Article title:

Effects of low-level prenatal exposure to dioxins on cognitive development in Japanese children at 42 months

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Abstract

[Background] Prenatal exposure to polychlorinated dibenzo-p-dioxins (PCDDs) or polychlorinated dibenzofurans (PCDFs) and dioxin-like polychlorinated biphenyls (dioxin-like compounds [DLCs]) through environmental chemicals may affect the neurodevelopment of children. In our previous study, an inverse association was observed between prenatal DLCs and neurodevelopment of infants aged 6 months in both sexes. However, studies are yet to determine how long these adverse effects last.

[Objective] To examine whether the effects of DLCs on cognitive development remains at 42 months.

[Methods] In this prospective cohort study conducted in Sapporo, Japan, pregnant mothers' blood was analyzed for the congener level of DLCs. The Kaufman Assessment of Battery for Children (K-ABC) was used to test their children's cognitive development at 42 months. A total of 141 mother-child pairs were included in the final analysis. The multiple linear regression analysis was used to examine the association between the K-ABC scores and DLC levels in the maternal blood.

[Results] Seven isomers (1,2,3,6,7,8-HxCDD, 2,3,4,7,8-PeCDF, 33'44'55'-HxCB(#169), 2344'5-PenCB(#114), 233'44'5-HexCB(#156), 233'44'5'-HexCB(#157), 23'44'55'-HexCB(#167), total PCDF, and TEQ-PCDD, PCDF, PCDD/DFs levels were positively associated with the achievement score (AS) of K-ABC. However, total non-ortho PCBs were negatively associated with the Mental Processing Composite Score (MPCS) of K-ABC in males. In females, increased TEQ-dl PCB and TEQ-PCDD/F/dl-PCB were also associated with increasing AS score.

[Conclusions] This study suggests that the negative effects of prenatal DLC exposure on children's cognitive development at 6 months were not observed in children aged 42 months. Regarding the sex-specific effects, AS and DLCs were positively correlated in females, whereas those of MPCS and DLCs were significantly negative in males.

Keywords: Birth-cohort study, Dioxin-like compounds, Prenatal exposure, Cognitive development, Kaufman Assessment of Battery for Children

1. Introduction

Endocrine-disrupting substances (EDCs) such as polychlorinated dibenzo-p-dioxins (PCDFs), polychlorinated dibenzofurans (PCDFs), and dioxin-like polychlorinated biphenyls (PCBs) (dioxin-like compounds [DLCs]) are considered as health hazards through various routes (e.g., fish intake, air waste pollution from an incinerator, etc.). Mother's exposure to environmental chemicals containing DLCs during and after pregnancy can affect children's neurological development. DLCs combined with the aromatic hydrocarbon receptor (AhR) are toxic, which can change the synapse pattern and affect the internal secretion and metabolism of hormones, such as the thyroid hormone, and can eventually lead to neurodevelopment (Schug et al., 2015), because thyroid hormones are important for fetal brain development.

Only three epidemiological cohort studies have been reported the association between DLCs and neurodevelopment of children (Patandin et al., 1999, Lanting et al., 1998; Wilhelm et al., 2008; Nakajima et al.,2006). In the Rotterdam study, PCB and DLCs in the breast milk, an indirect measurement of prenatal exposure, were analyzed in the breastfeeding group (n=209; dioxin toxicity equivalency quantity (TEQ) of mother's milk, 33.4 ng/kg milk fat). This study examined the cognitive development of 395 children using the K-ABC at 42 months. The K-ABC tests the intelligence and achievement of children aged 2.5 to 12.5 years (Kaufman and Kaufman, 1983) using the Mental Processing Composite Scale (MPCS) and Achievement Scale (AS) components,

respectively. Association between DLCs in the breast milk and children's cognitive development at 42 months was not observed (Patandin et al., 1999). In same study, the effects of PCB exposure in the maternal and cord blood were examined using the Touwen/Hempel neurological test. The results showed that adverse neurological effects of PCB exposure at 18 months disappeared at 42 months (Lanting et al., 1998). However, prenatal effects of DLCs remained unclear because the DLC concentrations in the maternal blood during pregnancy were not measured in the Rotterdam cohort study. In the Duisburg study, PCDD/DF and dioxin-like PCB concentrations in the maternal blood during pregnancy and breast milk of the 232 samples were measured (dioxin total TEQ of the mother's blood: median 19.3 pg/g lipid base, dioxin TEQ of mother's milk: median 19.7 pg/g lipid base). The study examined children's development using the Bayley Scales of Infant Development (BSID), which tests the mental development index (MDI) and motor development index (PDI) (Bayley, 1965) at 12 and 24 months. This study concluded that DLC concentrations in the maternal and breast milk and developmental indexes were not associated at 12 and 24 months (Wilhelm et al., 2008). However, in the Duisburg study, the effects of prenatal exposure to DLCs on developmental index at 42 months were not evaluated.

In the Sapporo cohort study, we evaluated the DLC concentration, including those of each isomer, in the maternal blood. At 6 months after birth, the BSID-II was used to evaluate the effects of prenatal exposure to DLCs. Total PCDDs, total PCDD/DFs, and one PCDD isomer were

significantly negatively associated with the MDI, and two PCDD and three PCDF isomers were significantly negatively associated with the PDI (Nakajima et al., 2006). Additionally, we found significant sex differences on the effects of prenatal exposure to PCDD, PCDF, and PCB congeners on the PDI and MDI score at 6 and 18 months (Kishi et al., 2013; Nakajima et al., 2017). Children will usually start to understand numbers, language, and hand motions at the age of 3 to 4 years and these stages are important for later development. In addition, signs of developmental disorder might appear in this stage. Miniscalo et al. (2006) prospectively studied the speech and language problems of 105 nearly 30-month-old children. Children with speech and language problems before 3 years were high risk for autism spectrum disorders or attention-deficit hyperactivity disorder, or both at 7 years of age. Clegg et al. (2004) also reported that men with developmental language disorder in middle childhood had worse social adaptation and other language-related problems. Therefore, the association between prenatal exposure to DLCs and children's development at 42 months should be evaluated. However, there is no previous study evaluating the association between DLC and isomer concentrations in the maternal blood, which is a direct indicator of prenatal exposure and children's development at 42 months. This study aimed to examine whether the negative effects of DLCs on children's cognitive development remains at 42 months using the K-ABC including sex difference.

2. Materials and Methods

2.1. Study population

We recruited pregnant women between July 2002 and October 2005 from the Sapporo Toho Hospital in Hokkaido, Japan. Details regarding the study participants and data collection based on the baseline questionnaires and medical records at birth have been previously described (Kishi et al., 2011, 2013, 2017). Among the 514 women who agreed to participate in the study, 426 women's maternal blood were analyzed for DLC concentration. A total of 333 women (66.1%) agreed to participate in the follow-up study of their children's neurodevelopment. At 6 and 18 months, children were assessed using the BSID-II (Kishi et al., 2013; Nakajima et al., 2006, 2017). Then, 151 mother–child pairs were examined using the K-ABC (45.3% of the follow-up study participants). The following eligibility criteria were used: mothers with no serious illnesses or complications during pregnancy and delivery, singleton full-term babies (37–42 weeks), infants with an Apgar score of >7 at 1 min, infants without congenital anomalies or diseases, and infants who completed the K-ABC. A total of 141 mother–infant pairs met the eligibility criteria for this study. All subjects were native Japanese residents and resided in Sapporo and the surrounding areas.

2.2. Measurement of DLC concentrations in the maternal blood

A 40-mL blood sample was taken from the maternal peripheral vein after the second

trimester during the woman's last pregnancy. Blood sampling details from mothers and specimen storage have been previously described (Nakajima et al., 2006). DLC and PCDD/DF concentrations in the maternal blood were measured using a high-resolution gas chromatography/high-resolution mass spectrometry equipped with a solvent-cut large-volume injection system (SGE Ltd., Victoria, Australia) at the Fukuoka Institute of Health and Environmental Sciences. The DLC levels were measured for each isomer (7 PCDDs, 10 PCDFs, 4 non-ortho PCBs, and 8 mono-ortho PCBs), and the total TEQ levels were calculated (Iida and Todaka, 2003; Todaka et al., 2003). TEQs are determined by summing up the toxicity levels of each dioxin compound based on the corresponding toxic equivalency factor (TEF). TEFs are estimates of dioxin toxicity relative to 2,3,7,8-TCDD toxicity, which is assigned with 1 TEF. Regarding the values below the detection limit, we inputted a value equal to half the detection limit (Longnecker et al., 2003) for each isomer's concentration.

2.3. Measurement of cognitive development

We used the Japanese version of the K-ABC to assess children's cognitive development at 42 (mean, 42.7±0.9) months of age, which was translated and standardized for Japanese children by Matsubara et al. (1993a, 1993b). This test is based on neuropsychological and information-processing theories. Intelligence or problem-solving abilities are measured using two mental processing scales: sequential and simultaneous. MPCS unifies these scales and is intended to measure total intelligence. AS provides an estimate of previous learning using subtests that

measure acquired knowledge and application of skills, such as arithmetic and reading. Both MPCS and AS were used in this study.

The children were brought to the community center in Sapporo where one examiner tested them in a quiet, private room together with their parent(s). The developmental evaluation was performed by three occupational therapists who had clinical experience in the field of developmental disabilities. The examiners were unaware of the infants' exposure levels to DLCs. For all examined children, the scoring was firstly initiated by the examiner who performed the examination and subsequently double-checked by the two other examiners based on a video recording of the examination.

2.4. Assessment of mothers' intelligence level and nursery environmental condition

When children reached 42 months old, the mothers' intelligence score and nursery environmental condition were investigated. To assess the mothers' intelligence level, the short version of the Wechsler Adult Intelligence Scale-Revised version (WAIS-R) in Japanese was used (Kobayashi et al., 1993), the most popular method in measuring one's intelligence level. The short version consists of three items, and the score for each item is summed up. To evaluate the children's nursery environmental conditions, the Index of Child Care Environment (ICCE) devised by Anme et al. (1997) was used.

2.5. Data analysis

The Spearman rank correlation coefficients and Mann–Whitney U test were used for data analysis and to determine the statistical significance, respectively. A multiple linear regression model was used to examine the association between the K-ABC scores (MPCS and AS) and DLC levels in the maternal blood. Congeners with <50% of the detection rate were excluded from a multiple linear regression model. Confounders related to K-ABC scores or total DLC concentrations were selected. The model was adjusted based on the maternal age, smoking status during pregnancy (never smoking=0, smoking/quit smoking=1), gestational age (weeks), birth weight (g), children' s age (month at examination of K-ABC), mother's WAIS-R short-version score, annual income (>5 million yen = 0, <5 million yen=1), ICCE, and blood sampling time (during pregnancy=0, after birth=1). Blood sampling time obtained during pregnancy or after delivery that caused anemia to the mothers influenced the DLC concentrations. Only 121 subjects were included in this multiple linear regression model because of some missing covariate values. Results were considered significant if $p<0.05$. All analyses were conducted using the SPSS (version 22.0; SPSS Inc., Chicago, IL, USA).

2.6. Ethical considerations

All mothers' written informed consents were obtained, and this study was approved by the Institutional Ethical Board for Epidemiologic Studies of the Hokkaido University Graduate School of Medicine, Center for Environmental and Health Sciences.

3. Results

The mothers of the subjects had the following characteristics: maternal mean age, 31.6 years old (SD=4.8); first born, 50.4%; >5-million-yen annual income, 56.7%; >13 years educational level, 34.0%; score of WAIS-R short-version of mothers, 21.4 (SD=4.0); male children, 46.8%; gestational age, 39.1 (SD=1.1) weeks; birth weight, 3130 (SD=315) g and children's age during examination, 42.7 (SD=0.9) months. The difference by sex was observed in the gestational age (weeks) ($p<0.05$) (Supplemental Table 1).

Table 1 shows PCB and PCDD/DF (picogram per gram lipid and WHO-05 [TEQ]) concentrations in the maternal blood. Concentrations of 9 congeners in the maternal blood were at <50% of the detection rate. These 9 congeners included 2,3,7,8-TCDD, 1,2,3,4,7,8-HxCDD, 2,3,7,8-TCDF, 1,2,3,7,8-PeCDF, 2,3,4,6,7,8-HxCDF, 1,2,3,7,8,9-HxCDF, 1,2,3,4,7,8,9-HpCDF, OCDF, and 344'5-TCB (#81).

Table 2 shows the relationship between K-ABC scores and maternal and child characteristics. The <5-million-yen annual income group had higher MPCS and AS scores than the >5-million-yen annual income group ($p<0.05$). The WAIS-R short-version scores of the mothers were positively related to the MPCS and AS ($r=0.340$ and $r=0.150$, respectively). Regarding the smoking status of pregnant women, the AS scores in never smoking were higher than smoking/quit

smoking ($p<0.05$). The ICCE was positively related to the MPCS ($r=0.176$).

Table 3 shows the relationship between DLC levels and K-ABC scores using the Spearman's correlation coefficient test. The relationship between MPCS and DLCs was not observed. Regarding the relationship between AS and DLCs in all subjects, 2 isomers of PCDD, 3 isomers of PCDF, 2 isomers of non-ortho PCB, 8 isomers of mono-ortho PCB, total PCDF, total non-ortho PCBs, total mono-ortho PCBs, total dioxin, and all WHO-05 [TEQ] were positively associated. In males, 1,2,3,6,7,8-HxCDD of PCDD was positively associated ($r=0.275$). In females, the result was similar in all subjects; however, the association was stronger than in all subjects, especially with mono-ortho PCB, total DLCs, and WHO-05 [TEQ].

Supplemental Table 2 shows the relationship between DLCs and subjects' characteristics. The concentrations of total PCDD, total PCDF, total PCDD/DF, total non-ortho PCBs, total mono-ortho PCBs, total coplanar PCB, and total dioxin were significantly associated with maternal age ($r=0.198$, $r=0.175$, $r=0.206$, $r=0.371$, $r=0.467$, $r=0.468$, $r=0.469$, respectively). The total PCDF was positively associated with annual income and inshore and deep-sea fish intake ($r=0.181$, $r=0.251$, $r=0.178$, respectively). However, the total PCDF was negatively associated with delivery order ($r=-0.203$). The total non-ortho PCBs, total mono-ortho PCBs, total coplanar PCB, and total dioxin were negatively related to smoking status during pregnancy ($r=-0.336$, $r=-0.312$, $r=-0.314$, respectively).

Table 4 shows the results of the multiple linear regression model for AS of K-ABC. 1,2,3,6,7,8-HxCDD of PCDD, 2,3,4,7,8-PeCDF of PCDF, 33'44'55'HxCB (#169) of non-ortho PCB, 4 isomers of mono-ortho PCB (#114, #156, #157, #167), total PCDF, PCDD-TEQ, PCDF-TEQ, and PCDD/DF-TEQ were positively associated with the AS score after adjustment. Regarding the sex difference, the effects of prenatal exposure to DLCs on the AS score in females, 2 isomers of PCDD (1,2,3,7,8-PeCDD, 1,2,3,6,7,8-HxCDD), 4 isomers of PCDF (2,3,4,7,8-PeCDF, 1,2,3,4,7,8-HxCDF, 1,2,3,6,7,8-HxCDF, 1,2,3,4,6,7,8-HpCDF), 3 isomers of non-ortho PCB (#77, #126, #169), 7 isomers of mono-ortho PCB (#105, #114, #118, #123, #156, #157, #167), total PCDF, total non-ortho PCBs, total mono-ortho PCBs, total coplanar PCB, total dioxin, and all of WHO-05 [TEQ] levels were also strongly associated. However, this relationship was not observed in males.

Table 5 shows the results of the multiple linear regression model for MPCS of K-ABC. In males, the total non-ortho PCBs was negatively associated (B=-20.250, 95% CI=-39.721; -0.779). Each congener of non-ortho PCB and mono-ortho PCB was also negatively related in males, but not significant. Association was not observed in all subjects and females.

4. Discussion

Inverse association between prenatal DLCs exposure and cognitive development in

children aged 6 months was not observed in children follow up until 42 months of age. In the present analysis, DLC isomers positively affected the AS scores of K-ABC. Additionally, sex difference was observed in the relationship between DLCs and K-ABC. AS scores show that females were strongly and positively associated. However, MPCS scores and total non-ortho PCBs show that males were negatively associated.

4.1. Effects of the DLC levels on the AS score in all subjects and females

DLC levels and AS scores remained positively associated even after adjustment (Table 4). DLC levels seemed to positively affect children's health. We speculated that the AS score of the K-ABC indicates the cognitive ability to work and resolve new challenges to process information, reflecting the degree of knowledge and skills acquired from the environment (Kaufman et al., 1993). In other words, AS scores indicate that cognitive ability was taken from a posteriori environment. The AS score was associated with the annual income, mother's WAIS-R short-version score, and maternal smoking status (Table 2). Based on the result of multiple linear regression model before and after adjusting all subjects, the ICEE was weakly attributed. In females, the annual income and birth weight were significantly attributed to AS (Supplemental Table 3). These results are similar to that reported in the previous studies that early childhood socioeconomic status and nursing environment influence the cognitive development (Linver et al., 2002; Schoon et al., 2012).

Total PCDF was significantly related, but total PCDD and PCDD/DF were not. Total

PCDF were significantly correlated to annual income, delivery order, inshore fish intake, deep-sea fish intake, except for total PCDD and PCDD/DF (Supplemental Table 2). Although we tried to analyze a multiple regression model after adjusting for these factors; the results did not change significantly.

In the Rotterdam study, no obvious adverse effects on the K-ABC at 42 months were reported (Patandin et al., 1999), which was inconsistent with our results because of different specimens. The Rotterdam study uses DLC concentrations of the breast milk as an indirect indicator of prenatal exposure; however, our study directly measures DLC concentrations in the maternal blood during pregnancy, which could provide reliable evidence. In the Duisburg study, maternal DLC and BSID scores at 12 and 24 months were not associated, and thyroid hormone levels among offspring (Wilhelm et al., 2008) suggested that adverse effects of prenatal exposure to DLCs may disappear at >12 months of age because of the current low DLC exposure.

Regarding the sex difference, DLC levels between the mothers with male children and mothers with female children were not significantly associated. We reported that 1 isomer in a 6-month-old and 6 isomers in a 18-month-old in female were significantly and positively associated with the MDI of BSID-II (Kishi et al., 2013; Nakajima et al., 2017). The New Bedford study has also reported a positive association between PCBs and continuous performance test in females (Sagiv et al., 2012). The mechanism considered, i.e., PCDD/DFs and PCBs, act as endocrine

disruptor chemicals, which might affect the steroid hormone system (Brouwer et al, 1999; Winneke et al, 2002). The relationship between umbilical cord blood testosterone levels and language delay was reported by Whitehouse (Whitehouse et al., 2012). They analyzed the bio-available testosterone (BioT) concentration of umbilical cord blood samples of 861 births and concluded that high prenatal testosterone levels are a risk factor for language delay in male, but may be a protective factor for female. Another possible reason was that AS scores including the idea on numbers and language affect more prominently in females than in males at 42 months (Yamashita et al, 1994), even after performing the standard calculation of K-ABC scores. Therefore, adverse effects of DLC exposure at low levels may be influenced by sex-hormone levels while growing in utero or other potential strong confounders, such as the nutritional information during pregnancy and after birth.

4.2. Relationship between DLC concentration and MPCS score

Relationships between DLC concentrations and MPCS scores were observed after adjustment only in males. Total non-ortho PCBs was negatively associated (B=-20.250, 95% CI=-39.721;0.779) (Table 5). In the same cohort study, 10 DLC isomers and psychomotor developmental index of BSID-II in males aged 6 months were negatively associated (Kishi et al., 2013, Nakajima et al., 2017).

Sex-specific effects of prenatal DLC exposures were reported in some studies. Regarding

the cognitive development, in Tohoku study, negative effects were observed between PCBs and K-ABC in males at 42 months (Tatsuta et al., 2014). Moreover, in the New Bedford study, PCBs and neuropsychological measures of attention were negatively associated among 8-year-old boys (Sagiv et al., 2012). As mentioned, sex difference might affect the sex-hormone levels. In addition, Megham and Heather (2016) assessed the effects of sex-specific endocrine-disrupting compounds (EDCs) in animal's studies, with the results that gestational EDC exposure can alter fetal neurogenesis and gene expression throughout the brain. The result of the present study was consistent with these studies that males may be more vulnerable to prenatal DLC exposures than females. This suggests that evaluation on the influence is necessary after 42 months of age.

The negative effects of prenatal DLC exposure observed in our previous paper (Nakajima et al., 2006; Kishi et al., 2013) were weakly observed at 42 months of age. Compared with the subjects at 6 months from the previous study (Nakajima et al., 2006, 2017), our subjects were benefited by additional social and environmental factors for children's development, such as educational level, annual income, fish intake frequency, and smoking status during pregnancy. Therefore, the results of both studies cannot be compared directly.

4.3. Other factors associated with DLC exposure

To evaluate the effects of DLCs, many studies had considered MeHg. Furthermore, maternal fish intake is associated with DLCs and mercury exposure

during pregnancy (Miyashita et al., 2015a, 2015b; Grandjean et al., 1997). In our cohort study, the mercury level of mothers' hair (median 1.33 ppm [minimum-maximum 0.46–7.55 ppm) and the fatty acid level of maternal blood were examined (Kishi et al., 2013, 2015; Miyashita et al., 2015a). We have also examined the association between in utero exposure to PCBs, MeHg, and polyunsaturated fatty acids and the birth size, and consequently observed positive association with MeHg. The beneficial effect of essential nutrition may mask the adverse effects of MeHg on the birth size (Miyashita et al., 2015b). Other potential factors increasing the AS scores are maternal seafood intake, which might affect the neurodevelopment (Suzuki.et al, 2010). Fish contains a long-chain polyvalent unsaturated fatty acid, which is an important nutrient for nerve development during the early fetal period (Gil and Gil, 2015; Nyradi et al., 2013). In the present study, consumption of inshore and deep-sea fish was related to total PCDF (Supplemental Table 2). Therefore, we tried to analyze and adjusted the maternal fatty acid and mercury levels; however, slight changes in the DLC results including the isomers were insignificant (data not shown).

4. 4. Strengths and limitations of the study

Our study is one of the few that evaluated the effects of DLC exposure on the cognitive development in each isomer level. Additionally, our evaluation of the cognitive development was

examined objectively by three occupational therapists. To avoid tester bias, the examiners were unaware of the infants' DLC levels. The scoring performed by the examiner was double-checked by the other two examiners based on the video recording of the examination. We also examined the mother's intelligence and educational levels using the WAIS-R short-version score as a confounding variable. Lastly, we analyzed the fatty acid level of the maternal blood, even though significant effects to infants' cognition level were not observed.

One limitation of this study was the sample size. The original 333 subjects (66.1% of all who completed the questionnaire) agreed to participate in the follow-up study of their child's neurodevelopment at recruitment; however, only 151 mother–child pairs were examined using the K-ABC (45.3% of the follow-up study participants). The annual income of non-participants and participants at 42 months of age was significantly different. The 42-month-old subjects had <5,000,000 yen than those in non-participants. Therefore, this study population was biased for economic advantage and environmental conditions.

4.6. Conclusions

This study suggests that the negative effects of prenatal DLC exposure on children's cognitive development at 6 months were not observed at 42 months of age. However, the AS score of K-ABC and DLCs were positively associated. Regarding sex-specific effects, the relationships between AS and DLCs were strongly positive in females, whereas those of MPCS and DLCs were

significantly negative in males.

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Table 1. Concentration of dioxin-like PCB and PCDD/DF (pg/g lipid) in maternal blood in 42 months (N=141)

	Detection limit[a]	25th[b]	50th[b]	75th[b]
PCDD				
2,3,7,8-TCDD	1	ND	ND	1.4
1,2,3,7,8-PeCDD	1	3.1	4.1	5.4
1,2,3,4,7,8-HxCDD	2	ND	ND	2.3
1,2,3,6,7,8-HxCDD	2	10.3	13.9	18.5
1,2,3,7,8,9-HxCDD	2	ND	2.2	3.3
1,2,3,4,6,7,8-HpCDD	2	18.6	24.3	31.3
OCDD	4	333.4	437.7	581.3
PCDF				
2,3,7,8-TCDF	1	ND	ND	ND
1,2,3,7,8-PeCDF	1	ND	ND	ND
2,3,4,7,8-PeCDF	1	4.3	5.9	7.5
1,2,3,4,7,8-HxCDF	2	ND	2.4	3.3
1,2,3,6,7,8-HxCDF	2	2.1	2.7	3.6
2,3,4,6,7,8-HxCDF	2	ND	ND	ND
1,2,3,7,8,9-HxCDF	2	ND	ND	ND
1,2,3,4,6,7,8-HpCDF	2	ND	2.4	3.4
1,2,3,4,7,8,9-HpCDF	2	ND	ND	ND
OCDF	4	ND	ND	ND
Non-ortho PCB				
33'4'4'-TCB(#77)	10	5.0	12.5	15.6
344'5-TCB(#81)	10	ND	ND	ND
33'44'5-PenCB(#126)	10	24.1	38.0	58.6
33'44'55'-HxCB(#169)	10	19.7	26.8	36.1
Mono-ortho PCB				
233'44'-PenCB(#105)	10	996.3	1480.9	2232.6
2344'5-PenCB(#114)	10	238.7	361.9	544.0
23'44'5-PenCB(#118)	10	4041.6	6142.8	9263.6
2'344'5-PenCB(#123)	10	73.1	118.9	170.6
233'44'5-HexCB(#156)	10	1370.7	2045.1	2964.3

	233'44'5'-HexCB(#157)	10	330.4	511.9	748.6
	23'44'55'-HexCB(#167)	10	511.6	799.2	1110.7
	233'44'55'-HpCB(#189)	10	161.1	236.5	346.2
Total					
	Total PCDD		367.1	488.5	641.1
	Total PCDF		16.2	19.4	24.4
	Total PCDD/DF		383.7	508.3	666.0
	Total Non-ortho PCBs		59.2	84.6	113.2
	Total Mono-ortho PCBs		8028.6	12060.8	17656.4
	Total Coplanar PCB		8100.8	12119.9	17762.6
	Total Dioxin		8580.0	12577.2	18466.8
WHO-05					
	Total PCDD TEQ		5.4	7.3	9.5
	Total PCDF TEQ		2.0	2.6	3.4
	Total PCDD/DF TEQ		7.5	10.1	12.5
	Total non-ortho PCB TEQ		3.0	4.8	6.9
	Total mono-ortho PCB TEQ		0.2	0.4	0.5
	Total coplanar PCB TEQ		3.2	5.2	7.4
	Total Dioxin TEQ		11.0	15.4	20.3

Abbreviations: DLC, dioxin-like compound; PCDD, polychlorinated dibenzo-p-dioxin; PCDF, polychlorinated dibenzofuran; PCBs, polychlorinated biphenyls; WHO, World Health Organization; TEQ, toxic equivalent; ND, non-detectable

[a]For subjects with a level below the detection limit, we inputted a value equal to half the detection limit.

[b]Percentiles.

Table 2. The relationship between K-ABC scores, and maternal and child characteristics

		N	MPCS		AS	
			Mean ±SD, r[1)]	p[2)]	Mean ±SD, r[1)]	p[2)]
Maternal characteristics						
Age (years)		141	r=-0.127		r=0.147	
Delivery order						
	First child	71	102.9±12.2		99.8±14.0	
	Second child over	70	103.6±13.5		99.9±14.5	
Annual income (yen)						
	> 5,000,000	80	101.4±13.4	*	97.2±12.7	*
	< 5,000,000	61	105.7±11.7		103.3±15.4	
WAIS-R short-version score		129	r=0.340	***	r=0.150	*
Inshore fish						
	0–0.5 times/week	87	103.6±12.8		99.4±14.1	
	1–2 times/week	54	102.6±12.8		100.5±14.5	
Deep-sea fish						
	0–0.5 times/week	66	103.3±13.5		100.6±14.9	
	1–2 times/week	75	103.2±12.2		99.2±13.6	
Smoking during pregnancy						
	Never	71	103.0±13.1		102.3±13.8	*
	Smoking/quit smoking	70	103.5±12.5		97.3±14.3	
Child Characteristics						
Sex						
	Male	66	104.1±12.7		98.8±14.8	

	Female	75	102.5±12.9		100.8±13.7
Gestational age (weeks)		141	r=0.085		r=0.037
Birth weight (g)		141	r=0.063		r=0.008
ICCE		137	r=0.176	*	r=0.148
Children's months age at examination		134	r=0.094		r=0.013
K-ABC score		141	103.2± 12.8		99.8 ± 14.2

Abbreviation: K-ABC, The Kaufman Assessment of Battery for Children; MPCS, Mental processing composite scale; AS, Achievement scale; WAIS-R, Wechsler Adult Intelligence Scale-Revised version; ICCE, Index of Child Care Environment

1) r=Spearman's rank correlation coefficient test.

2) p-value were calculated by Spearman’s rank correlation test, Mann–Whitney U-test.

*p<0.05, **p<0.01; ***p<0.001

※Educational level, Alcohol intake during pregnancy, Blood cotinine level before pregnancy, Duration of breast milk were not related with MPCS and AS.

Table 3 The relationship between DLC levels and K-ABC scores used by Spearman's correlation coefficient test

	All N=141		Male N=66		Female N=75	
	MPSC	AS	MPSC	AS	MPSC	AS
PCDD						
1,2,3,7,8-PeCDD	-0.006	0.242 **	0.119	0.125	-0.108	0.349 **
1,2,3,6,7,8-HxCDD	0.022	0.277 **	0.104	0.275 *	-0.054	0.283 *
1,2,3,7,8,9-HxCDD	0.048	0.157	0.205	0.145	-0.103	0.176
1,2,3,4,6,7,8-HpCDD	-0.043	0.081	0.024	0.029	-0.104	0.146
OCDD	-0.084	0.103	-0.026	0.054	-0.121	0.171
PCDF						
2,3,4,7,8-PeCDF	0.010	0.273 **	0.062	0.221	-0.022	0.320 **
1,2,3,4,7,8-HxCDF	0.042	0.190 *	0.165	0.163	-0.062	0.215
1,2,3,6,7,8-HxCDF	0.019	0.179 *	0.056	0.144	-0.012	0.221
1,2,3,4,6,7,8-HpCDF	0.077	0.138	0.066	0.025	0.094	0.281 *
	0.110	0.145	0.140	0.212	ND	ND
Non-ortho PCB						
33'4'4'-TCB(#77)	-0.104	-0.027	-0.048	-0.198	-0.141	0.146
33'44'5-PenCB(#126)	-0.036	0.171 *	-0.015	-0.037	-0.018	0.359 **
33'44'55'-HxCB(#169)	-0.046	0.309 ***	-0.068	0.160	-0.017	0.429 ***
Mono-ortho PCB						
233'44'-PenCB(#105)	-0.027	0.182 *	-0.008	-0.047	-0.021	0.384 **
2344'5-PenCB(#114)	-0.026	0.268 **	-0.034	0.098	-0.012	0.425 ***

	23'44'5-PenCB(#118)	-0.002	0.225	**	0.030	0.046	-0.011	0.401	***
	2'344'5-PenCB(#123)	0.016	0.236	**	0.054	0.047	-0.019	0.401	***
	233'44'5-HexCB(#156)	-0.062	0.292	***	-0.130	0.132	-0.001	0.431	***
	233'44'5'-HexCB(#157)	-0.043	0.289	**	-0.111	0.130	0.013	0.442	***
	23'44'55'-HexCB(#167)	-0.023	0.277	**	-0.081	0.082	0.027	0.455	***
	233'44'55'-HpCB(#189)	-0.122	0.206	*	-0.177	0.131	-0.079	0.312	**
Total									
	Total PCDD	-0.074	0.112		-0.006	0.057	-0.118	0.184	
	Total PCDF	0.055	0.254	**	0.075	0.126	0.044	0.362	**
	Total PCDD/DF	-0.071	0.120		-0.003	0.058	-0.113	0.201	
	Total Non-ortho PCBs	-0.073	0.216	*	-0.078	0.019	-0.040	0.384	**
	Total Mono-ortho PCBs	-0.028	0.244	**	-0.042	0.054	-0.004	0.418	***
	Total Coplanar PCB	-0.029	0.244	**	-0.043	0.051	-0.005	0.420	***
	Total Dioxin	-0.032	0.241	**	-0.047	0.054	-0.007	0.412	***
WHO-05									
	Total PCDD TEQ	-0.002	0.271	**	0.117	0.184	-0.115	0.353	**
	Total PCDF TEQ	0.010	0.262	**	0.053	0.184	-0.030	0.328	**
	Total PCDD/DF TEQ	0.004	0.281	**	0.110	0.195	-0.093	0.368	**
	Total non-ortho PCB TEQ	-0.052	0.198	*	-0.031	0.003	-0.033	0.380	**
	Total mono-ortho PCB TEQ	-0.028	0.244	**	-0.042	0.054	-0.004	0.418	***
	Total coplanar PCB TEQ	-0.054	0.204	*	-0.038	0.010	-0.030	0.392	**
	Total Dioxin TEQ	-0.034	0.256	**	0.028	0.121	-0.079	0.358	**

Abbreviation: K-ABC, The Kaufman Assessment of Battery for Children; MPCS, Mental processing composite scale; AS, Achievement scale; DLC, dioxin-like compound; PCDD, polychlorinated dibenzo-p-dioxin; PCDF, polychlorinated dibenzofuran; PCBs, polychlorinated biphenyls; WHO, World Health Organization; TEQ, toxic equivalent

Table 4. The results of multiple linear regression model for AS of K-ABC

		All				Male				Female			
		B[a]	95% CI			B[a]	95% CI			B[a]	95% CI		
PCDD	1,2,3,7,8-PeCDD	13.486	-0.407	; 27.378		4.953	-21.444	; 31.350		23.784	8.014	; 39.553	**
	1,2,3,6,7,8-HxCDD	17.066	4.731	; 29.401	**	22.366	-0.941	; 45.673		18.940	4.388	; 33.492	*
	1,2,3,7,8,9-HxCDD	8.202	-2.092	; 18.496		8.154	-9.424	; 25.732		11.999	-1.344	; 25.341	
	1,2,3,4,6,7,8-HpCDD	8.404	-8.953	; 25.761		4.359	-24.502	; 33.221		17.331	-5.160	; 39.823	
	OCDD	11.477	-5.836	; 28.789		10.223	-21.255	; 41.701		19.870	-1.794	; 41.534	
PCDF	2,3,4,7,8-PeCDF	14.373	0.996	; 27.750	*	7.178	-21.239	; 35.595		25.278	10.683	; 39.873	**
	1,2,3,4,7,8-HxCDF	8.877	-1.990	; 19.744		9.940	-10.401	; 30.281		17.207	3.803	; 30.610	*
	1,2,3,6,7,8-HxCDF	10.395	-0.626	; 21.416		9.451	-10.789	; 29.691		18.014	4.680	; 31.348	**
	1,2,3,4,6,7,8-HpCDF	8.798	-0.348	; 17.945		8.659	-7.307	; 24.626		15.799	4.183	; 27.415	**
Non-ortho	33'4'4'-TCB(#77)	0.476	-12.286	; 13.237		-17.031	-38.787	; 4.726		18.171	2.180	; 34.162	*
PCB	33'44'5-PenCB(#126)	5.747	-4.880	; 16.374		-11.083	-31.619	; 9.454		18.097	6.362	; 29.832	**
	33'44'55'-HxCB(#169)	17.107	4.383	; 29.831	**	4.392	-22.739	; 31.523		27.374	14.226	; 40.522	***
mono-ortho	233'44'-PenCB(#105)	5.927	-6.563	; 18.416		-12.674	-34.916	; 9.569		21.998	7.516	; 36.479	**
PCB	2344'5-PenCB(#114)	15.867	1.792	; 29.942	*	-1.922	-28.885	; 25.041		29.109	13.660	; 44.559	***
	23'44'5-PenCB(#118)	9.507	-3.080	; 22.094		-8.192	-32.282	; 15.898		22.968	8.985	; 36.951	**
	2'344'5-PenCB(#123)	8.745	-3.088	; 20.577		-6.382	-30.507	; 17.743		19.419	6.221	; 32.616	**
	233'44'5-HexCB(#156)	17.211	2.981	; 31.441	*	0.382	-27.850	; 28.614		31.432	16.230	; 46.634	***
	233'44'5'-HexCB(#157)	19.131	5.015	; 33.247	**	0.288	-29.695	; 30.271		31.572	17.075	; 46.068	***
	23'44'55'-HexCB(#167)	15.349	2.064	; 28.634	*	-5.928	-33.551	; 21.696		29.912	16.025	; 43.799	***
	233'44'55'-HpCB(#189)	10.027	-2.272	; 22.325		7.812	-19.422	; 35.046		13.502	-0.646	; 27.650	

Total	Total PCDD	12.381	-5.272	;	30.035		10.847	-21.025	;	42.720	21.184	-0.842	;	43.210	
	Total PCDF	21.011	1.957	;	40.064	*	15.644	-17.649	;	48.936	41.691	18.057	;	65.325	**
	Total PCDD/DF	12.896	-5.017	;	30.809		11.168	-21.222	;	43.558	22.216	-0.059	;	44.491	
	Total Non-ortho PCBs	11.640	-3.194	;	26.475		-10.785	-38.996	;	17.427	30.079	13.836	;	46.322	***
	Total Mono-ortho PCBs	12.190	-1.508	;	25.888		-7.868	-34.879	;	19.144	26.715	11.881	;	41.549	**
	Total Coplanar PCB	12.199	-1.518	;	25.915		-7.907	-34.950	;	19.137	26.769	11.917	;	41.621	**
	Dioxin	12.521	-1.637	;	26.679		-8.226	-36.205	;	19.752	27.456	12.191	;	42.721	**
WHO-05	Total PCDD TEQ	15.858	0.813	;	30.902	*	9.628	-18.271	;	37.528	25.740	8.327	;	43.153	**
	Total PCDF TEQ	16.471	0.722	;	32.220	*	8.714	-21.559	;	38.986	31.527	13.380	;	49.674	**
	Total PCDD/DF TEQ	16.727	1.165	;	32.288	*	9.868	-19.269	;	39.004	28.183	10.241	;	46.124	**
	Total non-ortho PCB TEQ	7.939	-3.603	;	19.481		-10.166	-32.932	;	12.600	21.126	8.616	;	33.637	**
	Total mono-ortho PCB TEQ	12.190	-1.508	;	25.888		-7.868	-34.879	;	19.144	26.715	11.881	;	41.549	**
	Total coplanar PCB TEQ	8.228	-3.561	;	20.016		-10.349	-33.641	;	12.943	21.678	8.920	;	34.436	**
	Total Dioxin-TEQ	15.515	-0.035	;	31.065		1.310	-28.400	;	31.020	30.335	12.953	;	47.717	**

Abbreviation: K-ABC, The Kaufman Assessment of Battery for Children; AS, Achievement scale; DLC, dioxin-like compound; PCDD, polychlorinated dibenzo-p-dioxin; PCDF, polychlorinated dibenzofuran; PCBs, polychlorinated biphenyls; WHO, World Health Organization; TEQ, toxic equivalent; WAIS-R, Wechsler Adult Intelligence Scale-Revised version; ICCE, Index of Child Care Environment;

[a] B was adjusted for, mother's age, smoking status(non-smoking=0), gestational age(weeks), birth weight(g), annual income (under 500 million yen=0), mother's WAIS-R short-version scores, ICEE score, children's month age at examination, blood sampling time(during pregnancy=0).

DLCs were transferred to logarithm. The model was also adjusted to determine the influence of sex differences in all subjects.

*p<0.05, **p<0.01, ***p<0.001

Table 5. The results of multiple linear regression model for MPCS of K-ABC

		All		Male		Female	
		B[a]	95% CI	B[a]	95% CI	B[a]	95% CI
PCDD	1,2,3,7,8-PeCDD	0.660	-11.060 ; 12.381	2.733	-16.241 ; 21.707	-2.411	-17.904 ; 13.082
	1,2,3,6,7,8-HxCDD	2.846	-7.721 ; 13.414	5.659	-11.681 ; 22.999	-1.837	-15.870 ; 12.197
	1,2,3,7,8,9-HxCDD	3.059	-5.561 ; 11.679	8.130	-4.383 ; 20.642	-1.910	-14.392 ; 10.572
	1,2,3,4,6,7,8-HpCDD	1.425	-13.037 ; 15.888	7.653	-12.973 ; 28.278	-6.384	-27.211 ; 14.443
	OCDD	-4.080	-18.540 ; 10.379	2.454	-20.253 ; 25.161	-5.147	-25.406 ; 15.112
PCDF	2,3,4,7,8-PeCDF	3.638	-7.669 ; 14.946	-2.500	-22.957 ; 17.958	4.420	-10.209 ; 19.050
	1,2,3,4,7,8-HxCDF	2.526	-6.586 ; 11.639	5.586	-9.087 ; 20.258	-1.687	-14.593 ; 11.220
	1,2,3,6,7,8-HxCDF	-0.519	-9.809 ; 8.771	1.811	-12.860 ; 16.482	-4.899	-17.752 ; 7.955
	1,2,3,4,6,7,8-HpCDF	3.015	-4.678 ; 10.709	5.511	-5.991 ; 17.012	-1.548	-12.807 ; 9.711
Non-ortho PCB	33'4'4'-TCB(#77)	-5.216	-15.761 ; 5.330	-14.420	-29.880 ; 1.039	-3.367	-18.563 ; 11.830
	33'44'5-PenCB(#126)	1.350	-7.511 ; 10.211	-13.228	-27.635 ; 1.179	6.033	-5.427 ; 17.493
	33'44'55'-HxCB(#169)	1.465	-9.427 ; 12.356	-12.604	-31.746 ; 6.537	5.922	-7.733 ; 19.577
Mono-ortho PCB	233'44'-PenCB(#105)	-0.366	-10.772 ; 10.040	-14.678	-30.277 ; 0.920	7.112	-7.010 ; 21.234
	2344'5-PenCB(#114)	3.049	-8.880 ; 14.978	-8.793	-27.985 ; 10.398	5.699	-10.021 ; 21.419
	23'44'5-PenCB(#118)	2.598	-7.943 ; 13.138	-11.472	-28.522 ; 5.577	7.758	-6.015 ; 21.530
	2'344'5-PenCB(#123)	2.600	-7.303 ; 12.503	-11.653	-28.681 ; 5.376	6.498	-6.312 ; 19.308
	233'44'5-HexCB(#156)	0.895	-11.218 ; 13.008	-11.862	-31.827 ; 8.102	5.554	-10.246 ; 21.354
	233'44'5'-HexCB(#157)	3.383	-8.692 ; 15.459	-12.023	-33.256 ; 9.210	6.211	-9.029 ; 21.451

	23'44'55'-HexCB(#167)	3.319	-7.948	;	14.586	-14.967	-34.336	;	4.402		9.458	-4.960	;	23.876
	233'44'55'-HpCB(#189)	-3.921	-14.221	;	6.380	-8.003	-27.490	;	11.485		-2.911	-16.182	;	10.359
Total	Total PCDD	-3.730	-18.492	;	11.032	3.131	-19.864	;	26.125		-5.700	-26.349	;	14.949
	Total PCDF	5.245	-10.876	;	21.367	2.908	-21.228	;	27.044		1.593	-22.254	;	25.440
	Total PCDD/DF	-3.562	-18.550	;	11.426	3.130	-20.242	;	26.502		-5.615	-26.551	;	15.321
	Total Non-ortho PCBs	-0.765	-13.210	;	11.680	-20.250	-39.721	;	-0.779	*	6.462	-9.993	;	22.916
	Total Mono-ortho PCBs	1.685	-9.838	;	13.208	-14.421	-33.410	;	4.569		7.378	-7.516	;	22.272
	Total Coplanar PCB	1.672	-9.867	;	13.210	-14.487	-33.496	;	4.523		7.384	-7.531	;	22.299
	Total Dioxin	1.479	-10.430	;	13.389	-15.101	-34.761	;	4.559		7.363	-7.968	;	22.694
WHO-05	Total PCDD TEQ	0.320	-12.410	;	13.051	3.912	-16.202	;	24.025		-4.552	-21.581	;	12.476
	Total PCDF TEQ	3.550	-9.756	;	16.855	-1.567	-23.388	;	20.255		3.369	-14.870	;	21.607
	Total PCDD/DF TEQ	1.176	-12.000	;	14.353	2.839	-18.181	;	23.859		-2.855	-20.588	;	14.878
	Total non-ortho PCB TEQ	1.035	-8.622	;	10.692	-15.521	-31.349	;	0.306		6.425	-5.977	;	18.828
	Total mono-ortho PCB TEQ	1.685	-9.838	;	13.208	-14.421	-33.410	;	4.569		7.378	-7.516	;	22.272
	Total coplanar PCB TEQ	1.056	-8.810	;	10.921	-15.813	-32.011	;	0.385		6.542	-6.120	;	19.204
	Total Dioxin-TEQ	0.906	-12.226	;	14.037	-7.053	-28.291	;	14.184		1.402	-16.100	;	18.905

Abbreviation: K-ABC, The Kaufman Assessment of Battery for Children; MPCS, Mental Processing composite scale; DLC, dioxin-like compound; PCDD, polychlorinated dibenzo-p-dioxin; PCDF, polychlorinated dibenzofuran; PCBs, polychlorinated biphenyls; WHO, World Health Organization; TEQ, toxic equivalent; WAIS-R, Wechsler Adult Intelligence Scale-Revised version; ICCE, Index of Child Care Environment;

[a]B was adjusted for, mother's age, smoking status(non-smoking=0), gestational age (weeks), birth weight (g), annual income (under 500 million yen=0), mother's WAIS-R short-version scores, ICEE score, children's month age at examination, blood sampling time (during pregnancy=0).

DLCs were transferred to logarithm. The model was also adjusted to determine the influence of sex differences in all subjects.

*p<0.05, **p<0.01, ***p<0.001