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

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Abstract

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

Introduction

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

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

neurobehavioral status with total PCBs in cord blood, maternal hair mercury (hair Hg), and maternal fish intake.

Materials and Methods

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

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

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

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

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

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

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

Results and Discussion

The mean total PCB concentration in cord blood was 55.9 ng/g-fat (SD 35.3) (median 48.4), the mean total hair Hg level was 2.2 μg/g (SD 1.1) (median 2.0), and total fish intake was 25.9 kg/year (SD 17.8) (median 22.7). Table 1 shows the results of multiple regression

analyses. The total PCBs in cord blood and the total fish intake were not significantly associated with any seven clusters of the NBAS. The total hair Hg was negatively associated with the motor score, and positively associated with the range of state and the reflex scores. For the motor and the range of state, higher scores mean more optimal behavioral status, and for the reflex, lower scores mean more optimal status because the reflex score indicates the number of unusual reflexes. Thus, the results suggested that prenatal MeHg exposure adversely affected neonatal neurobehavioral status. In early studies, an adverse effect of prenatal MeHg exposure on neurodevelopment was found in the Faroe Islands[5] and Boston[6], but not in the Seychelles[7]. Our findings are in line with the former two, although the types of examination were different.

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

Acknowledgments

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

Table 1. Results of multiple regression analyses

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

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

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

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

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

Introduction

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

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

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

thyroid axis is a hypothetic mechanism for POPs-induced adverse effects.

Materials and Methods

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

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

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

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

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

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

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

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

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

** $p<0.001$

Results and Discussion

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

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

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

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

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

Acknowledgements

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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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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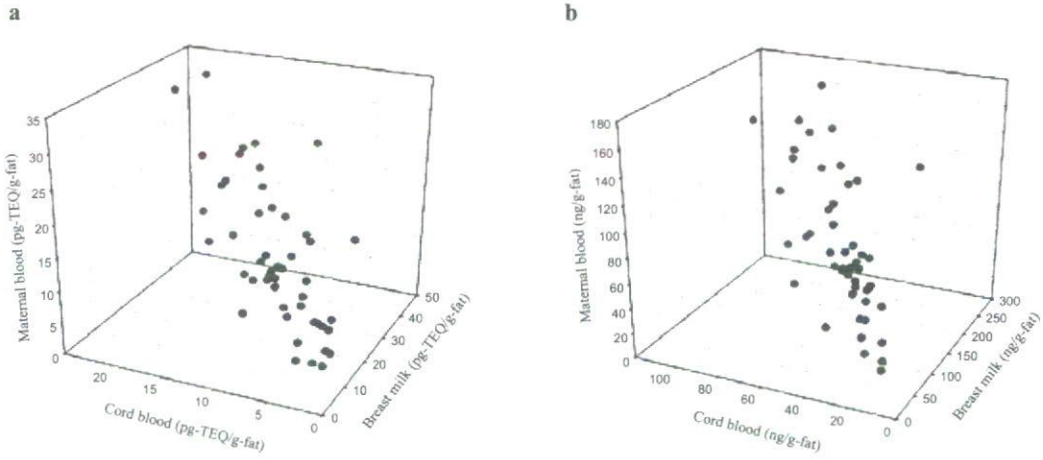

Fig. 1 Relationship of TEQ (a) and PCBs (b) among pair samples of breast milk, cord blood and maternal blood.

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. * $p<0.05$

Tohoku J. Exp. Med., 2008, **214**, 69-78

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.
Tohoku J. Exp. Med., 2008, **214** (1), 69-78.

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

Received October 9, 2007; revision accepted for publication December 3, 2007.
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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.