.024	-0.126	0.009
Total mercury concentration (ng/g)	-0.017	-0.089	0.051
Infant gender (Boys)	0.014	0.139	0.001
Parity (2nd or more)	0.012	0.058	0.244
Smoking status (nonsmoker)	0.006	0.033	0.461
Alcohol drinking (No)	-0.005	-0.036	0.411

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Permanent Waving Does not Change Mercury Concentration in the Proximal Segment of Hair Close to Scalp

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Mercury in hair is a generally accepted biomarker of methylmercury exposure, and permanent waving has been reported to affect the mercury concentration in hair. We conducted an experimental-field study to examine the changes in the mercury concentration in hair induced by treatments such as permanent waving, straightening and coloring. Hair samples were collected from 19 female subjects enrolled before and after hair treatment by a beautician during each visit to a beauty saloon. A total of 38 pair samples were cut in 1-cm segments from the proximal end up to 10 cm, and then as 2-cm segments up to the distal end thereafter. Each segment was analyzed for total mercury concentration by cold-vapor atomic absorption spectrometry. Permanent waving decreased mercury concentration for most of the segments except for the proximal two segments and the 8-9 cm segment from the proximal end. Nevertheless the average mercury concentration of 3-cm segments from the proximal end showed no significant decrease by permanent waving. Since females usually have hair longer than 3 cm, hair samples subjected to permanent waving may give lower mercury exposure estimates when the full-length hair strands are analyzed. However, analyzing the proximal 3-cm segment of hair samples does not give lower mercury exposure estimates. Assuming that hair samples are collected from puerperal women around the time of delivery, the 3-cm segments represent fetal exposure to methylmercury during the third trimester when fetuses are most vulnerable to methylmercury exposure. Therefore, mercury concentrations in the proximal segment of maternal hair collected in the right time can be a good biomarker of fetal methylmercury exposure. ——— methylmercury; fetal exposure; permanent waving; hair straightening; exposure biomarker.
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Hair is a biological sample that is easy to collect and preserve. Mercury in hair is generally accepted as a biomarker of methylmercury exposure (WHO 1990; ATSDR 1999; Food Safety Commission 2005). In several studies, including those in New Zealand (Kjellstrom et al. 1986, 1989), the Faroe Islands (Grandjean et al. 1997) and the Republic of the Seychelles (Davidson et al. 1995; Myers et al. 2003) the developmental effects of prenatal exposure to methylmercury

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have been investigated using the hair mercury concentration as the exposure marker.

More than 90% of mercury in the hair of people with no known exposure to inorganic mercury is methylmercury (Akagi et al. 1995). The total mercury concentration in hair is presumed to reflect the methylmercury concentration in blood when hair is produced by hair follicles (Cernichiari et al. 1995). Therefore, an analysis of mercury in segmentally cut hair samples provides the history of methylmercury exposure in the past depending on the distance of the segment from the scalp (Cox et al. 1989; Murata et al. 2007). The analytical imprecision of hair mercury concentration in the laboratory is less than 5% (Grandjean et al. 2002); this is much smaller than the analytical imprecision of blood methylmercury concentration.

In spite of the above advantages of hair mercury as a biomarker of methylmercury exposure, permanent waving (Yamamoto and Suzuki 1978; Yasutake et al. 2003; Dakeishi et al. 2005) has been reported to affect the mercury concentration in hair, thus giving imprecision in the evaluation of methylmercury exposure. Therefore, we conducted an experimental-field study in which hair samples were collected before and after hair treatments such as permanent waving and straightening. Because hair coloring has become popular in Japan recently, the effects of this treatment were also examined.

Materials and Methods

Subjects and hair samples

Nineteen women (19-39 years old) were recruited for this study. They gave informed consent after we explained the study plan to them. Hair samples from each subject were collected before and after treatment such as permanent waving or straightening by a beautician during each visit to a beauty saloon. Hair coloring was usually conducted after permanent waving. When permanent waving and coloring were carried out consecutively, hair samples were also collected after coloring. In this case, hair samples collected after permanent waving served as the "before" samples for examining coloring effects. An adequate number of hair strands were cut as close to the scalp of the occipital area as possible with stainless steel scissors. They were kept in clean plastic bags, taking strict measures to label the proximal and distal ends. The types of treatment were reported by the subjects upon submission of their hair samples.

During the study period the subjects submitted 17 pair samples before and after permanent waving and 10 pair samples before and after straightening. Eleven hair samples were collected after coloring that were carried out subsequently to permanent waving and submitted with pair samples before and after permanent waving. Thus, a total of 38 comparisons were made before and after one of the treatments. Although several subjects submitted more than one pair samples, these were treated independently, because the purpose of this study is to examine the change of mercury concentration in hair after hair treatments.

Mercury analysis

A number of hair strands were cut in 1-cm segments from the proximal end up to 10 cm, and then as 2-cm segments up to the distal end thereafter. Each hair segment was weighed and wet-ashed in a PYREX® tube with a mixture of nitrate/sulfate/perchloric acid (1 : 4 : 1 v/v) at 160°C for 30 min (Akagi and Nishimura 1991). Total mercury concentration was determined by cold-vapor atomic absorption spectrometry. Stannous chloride was used as the reducing agent. All the chemicals used were of analytical grade. To ensure the accuracy of the determination, a reference human hair (NIES CRM No. 13; National Institute for Environmental Studies, Tsukuba) was used. The determined values were within the range of the certified values (Yoshinaga et al. 1997).

Statistical analysis

All data were presented *en masse* as mean ± S.D. Comparisons between the mercury concentrations in each 1-cm or 2-cm segment before and after treatments were carried out by a paired *t*-test. Also, the total mercury concentrations in consecutive segments from the proximal end toward the distal end were averaged for predetermined length, and comparisons of these concentrations were made before and after treatments. Since the length of hair samples differed among individual subjects, numbers of samples decreased as the distance from the proximal end increased. Therefore, the group basis comparison by a paired *t*-test was made when the numbers of samples were five or more. A p value of < 0.05 was considered significant. All statistical analyses were carried out using JMP version 5.0.1 (SAS Institute Inc. 2002).

Results

Comparisons between corresponding segments en masse

In Table 1 the mean ± S.D. of each segment of pair samples before and after permanent waving from individuals are shown. The paired *t*-test revealed a statistically significant difference in mercury concentration for most of the comparisons before and after permanent waving except for the first two segments and the 8-9 cm segment. As shown in Table 2, however, the comparison by a paired *t*-test revealed significant decreases after straightening except for the first 1-cm. No significant difference in hair mercury concentration was observed between the hair samples before and after hair coloring (Table 3).

Comparison of average values from the proximal end toward the distal end for predetermined segments

Because hair mercury has generally been analyzed using full-length hair strands in previous studies, the average mercury concentration of each segment of hair strands from each subject was calculated. Although the number of segments differed among the individuals, this average represents the mercury concentration when full-length hair strands are analyzed. Mean ± S.D. of all individual subjects are shown in the right most column of the bottom half in Tables 4 and 5. The average mercury concentration in full-length hair strands showed a significant difference by 12% after permanent waving (Table 4). After straightening the average mercury concentration in full-length hair strands markedly and significantly differed by 38% (Table 5). Hair coloring did not affect hair mercury concentration averaged for the full length (Data not shown).

The mercury concentrations in hair halfway from the proximal end toward the distal end were also averaged; for example, the hair mercury concentration in a 3-cm-long hair strand was calculated by averaging those of the first, second and third 1-cm segments.

Table 4 shows that the average mercury concentrations in proximal segments up to 3 cm long did not significantly differ before and after permanent waving. However, the average mercury concentrations in the proximal 4-cm long and longer segments up to 12 cm long showed significantly differed by permanent waving. As for straightening, differences by treatment were statistically significant except for the first 1-cm segments (Table 5). Hair coloring did not affect hair mercury concentrations averaged from the proximal end toward the distal end for predetermined segments (Data not shown).

Discussion

Yamamoto and Suzuki (1978) demonstrated for the first time that waving lotion containing thioglycolate removes hair mercury effectively. Moreover, Yasutake et al. (2003) showed that more than 30% of hair mercury was removed by single treatment with waving lotion, and that repeated treatments further remove hair mercury. These *in vitro* experiments in which the entire length of each hair strand was immersed in waving lotion clearly demonstrated the diminishing effects of permanent waving lotion on hair mercury concentration.

In this study hair strands before and after the actual treatments in a beauty saloon were collected, cut in segments, and analyzed for mercury. Hair mercury concentration in segments close to the proximal end of the hair strands preserved the pretreatment mercury concentrations differently between permanent waving and straightening.

Permanent waving and hair straightening are essentially similar chemical (reducing and oxidizing) processes that involve splitting and re-bonding of S-S ligands in keratin (Zviak 1986). Their differences lie in the practical steps and the concentrations of the ingredients in the waving lotions used. During permanent waving hair strands are rolled onto curlers to make hair curly. Then reducing lotion is applied onto hair strands and the hair strands are left for a set time. Because hair strands contacting the lotion are rolled onto curlers, the proximal end of each strand is not completely covered by the lotion. In contrast, straightening makes hair straight and the lotion used is usually stronger and thicker than

TABLE 1. Changes in total mercury concentration induced by permanent waving: comparisons between corresponding segments.

	0-1 cm[b]	1-2 cm	2-3 cm	3-4 cm	4-5 cm	5-6 cm	6-7 cm	7-8 cm	8-9 cm	9-10 cm
n[a]	17	17	17	17	17	17	14	12	10	10
Before	1.66 ± 0.73[d]	1.67 ± 0.74	1.66 ± 0.78	1.62 ± 0.86	1.58 ± 0.86	1.54 ± 0.79	1.52 ± 0.88	1.59 ± 0.97	1.65 ± 0.93	1.68 ± 1.04
After	1.65 ± 0.78	1.55 ± 0.79	1.47 ± 0.76	1.34 ± 0.68	1.31 ± 0.72	1.26 ± 0.68	1.31 ± 0.80	1.43 ± 0.94	1.56 ± 0.88	1.45 ± 0.93
p value[c]	0.7404	0.0884	0.0157	0.0029	0.0029	0.0014	0.0016	0.0189	0.2105	0.0035

	10-12 cm	12-14 cm	14-16 cm	16-18 cm
n	8	4	3	1
Before	1.35 ± 0.71	0.78 ± 0.21	0.84 ± 0.03	0.86
After	1.18 ± 0.61	0.80 ± 0.27	0.92 ± 0.10	1.31
p value	0.0268	n.a.[e]	n.a.	n.a.

[a] Number of samples in each segment.
[b] Distance from proximal end of hair strands.
[c] A "*p* value" indicates statistical significance by paired *t*-test.
[d] Mean ± S.D. (ngHg/mg).
[e] Not applicable due to the small number of samples.

TABLE 2. Changes in total mercury concentration induced by hair straightening: comparisons between corresponding segments.

	0-1 cm[b]	1-2 cm	2-3 cm	3-4 cm	4-5 cm	5-6 cm	6-7 cm	7-8 cm	8-9 cm	9-10 cm
n[a]	10	10	10	10	10	10	10	9	7	6
Before	2.96 ± 1.51[d]	3.10 ± 1.61	3.09 ± 1.74	2.89 ± 1.68	2.79 ± 1.61	2.70 ± 1.52	2.45 ± 1.35	2.19 ± 1.24	1.81 ± 1.08	1.45 ± 0.97
After	2.67 ± 1.48	1.98 ± 1.13	1.74 ± 0.99	1.64 ± 0.99	1.52 ± 0.95	1.45 ± 0.85	1.40 ± 0.81	1.28 ± 0.68	1.06 ± 0.55	0.87 ± 0.53
p value[c]	0.0771	0.0042	0.0045	0.0027	0.0016	0.0012	0.0014	0.0081	0.0212	0.0270

	10-12 cm	12-14 cm	14-16 cm	16-18 cm	18-20 cm	20-22 cm
n	4	3	3	3	1	1
Before	1.08 ± 0.98	1.01 ± 0.77	1.00 ± 0.85	0.92 ± 0.79	0.58	0.53
After	0.75 ± 0.59	0.74 ± 0.52	0.67 ± 0.44	0.69 ± 0.49	0.51	0.54
p value	n.a.[e]	n.a.	n.a.	n.a.	n.a.	n.a.

[a] Number of samples in each segment.
[b] Distance from proximal end of hair strands.
[c] A "*p* value" indicates statistical significance by paired *t*-test.
[d] Mean ± S.D. (ngHg/mg).
[e] Not applicable due to the small number of samples.

TABLE 3. Changes in total mercury concentration induced by coloring: comparisons between corresponding segments.

	0-1 cm[b]	1-2 cm	2-3 cm	3-4 cm	4-5 cm	5-6 cm	6-7 cm	7-8 cm	8-9 cm	9-10 cm
n[a]	11	11	11	11	11	10	10	7	5	4
Before	1.53 ± 0.72[d]	1.42 ± 0.71	1.37 ± 0.80	1.36 ± 0.87	1.40 ± 0.91	1.39 ± 1.04	1.39 ± 1.10	1.40 ± 1.19	1.63 ± 1.17	1.20 ± 0.78
After	1.52 ± 0.74	1.49 ± 0.72	1.38 ± 0.71	1.33 ± 0.78	1.33 ± 0.84	1.43 ± 1.00	1.37 ± 0.94	1.32 ± 1.05	1.52 ± 1.26	0.99 ± 0.60
p value[c]	0.9878	0.3501	0.8995	0.6796	0.3594	0.4794	0.8483	0.3540	0.3002	n.a.[e]

	10-12 cm	12-14 cm	14-16 cm	16-18 cm
n	4	3	2	1
Before	1.28 ± 0.82	1.09 ± 0.80	1.14, 0.81	0.83
After	0.93 ± 0.50	0.84 ± 0.57	1.01, 0.70	0.86
p value	n.a.	n.a.	n.a.	n.a.

[a] Number of samples in each segment.
[b] Distance from proximal end of hair strands.
[c] A "*p* value" indicates statistical significance by paired *t*-test.
[d] Mean ± S.D. (ngHg/mg).
[e] Not applicable due to the small number of samples.

TABLE 4. Changes in total mercury concentrations induced by permanent waving: comparison of average values from the proximal end toward the distal end for predetermined segments.

	0-1 cm	0-2 cm[b]	0-3 cm	0-4 cm	0-5 cm	0-6 cm	0-7 cm	0-8 cm	0-9 cm	0-10 cm
n[a]	17	17	17	17	17	17	14	12	10	10
Before	1.67 ± 0.73[d]	1.67 ± 0.73	1.66 ± 0.74	1.65 ± 0.76	1.64 ± 0.78	1.62 ± 0.77	1.59 ± 0.84	1.67 ± 0.88	1.79 ± 0.89	1.78 ± 0.90
After	1.65 ± 0.78	1.60 ± 0.78	1.56 ± 0.77	1.50 ± 0.74	1.46 ± 0.73	1.43 ± 0.71	1.42 ± 0.76	1.50 ± 0.80	1.62 ± 0.83	1.60 ± 0.83
p value[c]	0.7404	0.2250	0.0748	0.0126	0.0071	0.0040	0.0163	0.0195	0.0248	0.0146

	0-12 cm	0-14 cm	0-16 cm	0-18 cm	Full length[e]
n	8	4	3	1	17
Before	1.58 ± 0.78	1.00 ± 0.37	1.07 ± 0.38	0.92	1.57 ± 0.75
After	1.41 ± 0.72	0.95 ± 0.36	1.04 ± 0.35	1.06	1.38 ± 0.70
p value	0.0407	n.a.[f]	n.a.	n.a.	0.0005

[a] Number of samples in each segment.
[b] Length from root up to the indicated segment.
[c] A "p value" indicates statistical significance by paired t-test.
[d] Mean ± S.D. (ng/mg).
[e] Mercury concentration of full-length hair strands calculated by averaging each segment, Mean ± S.D. (ng/mg).
[f] Not applicable due to the small number of samples.

TABLE 5. Changes in total mercury concentrations induced by hair straightening: comparison of average values from the proximal end toward the distal end for predetermined segments.

	0-1 cm	0-2 cm[b]	0-3 cm	0-4 cm	0-5 cm	0-6 cm	0-7 cm	0-8 cm	0-9 cm	0-10 cm
n[a]	10	10	10	10	10	10	10	9	7	6
Before	2.96 ± 1.51[d]	3.03 ± 1.56	3.05 ± 1.61	3.01 ± 1.62	2.96 ± 1.62	2.92 ± 1.60	2.85 ± 1.56	2.85 ± 1.57	2.77 ± 1.60	2.64 ± 1.66
After	2.67 ± 1.48	2.32 ± 1.28	2.13 ± 1.17	2.01 ± 1.12	1.91 ± 1.08	1.83 ± 1.04	1.77 ± 1.00	1.76 ± 1.00	1.64 ± 1.02	1.54 ± 1.05
p value[c]	0.0771	0.0078	0.0057	0.0043	0.0034	0.0027	0.0022	0.0040	0.0120	0.0251

	0-12 cm	0-14 cm	0-16 cm	0-18 cm	0-20 cm	0-22 cm	Full length[e]
n	4	3	3	3	1	1	10
Before	2.26 ± 1.84	2.55 ± 1.89	2.43 ± 1.80	2.32 ± 1.73	2.04	1.94	2.43 ± 1.27
After	1.51 ± 1.28	1.75 ± 1.29	1.66 ± 1.23	1.57 ± 1.17	1.47	1.41	1.50 ± 0.75
p value	n.a.[f]	n.a.	n.a.	n.a.	n.a.	n.a.	0.0023

[a] Number of samples in each segment.
[b] Length from root up to the indicated segment.
[c] A "*p* value" indicates statistical significance by paired *t*-test.
[d] Mean ± S.D. (ng/mg).
[e] Mercury concentration of full-length hair strands calculated by averaging each segment, Mean ± S.D. (ng/mg).
[f] Not applicable due to the small number of samples.

that used for permanent waving. Because hair straightening is carried out for customers with naturally curly hair, the lotion must be entirely spread onto hair strands from the proximal end close to the scalp. Furthermore, thermal reconditioning which may accelerate reaction between hair mercury and thiols in the lotion is often applied to straighten hair strands completely. These differences are presumably the causes of the different patterns of decrease in hair mercury concentration.

In an epidemiological study, Dakeishi et al. (2005) reported a 30% lower mercury concentration in hair samples from a group of mothers who had "artificial hair-waving treatments" than in hair samples from a group of mothers who did not. They analyzed full-length hair strands and their "with treatments" group included permanent waving and straightening. When the average mercury concentrations in full-length hair strands of the group in which permanent waving and straightening were combined was calculated in the present study, the 25% mercury concentration reduction was observed after the treatments. This is similar to the reduction observed in the "with treatments" group of Dakeishi et al. (2005).

In this study, no significant difference by permanent waving in average hair mercury concentration was observed for the first 3-cm segments. This is a great advantage of using hair mercury concentration as a biomarker of methylmercury exposure in studies of prenatal methylmercury exposure. Assuming that hair samples are collected from puerperal women around the time of delivery, the proximal segments 3 cm from the scalp reflect the methylmercury concentration in maternal blood and thus represent fetal exposure during the third trimester when fetuses are most vulnerable to methylmercury exposure. Unfortunately, this is not the case for hair straightening, because, as shown in this study, even the segments closest to the scalp showed a slight decrease, which, however, is not statistically significant.

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Determination of dioxins and polychlorinated biphenyls in breast milk, maternal blood and cord blood from residents of Tohoku, Japan

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Cord blood

ABSTRACT

Polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and polychlorinated biphenyls (PCBs) are bioaccumulative chemicals that are considered to be toxic contaminants based on several epidemiological studies. To elucidate exposure levels of these chemicals in the present study, concentrations of PCDD/DFs, dioxin-like PCBs (DL-PCBs) and PCBs in breast milk, maternal blood and cord blood obtained from the same participants registered in a birth cohort study in Tohoku, Japan, were measured. Congener-specific analysis revealed several differences in minor congeners of these compounds among the three specimen types, although major congeners were detected in the specimens. The toxicity equivalence quantity concentrations (1998 WHO-TEF) and PCBs in breast milk, maternal blood and cord blood on the whole and on a lipid basis were in the order of breast milk > maternal blood > cord blood. Pearson's correlation coefficients of TEQs and total PCBs among the three specimens were high, with the correlation coefficient of TEQ between breast milk and maternal blood being the highest ($r=0.94$, $p<0.001$). On the other hand, the TEQ between breast milk and cord blood was the lowest ($r=0.79$, $p<0.001$). Pearson's correlation coefficient between the TEQ and PCBs in each specimen was also high ($r=0.82$–0.95, $p<0.001$). The associations of chemical concentrations with maternal age, parity, fish intake, BMI and the rate of body weight increase during pregnancy were analyzed with multiple linear regression analysis. TEQ concentrations and PCBs were negatively associated with parity ($p<0.05$), and maternal age was positively associated with PCBs ($p<0.05$). However, the associations with BMI and fish intake during pregnancy were not significant. These results suggest that parity is an important factor affecting the concentrations of dioxins and PCBs in these specimens.

1. Introduction

Polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and polychlorinated biphenyls (PCBs) are considered to be bioaccumulative chemical toxins that are resistant to degradation, and are detected in almost all human biological samples such as breast milk and blood in industrialized countries (Schecter et al., 2006). The main source of

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