Cord blood BPA level and child neurodevelopment and behavioral problems: The Hokkaido Study on Environment and Children's Health

Machiko Minatoya[a], Atsuko Araki[a], Sonomi Nakajima[b], Seiko Sasaki[c], Chihiro Miyashita[a], Keiko Yamazaki[a], Jun Yamamoto[d], Toru Matumura[d], Reiko Kishi[a]

[a] Center for Environmental and Health Sciences, Hokkaido University, Kita 12, Nishi 7, Kita-ku, Sapporo 060-0812, Japan
[b] School of Health Sciences, Sapporo Medical University, Minami 1, Nishi 17, Chuo-ku, Sapporo 060-0061, Japan.
[c] Department of Public Health, Graduate School of Medicine, Hokkaido University, Kita 15, Nishi 7, Kita-ku, Sapporo 060-8638, Japan
[d] Institute of Environmental Ecology, Idea Consultants, Inc., 1334-5 Riemon, Yaizu, Shizuoka, 421-0212, Japan.

Correspondence
Reiko Kishi, MD, PhD, MPH
Tel.: +81-11-706-4746
Fax: +81-11-706-4725
Email: rkishi@med.hokudai.ac.jp
Mailing address: Kita 12, Nishi 7, Kita-ku, Sapporo 060-0812, Japan

Introduction

Child mental and behavioral disorders are growing concern in public health as the prevalence is increasing and its impact on child, family and community and even economy is enormous (Perou et al. 2013). The prevalence of childhood and adolescent mental disorders including behavioral problems has been reported around 20% (WHO 2005). There are multiple factors including socioeconomic and environmental factors that play a role in etiology of mental disorders.

Bisphenol A (BPA) is a ubiquitous environmental chemical used in manufacture polycarbonate plastics, epoxy resins, dental sealants and thermal paper (Vandenberg et al. 2007). A recent biomonitoring study reported that BPA was detectable in dust, air particles and water (Asimakopoulos et al. 2012). Even though BPA use in infant feeding bottles have been banned in the US and Europe, it is still used in many of manufactural products. A study suggested that canned food was responsible for 10-40% of the daily BPA intake (von Goetz et al. 2010). In the late 1990s, Japanese can manufacturers had developed “BPA reduced cans” by shifting of can coatings from epoxy resin to polyethylene terephthalate PET film or epoxy resin with lower BPA levels (Kawamura et al. 2014) and that led to a significant decrease in the levels of urine BPA (Matsumoto et al. 2003). It is difficult

to determine a level of BPA that has no risk to children, pregnant women and other populations based on current scientific knowledge. BPA is widely detected from different populations including children and pregnant women (Covaci et al. 2015; Yamamoto et al. 2016).

BPA has endocrine disrupting property and been recognized as a suspected agent causing developmental neurotoxicity (Grandjean and Landrigan 2014). Animal studies suggested that *in utero* exposure to BPA may produce adverse effects including impaired brain development, sexual differentiation, behavior (Kundakovic and Champagne 2011). Some evidences of changes in gene expression and DNA methylation in estrogen signaling pathways and estrogen receptors support these effects of BPA exposure on neurodevelopment (Kundakovic et al. 2013; Vandenberg et al. 2009; Wetherill et al. 2007).

Epidemiological studies reported that prenatal BPA exposures were associated with various health outcomes including behavioral problems in children (Braun et al. 2009; Braun et al. 2011; Casas et al. 2015; Harley et al. 2013; Miodovnik et al. 2011; Perera et al. 2012; Roen et al. 2015). Yet findings from these studies were inconsistent. Exposure assessment, the time window of exposure, the sex-specific directions of the associations, the specific behavioral domain and

sociodemographic of the populations varied from study to study (Mustieles et al. 2015). Previously, we reported that BPA level detected in our cohort population was relatively lower compared to other studies (Yamamoto et al. 2016). Relatively lower level exposure to BPA and its health effects on humans are still largely unknown. Based on animal studies, BPA exposure at a very sensitive time in development can lead long-lasting developmental adverse effects (Xu et al. 2010), and thus investigating association between early life exposure to BPA and child neuro and behavioral development is warranted.

In this study, we conducted various neuropsychological tests covering wide range of mental, psychomotor, intelligence and behavioral domains at different ages in early life. The aim of this study was to evaluate whether cord blood BPA level was associated with neural and behavioral development of children at 6, 18 and 42 months of age in a longitudinal birth cohort study.

Methods

Study population

A detailed description of the Hokkaido Study on Environment and Children's Health and study design can be found elsewhere (Kishi et al. 2011; Kishi et al. 2013). 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 (Sapporo Cohort). All subjects were residents in Sapporo City or surrounding areas. The participants completed the self-administered questionnaire at the time of enrollment regarding lifestyle and demographic characteristics including educational level, annual household income, smoking habit and alcohol intake, food consumption during pregnancy and medical history. Their medical records including maternal age, maternal anthropometric measures before and during pregnancy, pregnancy complications, parity were obtained. Child sex and gestational age were obtained from birth record. 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.

BPA measurement

Cord blood samples were obtained at birth and stored at −80 °C until analysis. Sample storage glassware was heated at 400 °C for 4 h to avoid potential environmental contamination of BPA. All glassware used in extraction procedure was washed with acetone. Potential contamination of samples with BPA from external

source was evaluated with additional control blank experiments. All the possible external sources of BPA contamination were eliminated by using only baked glassware for storage of samples, using glass cartridge instead of polypropylene one, which contained BPA. Free BPA concentration in cord blood samples (n = 10) were analyzed to verify no external BPA contamination. Thus, we confirmed total BPA in cord blood was a valid biomarker of BPA exposure.

The total BPA measured using solid-phase extraction coupled with isotope dilution liquid chromatography tandem mass spectrometry (ID-LC/MS/MS) at Idea Consultants, Inc. (Shizuoka, Japan). Briefly, to 1.0ml of sample, BPA-d_{16} β-glucuronidase spiking solution was added then incubated at 37 °C for 1.5 h followed by solid-phase extraction. BPA-d_4 was added to each sample prior to LC/MS/MS analysis. The detailed condition and peak can be found elsewhere (Yamamoto et al. 2016). The limit of quantification (LOQ) was 0.040 ng/ml. Concentrations below LOQ were given a value of one half of LOQ for statistical analysis.

Outcomes

Developmental assessment

We used Bayley Scale of Infant Development second edition (BSID-II)

(Bayley 1993) 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 1 and 42 months 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. Mental and psychomotor scores were based on the calibration scale from a raw score and are represented as index scores. The mean scores of MDI and PDI (± SD) are 100 (± 15). The developmental evaluation was performed by three occupational therapists who have clinical experience in the field of developmental disabilities (Nakajima et al. 2006).

Intellectual assessment

At child age of 42 months, we used the Kaufman Assessment Battery for Children (K-ABC) (Kaufman and Kaufman 1983). The K-ABC was designed to assess intelligence of children ages between 2.5 to 12.5 years. The Japanese version of K-ABC was translated and standardized by Matsubara et al (Matsubara et al. 1993a; Matsubara et al. 1993b). The K-ABC is comprised of four test scores including; simultaneous processing scales, sequential processing scales, mental processing composite and achievement scales. The simultaneous and sequential processing

scales are combined to comprise the mental processing composite. The mental processing composite score is considered the global estimate of a child's level of intellectual functioning. The achievement scales measure achievement and focus on applied skills and facts that were learned through the school or home environment. Subscale scores of K-ABC were based on the calibration scale from a raw score and are represented as index scores. The mean scores are 100 (± 15). The developmental evaluation was performed by two occupational therapists.

Behavioral problem assessment

The Japanese version of Child Behavior Checklist (CBCL/2-3) was sent by mail to the all participants in the follow up and completed by mothers to assess child behavioral problems at age 42 months (Nakata et al. 1998). CBCL/2-3 includes 100 items rated by mothers on a three-point scale; not true (0), somewhat true (1), very or often true (2). The CBCL provides eight syndrome scales (anxious/neurotic, withdrawn behavior, separation anxiety, sleep/eating, development, attention, oppositional, aggressive/destructive) grouped by three composite scales (Internalizing- sum of scores on the anxious/neurotic, withdrawn behavior and separation anxiety; Externalizing- sum of scores on the attention, oppositional and aggressive/destructive; and the Total- sum of scores of all eight syndrome scales

scores), reported as both raw scores and sex- and age- normalized T scores (Achenbach and Ruffle 2000; Nakata et al. 1998).

For MDI and PDI of BSID-II, the higher score indicated advanced development. Similarly, higher scores in K-ABC subscales indicated advanced intellectual development. For CBCL, higher scores indicated more parent-reported behavioral problems.

Statistical Analysis

Out of 514 participants in the Hokkaido Study (Sapporo cohort), 285 had cord blood BPA concentration measurements. Of these, 150 children successfully completed the BSID-II at 6 months of age, 105 BSID-II at 18 month of age, 86 K-ABC at 42 month of age, 181 CBCL at 42 month of age. 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), babies without congenital anomalies or diseases. BPA concentration was log 10 transformed for statistical analyses. According to the literature (Teramoto et al. 2005), for CBCL, either T score or abnormal range over certain T scores ($T \geqq 64$ for 3 composite scores, $T \geqq 71$ for 8 syndrome scores) was applied in multi-linear regression models and multi-logistic

regression models, respectively. Linear regression models were used to examine the associations between cord blood BPA concentrations and child neurobehavioral development test scores. Test scores were analyzed as continuous variables. Based on the previous related literatures, covariates were selected as follows; maternal smoking during pregnancy, parental education, family income, duration of breast feeding, child sex, index of child care environment (ICEE) (Anme et al. 1997) and testers (only for BSID-II and K-ACB). Additionally, Spearman's correlation was tested in association with neurobehavioral test scores and covariates with $p < 0.10$ as mentioned below were also included into each model (Table S3). For analyses of BSID-II, gestational age was added as a covariate. For analyses of CBCL, maternal pre-pregnancy BMI was additionally used as covariate. For K-ABC analyses, maternal alcohol consumption and smoking during pregnancy were added as covariates. In addition, testers of BSID-II and K-ABC were used as a covariate. Growth parameters such as birth weight and birth length were investigated in correlation with child developmental scores, however, none of the parameters were significantly associated with test score, thus these were not included as covariates in this study. Results were considered significant at $p < 0.05$. All analyses were conducted using SPSS (Version 22.0; SPSS, Chicago, IL, USA).

Results

Study population and exposure characteristics

Characteristics of participants included in this study were shown in Table 1. More girls were included in this study population than boys (55.4% vs. 44.6%). Maternal smoking during pregnancy and drinking during pregnancy were 16.1% and 34.4%, respectively. More than 70% were family income < 5 million yen/year. Both maternal and paternal education ≧ 13 years were more than half of this study population. The characteristics of participants at each behavioral test were presented in supplemental Table S2.

BPA was detected in 69.8% of cord blood samples. The median of cord blood BPA was 0.051 ng/ml (IQR:<LOQ-0.076 ng/ml).

Developmental, intellectual and behavioral problems

The distribution of BSID-II scores were shown in Table 2. The median scores of MDI and PDI at 6 months were 90 and 88, respectively, and at 18 months were 83 and 90, respectively. No associations were observed between cord blood BPA concentrations and MDI and PDI at 6 and 18 months of age (Table 2) and any subscale scores of K-ABC at 42 months of age (Table S4). The distribution of CBCL

scores were shown in Table 3. The median scores of all 8 syndrome scores were 50 and none of the participants had above abnormal range (T ≧ 71) scores except attention problems. Cord blood BPA concentration was positively associated with total problems score (β=4.77, 95% CI: -0.28, 9.82), internalizing problems score (β =4.35, 95% CI: -0.48, 9.18) and external problems score (β=4.33, 95% CI: -0.86, 9.25) of CBCL with marginal significance (Table 3). Further, cord blood BPA concentration was positively associated with development problems score (β=2.60, 95% CI: 0.15, 5.06) with significance and attention problems score (β=3.88, 95% CI: -0.14, 7.90) with marginal significance. No association was observed with other subscales.

Table 4 showed the associations between cord blood BPA concentration and CBCL scores by child sex stratification. The positive association between cord blood BPA and total problems score was significant only in girls (β=6.88, 95% CI: 0.65, 13.12). No significant association was found in 8 syndrome scores.

Discussion

The results from this study suggested that cord blood BPA level did not adversely affect children's mental and psychomotor development up to 18 months of age. However, cord blood BPA level may be associated with child behavioral

problems at 42 months of age.

Several studies also have determined cord blood BPA levels (Aris 2014; Chou et al. 2011; Kosarac et al. 2012). Compared to these studies, BPA concentration in cord blood was lower in this study. Considering these together, the exposure levels of BPA in this study population were considered to be relatively lower.

To date, very limited number of studies that evaluated infant mental and psychomotor development in association with maternal urine BPA levels. The previous study showed no association between maternal urine BPA concentrations and child cognitive scores at 1 and 4 years of age (Casas et al. 2015). In their study, the highest exposure to BPA showed adverse effects on psychomotor scores only at 1 year of age and diminished at age 4, however, regression analysis of BPA concentrations as continuous variables did not show the significance (Casas et al. 2015). Both their and our studies have used face-to-face developmental test by experts thus evaluation seemed to be more accurate than those conducted by parental reports. Findings from both studies indicate that prenatal exposures to BPA have null or very small impact on child cognitive or mental development.

Prenatal BPA exposures and child behavioral problems have been

investigated in several birth cohort studies. In this study, we found that cord blood BPA level was positively associated with total and internalizing problems scores and additionally development and attention scores of CBCL. Our result showed increased internalizing problems score, which may signal greater vulnerability to the subsequent developmental problems in adolescent and adulthood (Paus et al. 2008). Compared to the previous study using CBCL at the similar age range to our study (Perera et al. 2012), the findings were inconsistent. Perera et al. found that high maternal urine BPA level was associated with significantly higher CBCL scores on emotionally reactive and aggressive behavior syndromes among boys. Contrary, higher BPA level was associated with lower scores on anxious/depressed and aggressive behavior among girls (Perera et al. 2012). Braun et al. reported that adverse effects of prenatal BPA exposure measured in maternal urine predominantly in girls (Braun et al. 2009; Braun et al. 2011). Specifically, adverse effects on externalizing behavior in 2-year-old girls but not boys using the Behavioral Assessment System for Children (BASC) (Braun et al. 2009) and on anxiety, depression, and hyperactivity among girls but not boys at the age 3 years were found (Braun et al. 2011). Other epidemiological studies reported that BPA exposure was associated with internalizing problems (Evans et al. 2014; Harley et al. 2013;

Roen et al. 2015).

We should note that when we compare the results of these previous studies, timing of sample collection and specimen were completely different. They used maternal spot urine of average 34 weeks of gestation (Perera et al. 2012) and 16 and 26 weeks of gestation (Braun et al. 2009; Braun et al. 2011), respectively, while we used cord blood for BPA exposure assessment. Maternal urine samples during gestational period were used as fetal BPA exposure assessment in most of the prospective studies. Cord blood directly measures the fetal exposure to BPA however, only can reflect BPA exposure of perinatal period but not gestational period. Most of the previous studies found associations related to prenatal BPA exposure indicating a possible critical window of exposure, however, BPA exposure at delivery was obtained in this study. Our findings may indicate that perinatal exposure to BPA was associated with postnatal child behavioral problems. Additionally, characteristics including ethnicity, smoking habit, socio economic status (SES) such as educational levels, family income varied among studies. The studies with non-smoking mothers (Perera et al. 2012) or with better education and wealthier participants (Braun et al. 2009; Braun et al. 2011) may have contributed to the different findings on BPA exposures and child developmental outcomes. Most of these previous studies were

conducted in the US that was considered to have relatively higher BPA exposure compared to our study, which may also be able to explain differences in findings. In addition, smaller study population in this study might be one of the important reasons to observe different results from the previous finding.

There were prospective studies that have investigated child behavior in association with prenatal BPA exposure. Most of these studies reported increased behavioral problems in boys and decreased in girls (Casas et al. 2015; Evans et al. 2014; Harley et al. 2013; Roen et al. 2015). Studies showing increased behavioral problems in boys and decreased in girls assessed behavioral outcomes relatively older ages (school age; 6- to 10- year-old). In this study, we investigated CBCL scores in association with BPA levels stratified by child sex since some of the CBCL scores were associated with BPA levels overall. Significance between cord blood BPA and total problems score was only observed in girls, which may indicate sex-specific effects at 4 years of age. Child age in this study was relatively younger compared to these studies with sex-specific findings only among boys. This may imply that age at testing was important for assessing sex-specific effects on behavioral problems. Further study to elucidate whether sex-specific effects of prenatal BPA exposure could depend on the child developmental stage.

A number of animal studies reported associations between gestational BPA exposures and neurological systems including brain development and behaviors (Inadera 2015). BPA has various complex mechanisms of action to interfere with brain and behavior development. To the date, several main mechanisms of BPA action that may be related to neurodevelopment has been suggested. BPA binds to estrogen receptors (ERs) and exerts agonist/antagonist actions (Welshons et al. 2006). BPA is also an antagonist of the androgen receptor (AR) (Wetherill et al. 2007). BPA binds to sex hormone-binding globulin (SHBG), which may cause androgen-estrogen imbalance and interfere with neuroendocrine regulation of the hypothalamus-pituitary-axis (Gore 2010). Endocrine-related action mechanism may also involve the aryl hydrocarbon receptor (AhR) (Bonefeld-Jorgensen et al. 2007). Moreover, BPA binds to thyroid receptors (TRs), acting as an antagonist (Moriyama et al. 2002). BPA has been found to alter glucocorticoid-regulated responses, affecting the sexual differentiation of brain and behavior in rodents (Poimenova et al. 2010). Epigenetic mechanisms of action include the alternation of some DNA methylation patterns (Susiarjo et al. 2013). Together with our previous study of investigation on neonatal reproductive and thyroid hormone levels in association with cord blood BPA levels that found basically no association (Minatoya et al. 2017),

relatively lower level of BPA exposure maybe not be responsible for disrupting either reproductive and thyroid hormone levels or child neural development.

The strength of this study was that child neurodevelopmental assessments were conducted by trained experts using validated test tools and also at several different ages.

We should acknowledge several limitations as well. The small sample size in this study could be a major limitation because the reliability and the validity of findings were limited. The follow up rate of cohort participants was 83.9% at child age of 42 months. The number of participants included in the statistical analyses was reduced due to decrease in response rate (76.8% for CBCL) and some difficulties in conducting face-to-face developmental test of children. The other limitation is BPA exposure misclassification. We measured BPA level only once. Single measurement of cord blood sample may not represent long-term prenatal BPA exposure due to short half-lives of BPA and there might be a possibility of accidental exposure near blood drawing period. However, in this study, only vaginal birth was included and chances were small that medical equipment such as syringe, tubes which possibly contained BPA was used during the process of delivery. In addition, we used cord blood samples to assess BPA exposure instead of urine

samples as other studies. We did not collect maternal urine samples. BPA concentration in blood samples possibly be overestimated due to external contamination. However, BPA concentration in blood samples can be accurately assayed without contamination according to NIH round robin study (Vandenberg et al. 2014). And in fact, assay contamination is well controlled in most labs (vom Saal and Welshons 2014). In this study, we used glass cartridge to reduce background levels and no free BPA was detected, which was indication of null possible external contamination. Thus, using blood samples as BPA exposure measurement considered to be reasonable. There might be a concern that our study population were biased. Particularly, only very small percentages of children were above abnormal range in CBCL. Thus, the associations found in this study might be underestimated. However, in terms of other characteristics, previously reported predictors of higher BPA levels including smoking and maternal education (Arbuckle et al. 2015; Casas et al. 2013) were not significantly different between the populations included in this study and not (Table S1). Other characteristics that have been reported to be related to child development and behavioral problems such as gender (Wu et al. 2012) also did not differ between two groups. Therefore, we consider our population had null bias and findings from this study can be generalized

in Japanese pregnant women. Additionally, the sex ratio of this study population might possibly be biased to girl in this examination. However, we did not find any plausible reason to observed sex ratio differences from the original cohort population and considered this happened only by chance. Finally, we cannot eliminate the possible influence of other suspected environmental chemical exposures such as pesticides, lead, and cadmium.

Conclusion

In this study, we evaluated child developmental and behavioral outcomes using various assessment tools at different ages. There was no association between cord blood BPA level and child developmental outcomes. However, cord blood BPA level may be associated with behavioral problems of children.

Conflict of interest

The authors declare no conflict of interest.

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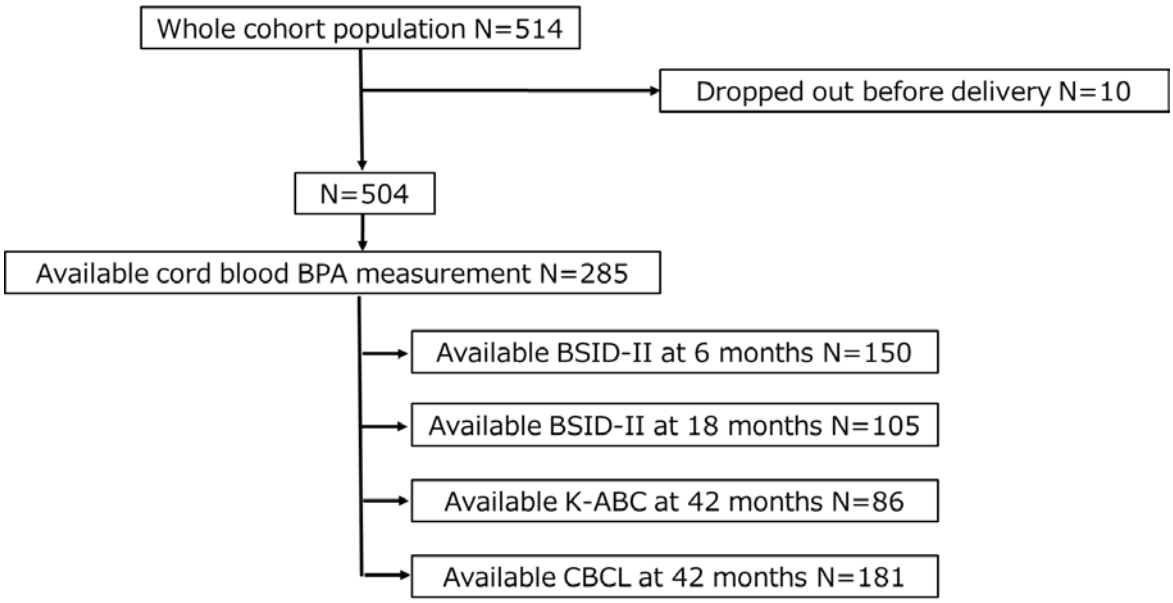

Figure 1 Selection of study population.

Table 1 Characteristics of the study population (n=285)

		N (%) or mean ± SD
Child		
Sex	Boys	127 (44.6)
	Girls	158 (55.4)
Mother		
Age (years)		30.4 ± 5.0
Pre-pregnancy BMI (kg/m^2)	< 18.5	46 (16.1)
	18.5-25.0	217 (76.1)
	≧ 25.0	21 (7.4)
Parity	0	147 (51.6)
	≧ 1	138 (48.4)
Education (years)	≦ 12	130 (45.6)
	≧ 13	155 (54.5)
Smoking during pregnancy	No	239 (83.9)
	Yes	46 (16.1)
Alcohol consumption during pregnancy	No	187 (65.6)
	Yes	98 (34.4)
Family income (million yen/year)	< 5	202 (70.9)
	≧ 5	81 (28.4)
Gestational age (days)		278 ± 7
Father		
Age (years)		31.8 ± 5.7
Education (years)	≦ 12	128 (44.9)
	≧ 13	156 (54.7)

The following categories had missing values; pre-pregnancy BMI (n = 1), family income (n = 2), paternal education (n = 1).

Table 2 Distribution of BSID-II scores and associations between cord blood BPA concentration and BSID-II scores

Age (n)	Subscale	Median (range)	β (95% CI)
6 months (n=150)	MDI	90 (72-107)	-1.29 (-5.22, 2.64)
	PDI	88 (53-114)	-0.08 (-6.98, 6.82)
18 months (n=105)	MDI	83 (55-109)	4.21 (-3.26, 11.67)
	PDI	90 (59-110)	2.43 (-6.11, 10.97)

Adjusted for maternal smoking during pregnancy, parental education, family income, gestational age, child sex, index of child care environment (ICEE), duration of breast feeding and tester.

BSID-II: Bayley Scales of Infant Development second edition, MDI: Mental development Index, PDI: Psychomotor development index.

Table 3 Distribution of CBCL scores and associations between cord blood BPA concentration and CBCL scores

Age (n)	Subscale	Median (range)	β (95% CI)
42 months (n=181)	**Composite scores**		
	Total problems	46 (25-68)	4.77 (-0.28, 9.82)$^{+}$
	Internalizing problems	47 (32-71)	4.35 (-0.48, 9.18)$^{+}$
	Externalizing problems	45 (29-69)	4.33 (-0.86, 9.25)$^{+}$
	Syndrome scores		
	Anxious/neurotic	50 (50-69)	0.50 (-1.77, 2.77)
	Withdrawn behavior	50 (50-74)	1.09 (-1.20, 3.37)
	Separation anxiety	50 (50-70)	0.48 (-2.12, 3.08)
	Sleep/eating	50 (50-69)	0.27 (-1.42, 1.95)
	Development	50 (50-70)	2.60 (0.15, 5.06)*
	Attention	50 (50-100)	3.88 (-0.14, 7.90)$^{+}$
	Oppositional	50 (50-69)	1.24 (-1.09, 3.57)
	Aggressive/destructive	50 (50-67)	1.69 (-0.43, 3.80)

Adjusted for maternal smoking during pregnancy, paternal education, family income, maternal pre-pregnancy BMI, child sex, index of child care environment (ICEE), duration of breast feeding.

* $P < 0.05$, $^{+}$ $p < 0.10$.

CBCL: Child behavior checklist

Table 4 Associations between cord blood BPA concentration and CBCL scores stratified by child sex.

	β (95% CI)	
	Boys (n=79)	Girls (n=102)
Composite scores		
Total problems	-0.19 (-9.15, 8.78)	6.88 (0.65, 13.12)*
Internalizing problems	1.37 (-6.67, 9.42)	5.07 (-1.11, 11.25)
Externalizing problems	0.72 (-8.12, 9.66)	5.84 (-0.21, 11.89)+
Syndrome scores		
Anxious/neurotic	0.10 (-3.93, 4.13)	-0.40 (-3.01, 2.22)
Withdrawn behavior	2.21 (-1.84, 6.25)	0.23 (-2.59, 3.04)
Separation anxiety	-0.29 (-4.57, 3.99)	0.72 (-2.58, 4.02)
Sleep/eating	0.04 (-3.20, 3.27)	0.02 (-1.82, 1.87)
Development	3.61 (-0.95, 8.17)	2.38 (-0.59, 5.35)
Attention	4.63 (-2.29, 11.54)	1.83 (-2.22, 5.88)
Oppositional	1.01 (-3.06, 5.09)	1.07 (-1.80, 3.93)
Aggressive/destructive	1.75 (-2.15, 5.64)	1.65 (-0.96, 4.25)

Adjusted for maternal smoking during pregnancy, paternal education, family income, maternal pre-pregnancy BMI, index of child care environment (ICEE), duration of breast feeding.

* $P < 0.05$, + $p < 0.10$.

CBCL: Child behavior checklist