Prenatal exposure to bisphenol A and phthalates and behavioral problems in children at preschool age: The Hokkaido Study on Environment and Children's Health

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Abstract

Studies reported adverse behavioral development including internalizing and externalizing problems in association with prenatal exposure to bisphenol A (BPA) and phthalates, however, findings were not sufficient due to using different assessment tools and child ages among studies. This study aimed to examine associations between maternal serum levels of BPA and phthalate metabolites and behavioral problems at preschool age.

The Strengths and Difficulties Questionnaire (SDQ) was used to assess behavioral problems at 5 years of age. BPA and phthalate metabolite levels in the 1st trimester maternal serum was determined by LC-MS/MS for 458 children. Variables used for adjustment were parental ages, maternal cotinine levels, family income during pregnancy, child sex, birth order and age at SDQ completed.

The median concentrations of BPA, MnBP, MiBP, MEHP and MECPP were 0.062, 26.0, 7.0, 1.40, and 0.20 ng/ml, respectively. BPA level was associated with increased hyperactivity/inattention risk among girls (OR=1.66, 95% CI: 0.95-2.90) and Σ DBP_m (MnBP + MiBP) level was associated with decreased total difficulties risk overall and among girls (OR=0.48, 95% CI: 0.20-1.13, OR=0.24, 95% CI: 0.06-1.03, respectively) without significance. MECPP level was associated with increase conduct problems risk (OR=2.78, 95% CI: 1.36-5.68).

Our analyses found no significant association between BPA or summation of phthalate metabolite levels and any of the behavioral problems at 5 years of age, however, suggested possible association

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between MECPP levels and increased risk of conduct problems.

Keywords: SDQ, bisphenol A, phthalates, prenatal exposure, birth cohort, behavioral problems

Introduction

It has been reported that developmental disabilities have increased in recent decades [1,2]. Childhood behavioral problems have influence on individual development, school performance and quality of life. BPA and phthalates are ubiquitous environmental chemicals that were detected from various specimen including urine, blood, breast milk and anomic fluid [3,4]. BPA is widely used in polycarbonate products, epoxy resins as coatings on the inside of many food and beverage cans [5]. There are variety of phthalates used in consumer products such as food packages, polyvinyl chloride floor materials, lotion and fragrances. Humans are exposed to phthalates by multiple routes. Exposures can be oral or dermal or can also be via inhalation [6]. Since BPA and phthalates can cross the placenta[7,8], exposure during critical period in fetal development is a concern [6,7].

Exposure to environmental chemicals such as bisphenol A (BPA) and phthalates may play roles in the development of child behavioral problems [9,10]. BPA and phthalates are both known as endocrine disruptors and there is a growing concern of exposure to these chemicals and adverse health outcomes on human. From laboratory studies, BPA has been shown to disrupt brain function and structure [11-13].

Previously several birth cohort studies have investigated associations between BPA and phthalates exposures and child behavioral problems. For example, maternal levels of BPA have been associated with various child behavioral outcomes including behavioral problems, internalizing and externalizing

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problems, cognitive development, anxiety and so on in early childhood [14-20]. Maternal levels of phthalate including di-2-ethylhexyl phthalate (DEHP), butylbenzyl phthalate (BBzP), and dibutyl phthalates (DBP) were associated with adverse child neurodevelopmental outcomes including internalizing and externalizing problems, however, findings from these studies were inconsistent as the age of children at testing, testing tools, and outcomes varied from study to study [20-24]. Additionally, some of these studies found association only in specific child sex.

The present study examined the association of maternal levels of BPA and phthalates with child behavioral problems at preschool age using Strength and Difficulty Questionnaire (SDQ), a widely-used assessment tool of child behavioral problems[25].

Methods

Study design and selection of study population

This study formed part of a prospective birth cohort study, the Hokkaido Study on Environment and Children's Health. The details of cohort profile can be found in elsewhere [26,27]. Briefly, the subpopulation consisted of cohort study participants who were born between April 2008 and June 2010 were included in this study. Total 3054 SDQ were distributed via mail between October 2014 and June 2015 to the subpopulation. 2032 SDQ was successfully filled and returned by the end of July 2015 (response rate =66.6%). Among 2032 children with valid completed SDQ, 1622 were classified

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into normal group and 411 were classified into borderline/clinical group based on total difficulties score of SDQ. Then we applied criteria for selecting participants to conduct exposure assessment. The criteria were follows; those who had maternal 1st trimester baseline questionnaire data, 1st and 3rd trimester maternal blood samples, maternal and cord blood samples at delivery, birth record, follow-up questionnaires data at ages 1,2, and 4 years of age to use as covariates. Further, we decided to include all the children in borderline/clinical group and randomly selected children in normal group (n=572). Finally, 14 children were excluded due to not enough serum volume for exposure assessment. This was nested case control study of 245 children in normal group as control and 213 children in borderline/clinical group as cases (Fig. 1).

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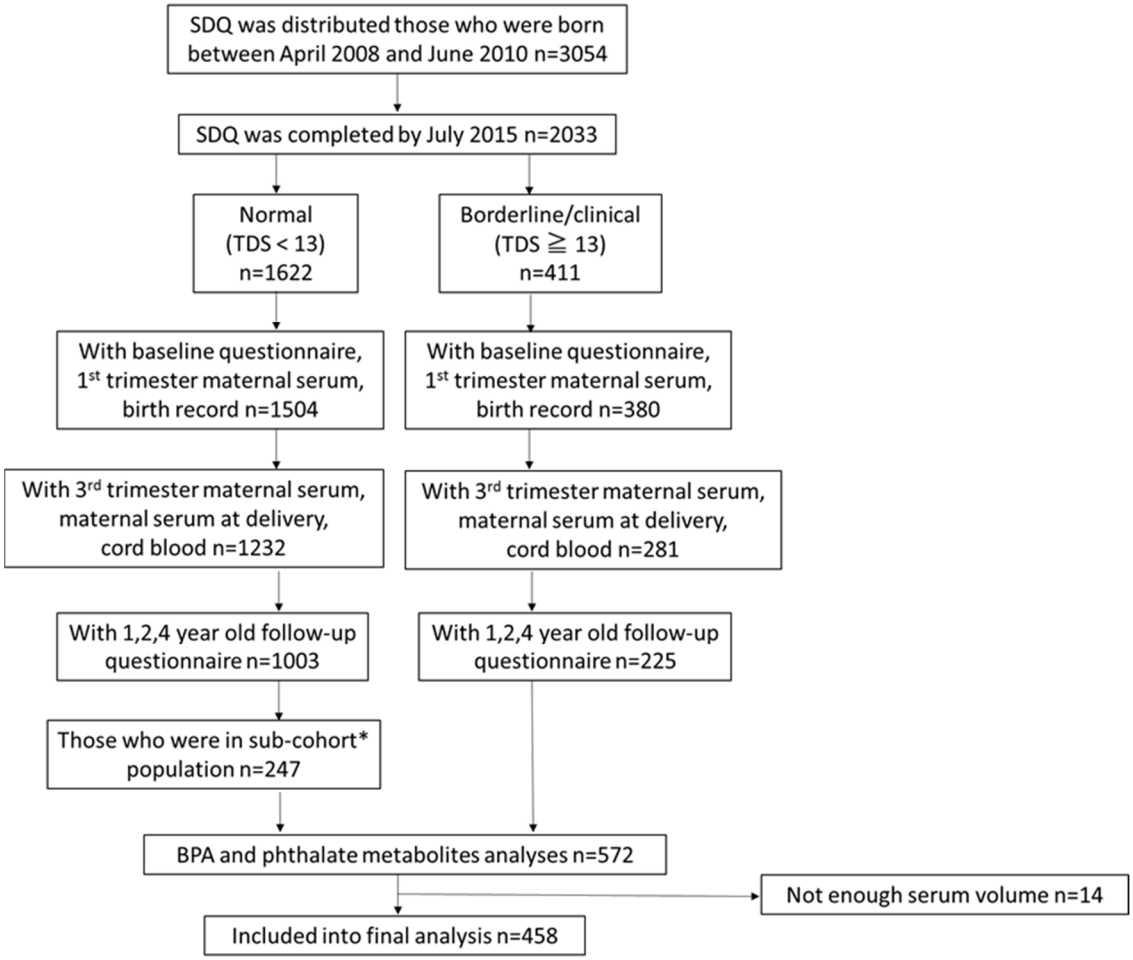

Figure 1 Selection of study population.

*The sub-cohort of 4869 participants, which corresponded to 23.3% of all participants (n=20926) in the Hokkaido study were established. In this sub-cohort, 500 participants who were randomly selected from each enrollment year between 2003 and 2011, and all 369 participants from the enrollment year 2012 were include. The sub-cohort population was supposed to be representing original cohort population. The aim of establishing the sub-cohort population was for effective exposure assessments.

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 and Hokkaido University Center for Environmental

and Health Sciences.

Assessment of child behavior

Japanese parent-report version of SDQ [28] were distributed via mail to the participants. Parents were asked to fill SDQ, which included 25 items on specific strengths and difficulties with an overall rating of whether their child had behavioral problems. SDQ was designed for a broad range of children, age 3 to 16 years and well validated tool of childhood mental health [25,29]. Each item has three response categories (0) not true, (1) somewhat true, (3) certainly true. It includes five subscales (conduct problems, hyperactive/inattention, emotional problems, peer problems and prosocial behavior). All subscale scores excluding prosocial behavior were summed as total difficulties score (ranged from 0 to 40 [29]) to assess the behavioral problems. Higher scores denote greater problems. We applied score bandings of the Japanese version of SDQ, children total difficulties with 0-12 were defined as normal, 13-15 were as borderline, and 16-40 were as clinical [28]. For the subscales, the following cut-offs were applied; Conduct problems: 0-3 = normal, 4 = borderline, 5-10 = clinical; Hyperactivity/inattention: 0-5 = normal, 6 = borderline, 7-10 = clinical; Emotional problems; 0-3 = normal, 4 = borderline, 5-10 = clinical; Peer problems: 0-3 = normal, 4 = borderline, 5-10 = clinical; Prosocial behavior; 6-10 = normal, 5 = borderline, 0-4 = clinical[28]. SDQ total and subscale scores were dichotomized comparing the children with borderline and clinical scores with normal children.

Exposure assessment

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Maternal serum of the 1st trimester was collected and stored at – 80 ℃ till analyses. Blood samples were analyzed for BPA and seven kinds of phthalate metabolites; mono-n-butyl phthalate (MnBP), mono-isobutyl phthalate (MiBP), mono-2-ethylhexyl phthalate (MEHP), mono-benzyl phthalate (MBzP), mono-2-ethyl-5-hydroxyhexyl phthalate (MEHHP), mono-2-ethyl-5-carboxypentyl phthalate (MECPP) and mono (4-methyl-7-carboxyheptyl) phthalate (cx-MiNP) by isotope-diluted liquid chromatography-tandem mass spectrometry (LC-MS/MS) for BPA analysis and ultra-performance LC-MS/MS for phthalate metabolites analysis. The method detection limits (MDLs) of BPA, MnBP, MiBP, MBzP, MEHP, MEHHP, MECPP, cx-MiNP were 0.011, 0.57, 0.44, 0.19, 0.31, 0.23, 0.11 and 0.12 ng/ml, respectively. All the analyses were conducted at Idea Consultants Inc. (Shizuoka, Japan). The detailed sample preparation for BPA analysis can be found from our previous report[30,31]. Briefly to each serum sample, BPA-d_{16} β-glucuronidase spiking solution was added and shaken then β-glucuronidase and 0.2 M acetate buffer solution (pH 5.0) were added. Samples were held in an incubator at 37 ℃ for 1.5 hrs followed by solid phase extraction. The detailed phthalate metabolites analyses are described in our previous article[31]. Briefly, serum samples for phthalate metabolites analyses were prepared as follows. MnBP-d_4, MiBP-d_4, MBzP-d_4, MEHP-d_4, MEHHP-$^{13}C_4$, MECPP-$^{13}C_4$, cx-MiNP-d_4 were added as surrogate and then 90 µL of 1M phosphoric acid was added to the serum sample (0.5 mL). After mixing by vortex and ultrasonic irradiated for 10 minutes and consequently, 940 µL of acetonitrile was added and centrifuged with 3,500 rpm for 5 min. Supernatants were transferred into new tubes

and added 1000 µL of ammonium acetate buffer solution (100 mM, pH 9.1), 3,000 µL of ammonium acetate buffer solution (100 mM, pH 6.5), and 10 µL of β-glucuronidase were added to each sample for the enzymatic hydrolysis of the phthalate metabolites conjugates, and 100 mM ammonium acetate solution were added. Samples were held in an incubator at 37 ℃ for 1.5 hrs followed by solid phase extraction by Oasis MAX 96 well plate (30mg, 30um, Waters, Milford, MA, USA). After solid phase extraction, a 500 uL of elution was transferred into sample vials and added 500 µL of ultra-pure water and analyzed by UPLC (ACQUITY UPLC H-Class, Milford, MA, USA) coupled to triple quadrupole tandem MS (QTRAP 6500, AB SCIEX, Framingham, MA). The insoluble particulates were filtered by in-line filters (2.1×5 mm, 1.7 um, Vanguard Phenyl column, Waters, Tokyo, Japan) preceding the BEH Phenyl column (2.1×50 mm, 1.7 um, Waters, Tokyo, Japan). The retention gap technique was used by installing retention gap columns Atlantis T3 (2.1×50 mm, 3 µm, Waters, Tokyo, Japan), which improved phthalate metabolites sensitivity by trapping mobile-phase phthalate metabolites (contaminants) in the retention gap column. The column temperature was 40℃. The total UPLC cycle time was 20 min including column re-equilibration. The calibration curve was linear over a concentration ranging from 0.02 to 20 ng/ml with a coefficient of correlation (r^2) greater than 0.999. The procedural blank levels were determined using 0.5 mL of ultrapure water. The MDLs of BPA and phthalate metabolites were calculated as follows according to the procedure of the manual of Analyses of Chemicals by the Ministry of Environment of Japan[32].

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Covariates

Parental factors including ages, educational levels, maternal pre-pregnancy BMI, parity, and family income were obtained from baseline questionnaire which was filled by participants during their pregnancy. Additionally, maternal smoking status was examined from cotinine levels of third trimester maternal blood measured by using high-sensitive enzyme-linked immunosorbent assay (ELISA). The limit of detection (LOD) was 0.12 ng/ml. According to previous finding[33], we defined cotinine levels $\leqq$ 0.21 ng/ml as non-smokers, 0.22-11.47 ng/ml as passive smokers, and $\geqq$ 11.48 ng/ml as active smokers. Gestational age, birth weight and gender of children were obtained from birth record.

Data analysis

Statistical analyses were performed using SPSS 22.0J (IBM Japan, Tokyo, Japan). Logistic regression models were used to calculate odds ratios (ORs) for having borderline/clinical scores (cases) in relation to maternal BPA and phthalates levels. The main analysis was case control study based on total difficulties scores. Then, 4 of the component subscales of total difficulties score (conduct problems, hyperactivity/inattention, emotional symptoms, and peer problems) were investigated as sub-analyses. Prosocial behavior was not considered as outcome because it is not the component subscales of total difficulties score, which was our main outcome. Maternal BPA and phthalates levels were $\log_{10}$ transformed and treated as continuous variables. The BPA and phthalates levels below

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MDL were replaced half the values of MDLs for statistical analyses. MEHP and MECPP were combined and expressed as the summation of DEHP metabolites (Σ $DEHP_m$). MEHHP was also a DEHP metabolite; however, in this study population, the detection rate was low, and thus, it was not included in the summation of DEHP metabolites. Similarly, MnBP and MiBP were combined and expressed as the summation of DBP metabolites (Σ DBP_m). To combine the metabolites, the summation of each metabolite expressed in molar concentration was multiplied with their respective parent molecular weight (MW) as follows:

$$\Sigma DEHP_m=((C_{MEHP}/MW_{MEHP})+(C_{MECPP}/MW_{MECPP}))*MW_{DEHP}$$

$$\Sigma DBP_m=((C_{MnBP}/MW_{MnBP})+(C_{MiBP}/MW_{MiBP}))*MW_{DBP}$$

where C is the measured concentration (ng/ml) and MW is the molecular weight (ng/nmol)

The ORs were given for one-unit increase on log_{10} scale. Covariate included in the final models were identified a priori using directed acyclic graph: parental ages (continuous), maternal cotinine levels ($\leqq$ 0.21ng/ml vs. 0.22-11.47ng/ml vs. $\geqq$11.48ng/ml), family income during pregnancy (< 5M vs. $\geqq$ 5M) and birth order (first vs. not first). In addition to above mentioned covariates, we included child sex and child age (months) at SDQ completed in the models based on previous literature. Further analysis was conducted for stratification of child sex. P-value of <0.05 was considered statistically significant.

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Results

Table 1 shows the comparison of characteristics of participants in two groups (normal vs. borderline/clinical). Both maternal and paternal ages were younger in borderline/clinical group compared to normal group. Maternal pre-pregnancy BMI was higher in borderline/clinical group. Percentage of family income during pregnancy < 5 million Japanese Yen was higher in borderline/clinical group. Percentage of maternal cotinine level ≧ 11.48 ng/ml (active smokers) was higher in borderline/clinical group. Child characteristics including gestational age, birth weight and age at SDQ completed were not different between two groups. The percentages of being first child and boy gender were higher in borderline/clinical group.

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245 Table 1 Basic characteristics of parents and their children.

Characteristics		Normal (n=245)	Borderline/clinical (n=213)
Maternal age (years)		31.5 ± 4.3	29.8 ± 4.8
Paternal age (years)		33.4 ± 5.5	31.3 ± 5.1
Maternal pre-pregnancy BMI (kg/m²)		20.7 ±2.5	21.4 ±3.3
Maternal cotinine levels at 3rd trimester	**≦ 0.21 (non-smoker)**	151 (61.6)	97 (45.5)
(ng/ml)	**0.22-11.47 (passive smoker)**	81 (33.1)	93 (43.7)
	≧ 11.48 (active smoker)	13 (5.3)	23 (10.8)
Maternal education (years)	**≦ 12**	88 (35.9)	92 (43.2)
	≧ 13	154 (62.8)	118 (55.4)
	Missing	3 (1.2)	3 (1.4)
Paternal education (years)	**≦ 12**	89 (36.3)	86 (40.4)
	≧ 13	154 (62.9)	123 (57.7)
	Missing	2 (0.8)	4 (1.9)
Family income during pregnancy (JPY)	**< 5M**	125 (51.0)	133 (62.4)
	≧5M	90 (36.7)	48 (22.5)
	Missing	30 (12.2)	32 (15.0)
Family income at SDQ completed (JPY)	**< 5M**	111 (45.3)	111 (52.1)
	≧5M	123 (50.2)	88 (41.3)
	Missing	11 (4.5)	14 (6.6)
Marital Status at SDQ completed	**Married**	236 (96.3)	198 (93.0)
Gestational age (days)		275.3 ± 8.2	275.4 ± 8.5
Birth weight (g)		3037 ± 339	3076 ± 383
Child Sex	**Boy**	122 (49.8)	128 (60.1)
	Girl	123 (50.2)	85 (39.9)
Birth order	**First child**	116 (47.3)	123 (57.7)
Age at SDQ completed (months)		67.3 ± 6.2	66.3 ± 6.3

246 Mean ± S.D. or n (%). JPY: Japanese Yen,

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Table 2 presents distribution of BPA and phthalates levels in maternal blood of all participants and of two groups. The median concentrations of BPA, MnBP, MiBP, MBzP, MEHP, MEHHP, MECPP and cx-MiNP were 0.062, 26.0, 7.0, <MDL, 1.40, <MDL, 0.20, and <MDL ng/mL, respectively. The detection rates of BPA, MnBP, MiBP, MBzP, MEHP, MEHHP, MECPP and cx-MiNP were 94.0%, 100.0%, 100.0%, 9.1%, 96.5%, 0.7%, 82.1% and 0.4%, respectively. The detection rates of MBzP, MEHHP and cx-MiNP were below 10%. Thus, these chemicals were excluded from the further analyses. The median concentration of BPA in borderline/clinical group was higher compared to that of the normal group. Contrary the median concentrations of MnBP and MiBP in borderline/clinical group were slightly lower compared to these of the normal group.

Table 2 Comparison of the distribution of BPA and phthalate metabolite levels in maternal blood between normal and borderline/clinical groups.

Exposure	MDL (ng/ml)	Detection rate (%)	Normal (n=245)		Borderline/clinical (n=213)	
			Median	IQR (25^{th},75^{th})	Median	IQR (25^{th},75^{th})
BPA	0.011	94.0	0.054	0.022, 0.207	0.086	0.032, 0.353
MnBP	0.57	100.0	26.7	17.7, 37.6	24.7	17.0, 34.0
MiBP	0.44	100.0	7.4	5.3, 9.9	6.7	5.1, 8.9
MBzP	0.19	9.1	<MDL	<MDL, <MDL	<MDL	<MDL, <MDL
MEHP	0.31	96.5	1.42	0.82, 9.07	1.35	0.71, 9.25
MEHHP	0.23	0.7	<MDL	<MDL, <MDL	<MDL	<MDL, <MDL
MECPP	0.11	82.1	0.20	0.11, 0.30	0.21	0.12. 0.33
cx-MiNP	0.12	0.4	<MDL	<MDL, <MDL	<MDL	<MDL, <MDL

ng/ml. MDL: method detection limit. IQR: Inter quartile range.

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Table 3 presents adjusted odds ratios for ten folds increase of maternal BPA and individual and summation of DBP and DEHP metabolite levels on having behavioral problems. BPA level was associated with increased hyperactivity/inattention risk among girls after adjustment (OR=1.66, 95% CI: 0.95-2.90) without statistical significance. MECPP level was significantly associated with an increased risk of conduct problems (OR=2.78, 95% CI: 1.36-5.68). This association remained after child sex stratification. MECPP level was also significantly associated with an increased risk of hyperactivity/inattention among girls (OR=5.71, 95% CI: 1.41-23.1). ΣDBP_m level was associated with decreased total difficulties risk overall and among girls (OR=0.48, 95% CI: 0.20-1.13, OR=0.24, 95% CI: 0.06-1.03, respectively) without statistical significance. There were no significant association between $\Sigma DEHP_m$ levels and any of the behavioral problem risks.

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Table 3 Adjusted odds ratios for ten folds increase of maternal BPA and phthalates levels on having behavioral problems.

	Number of children in borderline/clinical	BPA	MnBP	MiBP	MEHP	MECPP	$\sum DBP_m$	$\sum DEHP_m$
All					OR (95% CI)			
Total difficulties (≧13)	213	1.28 (0.94, 1.74)	0.51 (0.22, 1.18)	0.42 (0.17, 1.03)+	0.93 (0.65, 1.33)	1.13 (0.58, 1.13)	0.48 (0.20, 1.13)+	0.93 (0.63, 1.38)
Conduct problems (≧4)	142	1.15 (0.84, 1.58)	1.33 (0.55, 3.20)	1.37 (0.53, 3.58)	0.81 (0.56, 1.18)	2.78 (1.36, 5.68)*	1.34 (0.54, 3.33)	0.82 (0.55, 1.24)
Hyperactivity/inattention (≧6)	126	1.06 (0.75, 1.51)	1.10 (0.42, 2.84)	0.93 (0.33, 2.65)	1.22 (0.82, 1.84)	1.52 (0.71, 3.29)	1.04 (0.39, 2.80)	1.25 (0.81, 1.95)
Emotional symptoms (≧4)	116	0.92 (0.66, 1.27)	0.83 (0.35, 1.98)	0.57 (0.22, 1.45)	0.86 (0.59, 1.27)	0.65 (0.33, 1.31)	0.77 (0.31, 1.88)	0.85 (0.56, 1.28)
Peer problems (≧4)	64	0.99 (0.65, 1.52)	0.92 (0.30, 2.87)	0.45 (0.14, 1.49)	0.78 (0.47, 1.29)	0.90 (0.36, 2.25)	0.79 (0.25, 2.54)	0.76 (0.44, 1.44)
Boy					OR (95% CI)			
Total difficulties (≧13)	128	1.26 (0.82, 1.95)	0.54 (0.18, 1.61)	0.43 (0.13, 1.46)	0.82 (0.49, 1.35)	0.62 (0.24, 1.60)	0.50 (0.16, 1.58)	0.79 (0.46, 1.37)
Conduct problems (≧4)	83	1.32 (0.86, 2.03)	1.14 (0.36, 3.55)	0.95 (0.27, 3.30)	0.79 (0.48, 1.31)	2.85 (1.07, 7.57)*	1.09 (0.33, 3.56)	0.78 (0.45, 1.36)
Hyperactivity/inattention (≧6)	85	0.80 (0.50, 1.28)	1.03 (0.32, 3.32)	0.87 (0.24, 3.14)	1.05 (0.63, 1.76)	0.92 (0.35, 2.44)	0.98 (0.29, 3.31)	1.06 (0.61, 1.85)
Emotional symptoms (≧4)	77	0.89 (0.56, 1.42)	0.78 (0.24, 2.53)	0.52 (0.14, 1.86)	0.97 (0.57, 1.63)	0.65 (0.24, 1.75)	0.71 (0.21, 2.43)	0.95 (0.54, 1.68)
Peer problems (≧4)	40	0.96 (0.54, 1.72)	0.74 (0.17, 3.32)	0.50 (0.10, 2.53)	0.67 (0.34, 1.31)	0.68 (0.20, 2.37)	0.67 (0.14, 3.18)	0.64 (0.31, 1.33)
Girl					OR (95% CI)			
Total difficulties (≧13)	85	1.30 (0.83, 2.03)	0.26 (0.06, 1.06)+	0.25 (0.06, 1.09)+	1.10 (0.64, 1.88)	2.37 (0.87, 6.42)+	0.24 (0.06, 1.03)+	1.16 (0.65, 2.08)
Conduct problems (≧4)	59	1.03 (0.63, 1.67)	0.90 (0.19, 4.16)	1.46 (0.29, 7.40)	0.91 (0.50, 1.63)	4.04 (1.31, 12.5)*	0.98 (0.20, 4.78)	0.96 (0.51, 1.82)
Hyperactivity/inattention (≧6)	41	1.66 (0.95, 2.90)+	1.05 (0.17, 6.38)	0.95 (0.14, 6.50)	1.68 (0.84, 3.37)	5.71 (1.41, 23.1)*	0.99 (0.15, 6.41)	1.79 (0.84, 3.81)
Emotional symptoms (≧4)	39	0.93 (0.57, 1.51)	0.45 (0.10, 1.95)	0.34 (0.07, 1.64)	0.77 (0.43, 1.37)	0.84 (0.30, 2.33)	0.41 (0.09, 1.86)	0.76 (0.41, 1.41)
Peer problems (≧4)	24	1.08 (0.56, 2.09)	0.64 (0.09, 4.66)	0.18 (0.02, 1.33)+	1.06 (0.47, 2.39)	1.24 (0.30, 5.20)	0.47 (0.06, 3.54)	1.09 (0.46, 2.62)

Adjusted for parental ages, maternal cotinine levels, family income during pregnancy, child sex, birth order (first child or not), and child age at SDQ complete.

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

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278 **Discussion**

279 Recent reviews have shown that environmental chemicals may play a role in the etiology of
280 behavioral and developmental disorders[34,35]. In our study, prenatal exposure to BPA and phthalates
281 were measured in maternal blood of 1st trimester and child behavioral problems at 5 years of age
282 were assessed using the SDQ. Our analyses found no significant association between BPA or
283 summation of phthalate metabolite levels and an increased risk of any of the behavioral problems at
284 5 years of age, however, suggested possible association between MECPP levels and increased risk of
285 conduct problems. Stratification by child sex analyses found that maternal MECPP level was
286 associated with an increased risk of hyperactivity/inattention problems only in girls with a large
287 confidence interval. This could be due to a number of individual was too small in some categories of
288 the adjustment factors, since the crude model found no statistical significance (OR=1.32, 95% CI:
289 0.70-2.48). Thus, the interpretation of findings from adjusted model should be carried out cautiously.
290 SDQ scores of 2032 children in this study was 8.7 and was similar to the other previous studies in UK
291 (5-10 years old) and Japan (4-6 years old), which showed average scores of 8.3 and 8.6,
292 respectively[28,36]. The BPA level in this study was similar range to previous report of Japanese
293 pregnant women[30] and lower compared that of pregnant women in other studies[37-39].
294 There have been several prospective cohort studies that investigated associations between prenatal
295 exposure to BPA and child behavioral problems[14,15,17-20,40-43]. Our group assessed child behavioral

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problems at 3.5 years of age using CBCL and found that cord blood BPA level was positively associated with internalizing problem and development problem scores[43]. Braun et al. assessed child behavior at different ages using the prospective birth cohort in the US (HOME Study)[17,18,40]. In their study, among girls, higher maternal urinary BPA was associated with increased aggression and hyperactivity at age 2[17]. The follow-up of the same cohort at 3 years of age found that higher maternal urinary BPA was associated with more anxiety and depression of behavioral Assessment System for Children-Second Edition (BASC-2) and poorer emotional control of Behavior Rating Inventory of Executive Function-Preschool (BREIF-P) only among girls[18]. In our study, we did not find the statistical significance, however, increased odds of hyperactivity/inattention among girls in association with increased BPA level was consistent with findings from Broun et al[17,18]. Another birth cohort study in the US (CCCEH) also investigated association between maternal urinary BPA and child behavior[14,42]. The results of their study showed that higher levels of maternal BPA were associated with higher scores on emotionally reactive and aggressive behavior subscales of CBCL among boys at 5 years of age[14]. A follow-up of the same cohort at 7-9 years of age found that higher maternal BPA levels were associated with more anxiety and depression in boys[42]. Harley et al. investigated association between maternal urinary BPA and school aged child behavior in the birth cohort study (CHAMACOS)[15]. They found that higher maternal BPA was associated with higher depression and anxiety in boys. Evans et al. reported that higher maternal BPA was associated with higher level of

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aggression, anxiety, oppositional/defiant problems and conduct problems in boys using CBCL at ages 6-10 years in a birth cohort study (SFF II)[44]. Most of the previous studies found sex-specific effects of BPA exposure on child behavioral development and problems, while this study did not find any significant adverse effect of BPA exposure on the risk of child behavioral problems even after stratification of child sex. Inconsistent findings from the previous studies could be due to different exposure assessment timings among studies. The critical period of exposure to BPA during pregnancy on child neurobehavioral development is still not evident, thus using maternal blood samples of the 1st trimester may not well evaluate associations between prenatal exposures and outcomes. Braun et al. reported relationship between maternal urinary BPA and child behavior and the relationship was stronger with urine samples of ≦ 16 weeks of gestation compared to that of 26 weeks of gestation, which suggested a possible critical period for BPA exposure on neurobehavior development[17]. Our result indicated that the 1st trimester BPA level was associated with increased risk of hyperactivity/inattention among girls without significance, which is in line with the previous findings[17]. Further investigation is required to elucidate critical exposure period of BPA exposure and its influence on child behavioral development.

Various study population background may also be a reason for inconsistent findings. For example, maternal education levels > high school in this study was 62.8%, whereas it varied from low to high (21.6%[15] to 85%[44]) in the previous studies that found association between BPA exposure and child

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behavioral problems. It has been reported that maternal education level was a predictor of BPA levels [18,45]. Thus, it may have contributed to inconsistent findings. Similarly, income is inversely associated with BPA levels according to NHANES data [46] and thus, different cultural background such as poverty rate, ethnicity could be a reason for inconstancy.

There have been several reports from birth cohort studies regarding child behavioral development in association with prenatal phthalates exposure. Results from birth cohort studies have suggested that low molecular weight (LMW) phthalate such as DBP and DEP exposures might increase behavioral problems[20,21,41,47]. Whyatt et al. assessed child behavioral problems using CBCL at 3 years old in association with maternal urine phthalate levels[21]. In their study, MnBP, MiBP and MBzP were found to be associated with increased behavioral problems. However, no association was found between maternal urinary DEHP metabolites and child behavioral problems. Engel et al., investigated associations between maternal phthalate metabolites and child behavior at 4-9 years old using Behavior Assessment System for Children-Parent Rating Scale (BASC-PRS)[47]. Increased levels of LMW phthalate metabolites were associated with various behavioral problems including aggression, conduct problems, attention problems and depression. The same group also used Social Responsiveness Scale (SRS) to assess child behavior at ages 7-9 years of age[41]. It was found that LMW phthalates were also associated with poorer social cognition, social communication and social awareness. In our study, we did not find any association between LMW phthalates and child

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behavioral problems. Kobrosly et al. examined child neurobehavior using CBCL among children at 6-10 years of age[22]. They found increased 3rd trimester maternal urine MiBP was associated with attention problems and aggressive behavior and the association was mostly observed among boys. Lien et al. assessed child behavior at 8-9 years of age using CBCL[23]. In their study, 3rd trimester maternal MBP and MEOHP were associated with delinquent behavior and aggressive behavior scores at 8 years old. Recently, Gascon et al. assessed child behavioral problems using CBCL at 4 and SDQ at 7 years in the INMA-Sabadell birth cohort study[48]. They found that the average concentrations of the sum of 4 kind of DEHP metabolites (MEHHP, MEHP, MEOHP, and MECPP) in maternal urine of 1st and 3rd trimester were associated with increased social competence scores at 4 years. Contrary, they found that MEP concentrations were associated with a reduced risk of inattention symptoms at 4 years. One previous study reported that maternal MECPP level was inversely associated with child motor development at age 24-36 months only in girls [49]. In their study, not only MECPP but also the sum of DEHP metabolites and other DEHP metabolites (MEHHP, MEHP, MEOHP) were negatively associated with child motor development, which was inconsistent with our results. Overall, our findings from this study was not in line with these previous studies, as most of the studies reported effects of LMW phthalate exposures.

A number of factors including assessment tools for outcome measurements and age at assessment, timing of exposure assessment, and genetic and demographic variety of study populations, as well

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368 as other unknown factors could explain the inconstancies among studies. Different levels of exposure
369 among studies could also explain the different findings. Most of the previous studies used maternal
370 urine samples during pregnancy for exposure assessment, whereas we used maternal serum. Even
371 though a study reported correlation between serum and urine MECPP levels[50], direct comparison of
372 exposure levels with other studies were not possible. It also should be noted that measurable levels
373 are much higher in urine compared to blood samples for bisphenol A and phthalate metabolites.
374 Regarding BPA measurement using blood samples, it possibly be overestimated due to external
375 contamination. In this study, we used glass cartridge to reduce background levels and no free BPA
376 was detected[30], which was indication of null possible external contamination. Additionally,
377 background level was measured and confirmed that the influence of external contamination was null.
378 Hydrolytic enzymes are present in blood samples and may be responsible for diester to monoester
379 conversion after the blood sample is drawn[51]. Analysis of monoester may yield higher levels because
380 of monoester conversion of ex-vivo contamination during sampling, storage, and handling process.
381 To minimize the influence of enzyme activity, the blood samples were immediately stored at -80℃
382 and acid was added immediately after thawing. We still cannot rule out possible external
383 contamination during the process of sample drawing, storage and measurement. Using secondary
384 metabolites of phthalates was recommended. In this study, we found behavioral problems in
385 association with MECPP, which is a secondary metabolite of DEHP.

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386 Limitations of this study should also be discussed. First, our exposure assessment was based on the
387 single measurement which could not represent exposure of entire pregnancy period due to short
388 half-lives of BPA and phthalates. Thus, the critical period of exposure might not be well captured in
389 this study. Other limitation was that we had no information on factors that might have influence on
390 the outcomes such as family psychopathology, exposure to psychosocial environmental stressors.
391 Sample size can be another limitation of this study especially in sex specific analyses. Some of the
392 subscales of SDQ showed small number of children in borderline/clinical group (Table S2). This was
393 due to the study design. This was a nested case control study based on SDQ total difficulties score,
394 but not on subscale scores. Wide range of 95% CIs observed in sex-stratification analyses indicated
395 that the sample size was too small. It also should be noted that there might be a chance that
396 associations may possibly be identified due to the number of chemicals tested.
397 It should be noted that we did not measure postnatal exposures in this study. Some of the cross-
398 sectional and birth cohort studies reported associations between postnatal exposure to BPA or
399 phthalates exposures and child neurobehavioral development[52-57]. However, two of the prospective
400 studies revealed that only gestational but not childhood BPA was associated with child behavior[14,18].
401 Thus, we considered effects of prenatal exposure was more influential on child behavioral
402 development. The Characteristics of participants in this study (n=458) and those who completed SDQ
403 (n=2032) were compared in the Table S1. Population in this study showed higher percentage of non-

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404 smokers based on maternal cotinine levels and heavier mean birth weight. This implied that healthier
405 mothers and children tended to be included in this study and thus, the effect of prenatal exposure to
406 BPA and phthalates on child behavioral problems might have been underestimated and findings of
407 this study should be interpreted with caution.
408 In conclusion, we found no significant association between BPA or summation of phthalate
409 metabolite levels and any of the behavioral problems, however, suggested possible association
410 between MECPP levels and increased risk of conduct problems.
411

412 **Supplementary Materials**

413 Table S1: Characteristics of participants in this study (n=458) and those who completed SDQ (n=2032).
414 Table S2: SDQ score distribution stratified by child sex.
415

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422 **Author Contributions**

423 Conceptualization, R.K.; Formal Analysis, M.M.; Investigation, J.Y., Y.O., K.O., and T.M.; Writing –

424 Original Draft Preparation, M.M.; Writing – Review & Editing, S.I., K.Y., A.A., C.Y., N.T.; Supervision,

425 R.K.; Funding Acquisition, M.M, R.K.

426

427 **Conflicts of Interest**

428 The authors declare no conflict of interest.

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