Effects of prenatal phthalate exposure on thyroid hormone levels, mental and psychomotor development of infants: The Hokkaido Study in Environment and Children's Health

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Abstract

Di (2-ethylhexyl) phthalate (DEHP) is commonly used phthalates and concerns of adverse effects of prenatal DEHP exposure on neonatal thyroid hormone (TH) and neurodevelopment are increasing. However, there is no report regarding association between prenatal DEHP exposure and infant neurodevelopment including TH levels in Japanese population. Thus the aim of present study was to evaluate the associations between prenatal DEHP exposure and mental and psychomotor development of infants 6 and 18 months along with investigating influence on neonatal free thyroxine (FT4) and thyroid stimulating hormone (TSH) levels in the prospective birth cohort study.

Maternal blood samples collected between 23-41 weeks of gestation was analyzed for mono (2-ethylhexyl) phthalate (MEHP), metabolite of DEHP levels. Neonatal FT4 and TSH were obtained from mass screening data. Infant neurodevelopment was assessed by Bayley Scale of Infant Development second edition at 6 and 18 month of age. For the final analysis, 328 participants were included.

The median levels of maternal MEHP was 10.6 ng/ml, neonatal TSH and FT4 was 2.20 μU/ml and 2.03 ng/ml, respectively. We did not find any associations between prenatal DEHP exposure and neonatal TH levels or infant mental and psychomotor development at 6 and 18 month.

In this study, prenatal DEHP exposure did not show adverse effects on infant TH levels or mental and psychomotor development in early life stage. However, our previous study revealed negative effects of prenatal DEHP exposure on sex hormone levels, continuous investigation on neurodevelopment in later life in association with prenatal DEHP exposure is necessary.

Keywords:

Di (2-ethylhexyl) phthalate; Thyroid hormone; Neurodevelopment; Prenatal Exposure

Introduction

Phthalates are a group of chemicals widely used in consumer products including personal care products as well as in industry for primary plasticizers[1]. Phthalate can be inhaled through contaminated air or dust, ingested through food and dermally absorbed through care products[2] and detectable levels of phthalates have been reported worldwide[3-5]. In particular, Di-2-ethylhexyl phthalate (DEHP) represents one of the most important plasticizers used in industry[1]. DEHP is primarily metabolized to monoester, Mono-2-ethylhexyl phthalate (MEHP) then further metabolized to the secondary metabolites. Adverse health effects of phthalate exposure has been a growing issue, especially in populations such as pregnant women and infants[6] as maternal-fetal transmission to offspring have been reported[7, 8] and as detoxifying enzymes are not fully developed in fetus.

In animal studies, prenatal DEHP exposure were associated with adverse effects on neurodevelopment and behavior in their offspring[9]. In human, certain phthalate metabolites, especially metabolites of DEHP measured in maternal urine during pregnancy have been associated with adverse infant neurodevelopment[10-13]. Cross-sectional studies have reported that DEHP metabolites including MEHP were significantly associated with attention deficit hyperactivity disorder (ADHD)

symptom among school age children[14], reduced intelligence quotient (IQ) in school age children in Korea[15] and attention deficit disorder (ADD) symptom among children aged 6-15 years[16]. Some of these adverse effects on child neurodevelopment observed in previous studies were sex-specific and limited. Thus understanding impact of prenatal exposure to phthalates, especially most commonly used DEHP, is urgent.

Subtle changes in circulating levels of thyroid hormone (TH) may have permanent effect on child development[17]. TH is very important for fetal growth during pregnancy. Exposure to environmental chemicals including phthalates have been reported to cause thyroid disruption in experimental animals[18]. Several studies have indicated that phthalates may alter thyroid functions in human[19-21]. Especially, MEHP was inversely associated with serum free thyroxine (FT4) and total triiodothyronine (T3) levels in a cross-sectional study of 408 men attending a U.S. infertility clinic[20]. The same group reported significant inverse relationships between urinary DEHP metabolites including MEHP, MEHHP (Mono-2-ethyl-5-hydroxyhexyl phthalate), MEOHP (Mono-2-ethyl-5-oxohexyl phthalate) and MECPP (Mono-2-ethyl-5-carboxypentyl phthalate) and total thyroxine (T4), FT4, T3, and positive relationships with thyroid-stimulating hormone (TSH) among 1346 adult

population in U.S. (NHANES)[19]. Urinary DEHP metabolites were also inversely related to total and FT3 levels in a Danish cross-sectional study of 845 children[22]. Contrary, two of the previous studies observed no association between maternal DEHP exposure and thyroid function in their offspring[23, 24].

To our knowledge, there are no available data of investigating association between prenatal DEHP exposure and TH levels of neonates and effects of later infant neurodevelopment together. Thus the aim of this study was to investigate the association between prenatal DEHP exposure and infant mental and psychomotor development at two distinct time points of ages 6 and 18 month along with examining the influence of prenatal DEHP exposure on infant TH levels.

Materials and methods

Study population

This prospective birth cohort study was based on the Sapporo Cohort, Hokkaido Study on Environment and Children's Health[25, 26]. Briefly we recruited pregnant women at 23-35 weeks of gestation between July 2002 and October 2005 from the Sapporo Toho Hospital in Hokkaido, Japan. All subjects were residents in Sapporo City or surrounding areas. The participants completed the self-administered

questionnaire after the second trimester during their pregnancy. The questionnaire contained baseline information including their dietary habits, exposure to tobacco smoking history, alcohol consumption, caffeine intake, family income, educational levels of themselves and partners. The perinatal information of the mothers and their infants was collected from their medical records. We used the following eligibility for criteria for analyses of subjects; no serious illness or complications during pregnancy and delivery, no thyroid function diseases, singleton babies born at term (37 to 42 weeks of gestation), Apgar score of > 6 at 1 minute, babies without congenital anomalies or diseases, and Bayley Scale of Infant Development second edition (BSID-II)[29] completed. Among all 514 participants of Sapporo Cohort Study, 493 available maternal blood samples for MEHP measurements. Maternal blood samples collected after delivery were excluded from analysis due to the relatively short biological half-life of DEHP. Eventually 332 maternal blood levels of MEHP were available in this study. Of 332, 328 participants had available TH levels, among them, only 127 and 97 participants had available BSID-II scores at 6 month and at 18 month, respectively. This study was conducted with the informed consent of all participants in written forms. The protocol used in this study was approved by the Institutional Ethical Board for epidemiological studies at the Hokkaido University

Graduate School of Medicine, Hokkaido University Center for Environmental and Health Sciences and Ethics Review Committee of Nagoya University Graduate School of Medicine.

MEHP measurement

Approximately 40 mL of maternal blood samples were collected from each woman after the second trimester of their pregnancy. All samples were stored in uniform way at -80℃ until the analysis to avoid hydrolysis by enzyme activity. The concentrations of MEHP in maternal blood were measured by using gas chromatography-mass spectrometry (GC/MS) at Nagoya University under the analytical conditions mentioned previously[27]. 30 μl of blood samples were mixed with 120 μl of 1N HCl to deactivate the serum enzymes, 350 μl of saturated saline solution and 50 μl of 10 μM MEHP-d as an internal standard. Then MEHP was extracted two times with 500 μl of ethyl acetate after shaking for 15 minutes. There was no incubation process until extraction. The ethyl acetate layer was evaporated then the residue was dissolved into 40 μl of ethyl acetate. After addition of 20 μl of N-methyl-N-(tert-butyldimethylsilyl) trifluoroacetamide (GL Sciences, Tokyo, Japan), the reaction was left for 60 minutes at room temperature. The concentration of MEHP tertbutyldimethylsilyl derivative was measured by GC/MS (6890N, 5973N;

Agilent Technologies, CA, USA). Two ions, m/z 227 as quantification ion and 339 for confirmation ion, were used to detect MEHP[28]. The limit of detection (LOD) was 0.278 ng/ml (1 pmol/ml). For each sample, duplicate analysis was performed. To determine background levels, MEHP levels in a tube containing the same medium as the reaction vial were measured. All glass wares were heated at 200℃ for 2 hours to exclude the possibility of environmental contamination. Coefficient of variation (CV) of MEHP measurements within a day was 2.0-7.8 % for 6 days, and CV of day to day for 6 days was 6.2 % at 5 pmol/ml of concentration[27].

Thyroid hormone measurement

We obtained blood samples data of TSH and FT4 from Sapporo City Institute of Public Health which conducted the mass screening test for congenital diseases routinely. A heel-prick blood sample of newborns was obtained as spots on a filter paper for the Guthrie test. The blood samples were obtained from infants between 3 and 7 days after birth. Blood samples were applied to 0.3 cm filter disks and TSH and FT4 levels were measured using ELISA (TSH: Enzaplate N-TSH, Bayer Co., Tokyo, Japan; FT4: Enzaplate N-FT4, Bayer Co.). The FT4 levels were detected from all the samples. For samples with TSH levels below the detection limit (0.50 μU/mL), we used a value of half the detection limit (0.25 μU/mL).

Developmental assessment

We used BSID-II[29] to assess the infant mental (mental development index; MDI) and psychomotor (psychomotor development index; PDI) development at age 6 and 18 month. The BSID-II is considered most useful infant developmental test tool used between 0 to 3 years of age. The BSID-II mental scale assesses children's cognitive, language, and personal/social development and the psychomotor scale assesses fine and gross motor development. The developmental evaluation was performed by three occupational therapists who have clinical experience in the field of developmental disabilities[30]. The examiner was unaware of maternal MEHP levels. Additionally, the Index of Child Care Environment (ICCE) was used to investigate the child care environment of infants at 6 and 18 month of age[30].

Data analysis

Since the distributions of MEHP concentrations and TH levels were right skewed, these variables were transformed by the natural logarithms (ln) to improve their linear relation with MDI and PDI. To examine the relation between prenatal DEHP exposure and infant TH levels and infant neurodevelopment, mediation analyses were conducted by using bootstrapping confidence intervals. To select covariates to include in models, risk factors known or suspected of being associated with the

phthalates concentrations and/or infant neurodevelopment were reviewed in the literatures[10, 11]. The covariates used in this study were maternal age at delivery, maternal education, caffeine intake during pregnancy, family income, parity, blood sampling period, gestational age and infant sex. The covariates were included into the final model if their p-values were below 0.1. Results were considered significant at $p < 0.05$. All analyses were conducted using SPSS (Version 22.0; SPSS, Chicago, IL, USA).

Results

Table 1 shows basic characteristics of participants. There were no significant difference in maternal characteristics between infant sexes. 18% of mother smoked during pregnancy and 33% of mothers had alcohol consumption during pregnancy. Birth length was significantly longer in boys compared to girls ($p = 0.005$). The median concentration of MEHP was 10.6 ng/ml (Inter Quartile range (IQR); 6.3-17.1 ng/ml) and was detected in 100 % of the samples. The median concentration of TSH was 2.20 μU/ml (IQR; 1.40-4.00) and FT4 was 2.03 ng/ml (IQR; 1.79-2.29), respectively. The mean ± SD of MDI and PDI at 6 month were 90.2 ± 5.4 and 88.6 ± 10.4, respectively. The mean ± SD of MDI and PDI at 18

month were 84.6 ± 12.4 and 87.0 ± 11.5, respectively.

Table 2 shows maternal MEHP levels in relation to participants' characteristics. All the characteristics except blood sampling period were not associated with MEHP levels. MEHP levels were significantly higher among samples taken between 32-34 weeks of gestation. Maternal MEHP levels were not associated with infant characteristics.

Comparison between participants who completed BSID-II (N=127) and who did not (N=201) was shown in the Supplemental Table 1. Maternal and paternal education levels, family income, paternal age and MEHP level were significantly higher among those who included in final the analysis compared to those who were not included. Comparison between those who had two times of neurodevelopment assessments (both at 6 and 18 months, N=97) and those who had one assessment (only at 6 month, N=30), both maternal and paternal ages were significantly higher among those who had both assessments. Additionally, the percentage of maternal smoking during pregnancy was significantly lower among those who had both assessments. Furthermore PDI at 6 month was significantly higher among those who both assessments (Supplemental Table 2).

Table 3 shows association between infant TH levels and maternal MEHP levels. Both

TSH and FT4 levels were not associated with maternal MEHP levels.

Table 4 shows association between BSID-II score and 6, 18 month and maternal MEHP levels. Overall there was no significant association between BSID-II scores and maternal MEHP levels at both tested ages. Models included environmental chemicals; PCBs, dioxins and perfluorinated chemical (PFCs) that showed negative association with BSID-II in the same cohort [30, 31] did not change the results (data not shown).

Discussion

Maternal MEHP level was higher compared to previously reported values of pregnant women[32]. In our study, we found that maternal MEHP level was not associated with infant TH level nor infant neurodevelopment at ages 6 and 18 months. Our findings added evidence to the previous prospective study[23], which found no association between DEHP exposure and cord blood TH levels.

In several epidemiological studies, adverse health effects of DEHP exposure determined by urine metabolites on TH levels were reported[19, 21]. In Taiwanese study, high exposure to DEHP from foodstuffs were associated with decreased TSH levels in children, however, in their study, DEHP exposure assessment was only

based on interview, thus misclassification might exist[21]. Experimental evidence suggested that thyroid homeostasis disruption, peroxisome proliferator-activated receptors (PPAR) activation, and changing lipid metabolism maybe responsible for prenatal phthalate in association with fetal neurodevelopment[33]. Although these studies suggest DEHP metabolites may disrupt TH homeostasis among adults and children, how and which particular TH by which phthalate metabolite is still inconsistent. Additionally, adults and children may have different influence on thyroid functions. Contrary a prospective birth cohort study in Taiwan showed no significant association between DEHP exposure and TH levels in cord blood[23]. Our finding was consistent with the Taiwanese prospective study, however, given the fact that T4 and TSH levels in this study were within the normal ranges and the sample size was relatively small, thus possible significant relationships could easily have been missed.

In this study, we did not find any significant association between maternal MEHP levels and infant MDI and PDI at both 6 and 18 month. As described in previous publications from our group[30, 31], maternal caffeine intake, birth weight, birth length and gestational age for 6 month, family income and infant sex for 18 month were found to be correlated to BSID-II scores in this study population (Supplemental

Table 2) and thus even the study population was smaller compared to our original cohort, subpopulation in this study considered to have null bias. Our result was supported by previous report that socioeconomic status was associated with neurological functions such as language, memory, cognition and social development[34]. In this study, BSID-II score at 6 month were similar between boys and girls, however, girls showed higher BSID-II scores at 18 month. Previous study showed higher MDI and PDI among girls after first year of life but not earlier age[35] and our result was consistent with the previous report.

Kim et al.[10] found an inverse association between prenatal levels of MEHHP and MEOHP, metabolites of DEHP and MDI and PDI only in male. Tellez-Rojo et al.[12] evaluated the effects of maternal urinary concentrations of phthalate metabolites on MDI and PDI in children 24-36 months and found no significant association among all children, however, negative association between DEHP metabolites and MDI was observed only in girls. Polanska et al.[11] found that child motor development was inversely associated with sum of DEHP metabolites in maternal urine samples. A study examined infant neurodevelopment at 5 weeks newborn intensive care unit (NICU) Network Neurobehavioral Scale (NNNS) found that prenatal exposure to DEHP determined from maternal urine levels at 26 weeks of gestation was

associated with non-optimal reflexes in male infants[36]. The recent study in Taiwan[37] suggested that positive associations between maternal DEHP exposure and externalizing domain behavior problems in 8-year-old children by using Child Behavior Checklist (CBCL).

Although various epidemiological studies have been conducted on prenatal DEHP exposure and child neural and behavioral development, demographic of study population, timing of exposure and outcome measurements, assessment of outcome, and other factors have varied among studies and therefore, no clear conclusion have been found. The median concentration of MEHP was 10.6 ng/ml in this study. Compared to the study of serum MEHP measurements of pregnant women[32], the level was higher. However, the production and use of DEHP varied among countries, which could have caused differences in observed MEHP levels in blood. More than 50% of phthalate use in Japan is DEHP[38], and DEHP intake in Japanese population was higher than that of most other studies[39]. Additionally, the levels of DEHP in house dust in Japan were higher compared to the studies from other countries[40]. Previous report from our cohort population showed that prenatal DEHP exposure was not associated with infant birth size[27] suggested that MEHP levels we observed were not high enough to cause developmental adverse effects on

infant health.

No differences were found between boys and girls in relation to MEHP levels and infant neurodevelopment in this study. In several epidemiological studies, sex specific effects of prenatal phthalate exposure on child neurodevelopment were found[10, 12, 36, 41], yet results from these previous studies were inconsistent. Also the reason that we did not find significant association could be due to relatively smaller sample size of this study. Decreased PDI at 18 month among girls even it did not reach the significance may suggest there might be sex specific effect. Continuous neurodevelopmental evaluation and larger sample sizes were required to find out sex specific effects.

Maternal smoking and alcohol consumption during pregnancy, breast feeding were known important factors of infant neurodevelopment. Breast feeding also could possibly be an exposure source of DEHP. In this study, we examined the model including these factors, however, the results of analysis with and without these covariates did not show much change, and thus, those factors were excluded from the final model. We also investigated the association between maternal MEHP levels and TH of neonate and BSID-II with controlling other environmental chemical exposures including PCBs, dioxins and PFCs as they were found to be negatively

associated with neonatal TH levels and infant development[30, 31, 42]. However, the result remained unchanged and we did not find adverse effects of prenatal DEHP exposure on TH levels and BSID-II.

BSID-II mean scores in both 6 and 18 months were lower than standardized scores in this study. Since there are cultural and language differences between Japan and the United States, the BSID-II should be used with caution in Japan. Although we observed relatively lower mean BSID-II scores among our study population, Oka et al. reported high correlation between BSID-II and the Kyoto Developmental Test that was standardized in Japan[43]. Thus, BSID-II scores of our study population were considered to be validated and reasonable for using analyses.

The strength of our study was that we measured child neurodevelopment outcome by well-trained examiners and twice at different time points which allowed us to investigate the association of prenatal DEHP exposure and child neurodevelopment across the time. The limitations of this study need to be considered. In our study population, serum was used for measurements of phthalates instead of urine. Recently, some disadvantages of using serum instead of urine have had much attention[44]. It is known that hydrolytic enzymes are present in blood samples and may be responsible for diester to monoester conversion after the blood sample is

drawn[45]. Thus, levels of monoester phthalates may seem to be falsely elevated due to *ex-vivo* conversion of contamination. To reduce the influence of enzyme, blood samples were immediately stored at -80 ℃ and acid was added to samples immediately after thawing to inhibit enzyme activity. We cannot exclude the possibility of conversion of diesters from sample contamination happened between drawing of blood and analysis. In this study, all blood samples were collected at one hospital in the same way, and analyzed at the same laboratory, therefore, it is expected to be the same level of potential environmental contamination if any. Additionally findings from previous studies suggested that urinary DEHP metabolites in pregnant women were lower, particularly at the later stages of pregnancy than those in non-pregnant women, thus using urinary DEHP metabolites as exposure biomarkers needs to be cautiously implemented[46]. Other limitations were the follows. There have been concerns whether single drawing of maternal blood sample represent the long-term prenatal phthalate exposure due to short half-life of DEHP and there might be a possibility of accidental exposure near blood drawing period. Blood concentrations might change rapidly and therefore, repeated measurements would be desirable. Using secondary metabolites of phthalates was recommended due to hydrolytic enzyme activity in blood samples and several recent studies[47, 48]

successfully determined secondary metabolites of DEHP in blood samples though level of secondary metabolites in serum sample was lower than in urine sample[49]. Measurement of MEHP may be more relevant in studies investigating associations between DEHP exposure and adverse health effects as MEHP is known to be responsible for biological activities attributed to DEHP exposure[50]. Maternal blood samples were taken during the third trimester, thus, the effect of fetal exposure to DEHP during the earlier stages of fetal neurodevelopment have not been assessed in this study. Previously, influence on infant neurobehavior by phthalate was evident only with exposure measured at 26 weeks but not at 16 weeks of gestation[36], thus exposure assessment at late pregnancy might be more reasonable when comes to assessing neurodevelopment. Fetal growth is rapid during the third trimester. Biologically, little synapse formation occurs before the beginning of the third trimester, when it accelerates to approximately 40,000 synapses per minute[51] indicating that it was a relevant exposure period. There might be a chance of selection bias in this study as we only included participants with available maternal blood samples and BSID-II scores into the final analysis (N=127). Participants included in our final analysis showed higher percentage of college graduate level of education of parents, higher income level (≧ 5 million yen/year), compared to the

participants who excluded. Small sample size could be a possible reason for not observing association between prenatal DEHP exposure and neurodevelopment.

In conclusion, prenatal exposure to DEHP did not show adverse effects on neonate TH levels and infant MDI and PDI in early life stage, although prenatal PCBs, dioxins and PFCs exposure showed negative impact in the same cohort participants[30, 31, 42]. This was the first study of investigating prenatal DEHP exposure and TH levels of newborns and infant MDI and PDI at early stage of life. However, our previous study revealed negative effects of prenatal DEHP exposure on maternal fatty acids including omega 3[27] and sex hormone levels[28], continuous investigation on neurodevelopment in later life, especially peripuberty, in association with prenatal DEHP exposure is necessary.

Conflict of interest statement

None of the authors have any conflict of interest to report.

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Conflicts of Interest

None.

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Table 1 Characteristics of participants.

Parental characteristics		Mean ±SD or number (%) or Median (IQR)			p-value[b]
		All (N=328)	Boys (N=158)	Girls (N=170)	
Maternal age (years)		30.2 ± 4.7	30.5 ± 4.9	30.0 ± 4.6	0.524
Maternal education (years)	≦ 12	147 (44.8)	70 (44.3)	77 (45.3)	0.857
	> 12	181 (55.2)	88 (55.7)	93 (54.7)	
Parity	0	167 (51.1)	86 (54.8)	81 (47.6)	0.198
	≧ 1	160 (48.9)	71 (45.2)	89 (52.4)	
Maternal smoking during pregnancy	Yes	59 (18.0)	23 (14.6)	36 (21.2)	0.119
	No	269 (82.0)	135 (85.4)	134 (78.8)	
Alcohol consumption during pregnancy	Yes	108 (32.9)	105 (66.5)	53 (33.5)	0.819
	No	220 (67.1)	115 (67.6)	55 (32.4)	
Maternal work during pregnancy	Yes	31 (9.5)	15 (9.5)	16 (9.4)	0.980
	No	297 (90.5)	143 (90.5)	154 (90.6)	
Caffeine intake during pregnancy (mg/day)		143.0 ± 120.8	145.1 ± 101.2	141.1 ± 136.7	0.215
MEHP (ng/ml)		10.6 (6.3-17.1)	10.1 (6.5-15.6)	11.3 (5.9-17.8)	0.361
Maternal pre-pregnancy BMI (kg/m^2)		21.3 ± 3.3	20.9 ± 3.2	21.6 ± 3.4	0.030
Family income (million yen)	< 5	225 (68.8)	110 (69.6)	115 (68.0)	0.759
	≧ 5	102 (31.2)	48 (30.4)	54 (32.0)	
Paternal age (years)		31.9 ± 5.6	32.0 ± 5.5	31.7 ± 5.6	0.729
Paternal education (years)	≦ 12	146 (44.6)	67 (42.7)	79 (46.5)	0.491
	> 12	181 (55.4)	90 (57.3)	91 (53.5)	
Paternal smoke during pregnancy	Yes	237 (72.5)	111 (70.7)	123 (74.1)	0.490
	No	90 (27.5)	46 (29.3)	44 (25.9)	
Infant characteristics					
Birth weight (g)		3096 ± 366	3108 ± 381	3085 ± 352	0.614
Birth length (cm)		48.2 ± 1.9	48.4 ± 2.2	48.0 ± 1.5	0.005
Gestational age (days)		277.1 ± 8.9	276.2 ± 9.4	278.0 ± 8.4	0.115
Mode of delivery	Vaginal	278 (84.8)	130 (82.3)	148 (87.1)	0.229
	Cesarean section	50 (15.2)	28 (17.7)	22 (12.9)	
TSH (μU/ml)		2.20 (1.40-4.00)	2.21 (1.38-4.00)	2.30 (1.38-3.93)	0.790
FT4 (ng/ml)		2.03 (1.79-2.29)	2.02 (1.81-2.22)	2.03 (1.79-2.34)	0.437
MDI at 6 month	N=127	90.2 ± 5.4	90.3 ± 5.4	90.1 ± 5.4	0.898
PDI at 6 month	N=127	88.6 ± 10.4	87.3 ± 9.4	90.0 ± 11.3	0.132
MDI at 18 month	N=97	84.6 ± 12.4	82.2 ± 11.5	87.1 ± 12.9	0.053
PDI at 18 month	N=97	87.0 ± 11.5	84.4 ± 11.4	89.5 ± 11.1	0.029

p value obtained from Mann-Whitney U-test between boys and girls.
MEHP; Mono-2-ethylhexyl phthalate, TSH; Thyroid Stimulating Hormone, FT4; Free Thyroxine,
MDI; Mental Development Index, PDI; Psychomotor Development index

Table 2 Maternal MEHP levels in relation to participants' characteristics (N=328).

		MEHP levels (ng/ml)	
Maternal characteristics		mean ± SD	p-value
Maternal age (years)		ρ = -0.015	0.790
Maternal education (years)	≦ 12	14.3 ± 13.4	0.474
	> 12	13.4 ± 10.3	
Parity	0	13.6 ± 12.2	0.753
	≧ 1	14.0 ± 11.4	
Maternal smoking during pregnancy	Yes	15.4 ± 17.8	0.416
	No	13.4 ± 10.0	
Alcohol consumption during pregnancy	Yes	14.0 ± 12.2	0.818
	No	13.7 ± 11.6	
Maternal working during pregnancy	Yes	14.9 ± 9.4	0.585
	No	13.7 ± 12.0	
Caffeine intake during pregnancy (mg/day)		ρ = 0.080	0.148
Maternal pre-pregnancy BMI (kg/m^2)		ρ = -0.005	0.929
Family income (million yen)	< 5	13.9 ± 13.1	0.797
	≧ 5	13.6 ± 8.4	
Blood sampling period (weeks)	23-31	13.6 ± 10.1	0.002
	32-34	16.2 ± 15.0	
	35-41	12.0 ± 10.5	
Infant characteristics			
Sex	Boy	12.9 ± 11.0	0.181
	Girl	14.6 ± 12.5	
Birth weight (g)		ρ = -0.036	0.513
Birth length (cm)		ρ = -0.001	0.982
Mode of delivery	Vaginal	13.8 ± 12.1	0.971
	Cesarean section	13.7 ± 9.8	
Gestational age (days)		ρ = -0.007	0.906
TSH (μU/ml)		ρ = 0.041	0.464
FT4 (ng/ml)		ρ = 0.018	0.752

MEHP; Mono-2-ethylhexyl phthalate, TSH; Thyroid Stimulating Hormone, FT4; Free Thyroxine,

Mann-Whitney test or Kruskal-Wallis test and Spearman's coefficients.

Table 3. Association between infant thyroid hormone levels and maternal MEHP levels stratified by infant sex.

Thyroid hormone		N	β (95% CI)	p-value
TSH (μU/ml)	All	328	0.04 (-0.16, 0.23)[a]	0.719
	Boy	158	0.04 (-0.18, 0.26)[b]	0.732
	Girl	170	0.08 (-0.09, 0.25)[b]	0.381
FT4 (ng/ml)	All	328	-0.02 (-0.08, 0.03)[a]	0.361
	Boy	158	0.03 (-0.02, 0.07)[b]	0.277
	Girl	170	-0.01 (-0.05, 0.04)[b]	0.781

[a] Adjusted for infant sex, infant age (days) at hormone measurement and blood sampling period for MEHP measurement.
[b] Adjusted for infant age (days) at hormone measurement and blood sampling period for MEHP measurement.
TSH; Thyroid Stimulating Hormone, FT4; Free Thyroxine

Table 4. Association between MDI and PDI at 6 and 18 month and maternal MEHP levels stratified by infant sex.

BSID 6 month		**N**	**β** (95% CI)	**p-value**
MDI	All	127	0.64 (-0.75, 2.03)[a]	0.365
	Boy	64	-0.09 (-2.17, 1.98)[b]	0.929
	Girl	63	1.17 (-0.83, 3.17)[b]	0.247
PDI	All	127	-1.63 (-4.14, 0.88)[a]	0.202
	Boy	64	-1.99 (-5.44, 1.47)[b]	0.254
	Girl	63	-1.42 (-5.37, 2.53)[b]	0.474
BSID 18 month			**β (95% CI)[b]**	**p-value**
MDI	All	97	0.49 (-3.19, 4.17)[c]	0.791
	Boy	50	1.18 (-4.42, 6.78)[d]	0.674
	Girl	47	0.28 (-5.80, 5.25)[d]	0.920
PDI	All	97	-1.19 (-4.55, 2.17)[c]	0.483
	Boy	50	0.31 (-5.23, 5.86)[d]	0.810
	Girl	47	-2.86 (-7.54, 1.83)[d]	0.225

[a] Adjusted for infant sex, gestational age, mother's age at delivery, maternal education, blood sampling period for MEHP measurement, caffeine intake during pregnancy (only for PDI).
[b] Adjusted for gestational age, mother's age at delivery, maternal education, blood sampling period for MEHP measurement, caffeine intake during pregnancy (only for PDI).
[c] Adjusted for gestational age, mother's age at delivery, maternal education, blood sampling period for MEHP measurement, family income (only for PDI), parity (only for PDI).
[d] Adjusted for infant sex gestational age, mother's age at delivery, maternal education, blood sampling period for MEHP measurement, family income (only for PDI), parity (only for PDI).
BSID; Bayler Scale of Infant Development, MDI; Mental Development Index, PDI; Psychomotor Development index