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Posted Date: 29 August 2023

doi: 10.20944/preprints202308.1924.v1

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Exposure to Bisphenol A and Its Analogs among Thai School-Age Children

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Abstract: Bisphenol F (BPF) and bisphenol S (BPS) are increasingly used as substitutes for bisphenol A (BPA) in the plastic industry, driven by concerns over the adverse effects of BPA. However, there is a lack of information on the extent of exposure to these chemicals in children. This study aims to assess the extent of BPA, BPF, and BPS exposure and determine factors that influence such exposure. A group of Thai children (age 6-13 years, n=358) were recruited between October 2019-2020. Two first morning void was collected one week apart. Demographic and exposure-related information was gathered. Urinary concentrations of bisphenols were analyzed by liquid-chromatography and tandem mass spectrometry. Correlation between bisphenol concentrations and various factors was determined using generalized estimating equations with linear model. BPA, BPF, and BPS were detected at 79.6%, 31.0%, and 16.8%, with geometric mean (GM) concentrations of 1.41, 0.013, and 0.014 ng/mL respectively. Younger children aged < 10 years exhibited 1.3-1.6 times higher GM levels of all bisphenols compared to older children. Exposure to food stored in plastic containers were associated with higher levels of BPF and BPS. In conclusion, BPA was the most frequently detected bisphenol in urine samples from Thai children, followed by BPF and BPS.

Keywords: bisphenol A (BPA); bisphenol F (BPF); bisphenol S (BPS); liquid-chromatography and tandem mass spectrometry

1. Introduction

Bisphenol A (BPA), a major chemical previously used in the plastic industry, has been regulated to use in many countries due to its harmful effects on the endocrine system, including metabolic problems, obesity, and reproductive dysfunction [1–5]. Alternative bisphenol analogs such as bisphenol F (BPF) and bisphenol S (BPS) are now being used as substitutes for BPA [6]. These substances can be found in various everyday products, such as cleaning products, thermal paper, coatings, dental sealants, personal care products, canned food, and food packaging. BPF and BPS have similar structural characteristics to BPA and may have similar endocrine-disrupting effects [7].

After exposure, BPA, BPF, and BPS are metabolized by hepatic uridine 5′-diphosphate-glucuronosyltransferases (UGTs) to the corresponding glucuronides and subsequently eliminated in urine[8], therefore, urinary levels of these substances are the biomarker of exposure to bisphenols. Elimination of the conjugates into urine occurs mainly within 24 hours for BPA [9] and within 48 hours for BPS [10].

According to recent studies [9,10], exposure to BPA has decreased worldwide due to regulations and the prohibition of its use. However, there is a concerning trend of increasing detection rates for BPF and BPS in various parts of the world, suggesting their widespread presence. Despite this, in Thailand, there is limited information available on the extent of exposure to BPF and BPS.

 doi:10.20944/preprints202308.1924.v1

Understanding current levels of exposure to bisphenols and major exposure pathways is more important among children, as they are more vulnerable to exposure to endocrine disrupting chemicals [11,12]

This study aimed to determine the prevalence of BPA, BPF, and BPS exposure in Thai school-age children, and to identify factors that influence the urinary concentration of these chemicals. The result of this study can help understand the status of the exposure and develop mitigation measures for major bisphenols among children of Thailand.

2. Materials and Methods

A total of 358 children aged 6-13 years of age were recruited from an elementary school in Bangkok, Thailand, during October 2019 to October 2020. All participating children were measured for height, weight, and waist circumference. Two spot morning urine samples were collected one week apart and stored in cylindrical bisphenol-free containers in a refrigerator at -20°C until analysis.

Questionnaire was conducted with a help of their parents or guardians and asked for socioeconomic status, housing conditions, cosmetics use, canned food and beverages consumption, and plastic food container use. The study protocol was approved by the International Review Board of Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand (IRB 237/64).

Age and sex-specific BMI z-scores were calculated using WHO growth reference standard. All participants were classified into 4 groups: underweight, normal weight, overweight (Body mass index, BMI z-scores above 1 SD, Standard deviation) and obesity (BMI z-scores above 2 SD).

Measurement of bisphenols (BPA, BPF, BPS) in urine

Concentrations of BPA, BPF, and BPS in urine were determined using the liquid-chromatography and tandem mass spectrometry (LC-MSMS) technique. For the preparation of the urine sample, each 1 mL urine sample was centrifuged at 3,000 rpm for 10 minutes. Then, 500 µL of the urine sample was hydrolyzed by adding 100 µL of ammonium acetate and 20 µL (2,000 units) of β-glucuronidase enzyme (Sigma-Aldrich, St. Louis, USA). The mixture was vortexed, followed by incubation at 37°C for 3 hours. Subsequently, the samples were purified by protein precipitation using 500 µL of acetonitrile purchased from JT Baker. Bisphenol A-d16, d7 BPF, and d8 BPS (Sigma-Aldrich) were used as internal standards. The samples were vortexed again, centrifuged at 3,000 rpm for 10 minutes, and then subjected to LC-MSMS analysis. Bisphenol levels were determined using an Acquity UPLC® system (Waters, Illinois, USA) coupled with a XEVO-TQS mass spectrometer (Waters), with quantitation limits (LOQ) of 0.50, 0.01, and 0.01 ng/mL for BPA, BPF, and BPS, respectively.

Urinary creatinine and specific gravity

Urine creatinine (urine Cr) was analyzed using an enzymatic method with Alinity c Creatinine Reagent Kit 08P01 (Crea Enz) (Abbott Laboratories, Jinan, China). Urine specific gravity (SG) was measured using the urine specific gravity refractometer (MASTER-URC/NM, Atago, Japan) with the SG scale range of 1.000 to 1.050, refractive index range (nD) 1.333 to 1.356, minimum SG scale 0.001, minimum refractive index: 0.001.

To adjust for urine dilutional, urinary bisphenol concentration in ng/mL was either adjusted to Cr (mcg/g creatinine) or by SG [13].

Estimated daily intake (EDI) of bisphenols

Estimated daily intake (EDI) of bisphenols analog was calculated as follows.[14]

$$\mathbf{EDI} = \mathbf{UC} \times \mathbf{CE_{smoothed}} \times \frac{\mathbf{1}}{\mathbf{f}} \times \frac{\mathbf{1}}{\mathbf{BW}}$$

EDI; estimated daily intake of bisphenol (mcg/kg/day)

UC; creatinine-adjusted urinary concentrations of BPA, BPF, or BPS (mcg/g creatinine)

$\mathbf{CE_{smoothed}}$; the 24-h urinary creatinine excretion

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For the children, $\mathbf{CE_{smoothed}}$ corresponded to age and sex specific average creatinine excretion levels based on data provided by Remer et al. (2002)[15]:

Boys aged 6 to 8 years (0.49 g/ day) or 9–13 years (0.76 g/day)

Girls aged 6 to 8 years (0.45 g/ day) or 9–13 years (0.72 g/day)

F; the rate of urinary excretion of bisphenols after oral exposure. The F for BPA, BPF and BPS was close to 100%, 100% [9] and 70% [10] respectively.

Statistical analysis

The study population's characteristics were presented as mean (SD), median (IQR), and percentages. Bisphenol detection rates and concentration were compared across age, sex, and BMI groups using chi-square tests. Urine bisphenol concentration between groups was compared using a two-sample independent t-test. Generalized Estimating Equations (GEE) with a linear model identified factors associated with SG-adjusted bisphenol concentration, BPS/BPA ratio, and BPF/BPA ratio. Multivariate models adjusted for covariates with p-values < 0.1 in univariate models. Analyses were conducted using STATA version 13.1 (StataCorp, College Station, Texas, USA). Statistically significance was set at $p < 0.05$.

3. Results

3.1. Characteristics of the participants

The average age of the participating children was 9.4 ± 1.7 years with 52% of boys (Table 1). More than half of the participants had a normal BMI, had caregivers with high school or an equivalent level of education, and came from families with low monthly income.

Table 1. Baseline characteristics of the participants (N=358).

Characteristics	Value
Age (years; mean ± SD)	9.4 ±1.7
Sex, N (%)	
Boy	186 (52)
Girl	172 (48)
Age group, N (%)	
< 10 years	211 (58.9)
≥ 10 years	147 (41.1)
Anthropometry, median (IQR)	
Body weight (kg.)	32.7 (25.1-41.4)
Height (cm.)	134.2 (126-144)
Waist circumference (cm.)	60.5 (55-70.5)
BMI (kg/m²), median (IQR)	17.3 (15.4-20.9)
Underweight, N (%)	12 (3.3)
Normal, N (%)	209 (58.9)
Overweight, N (%)	56 (15.8)
Obese, N (%)	78 (22)
Caregiver Education, N (%)	
< High school	53 (18.3)
High school or equivalent	159 (54.8)
≥ Bachelor's degree	78 (26.9)
Family Income (THB/month), n (%)	
<30000	155 (54.2)
30000-50000	111 (38.8)
>50000	20 (7)
Residence, N (%)	

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Apartment/condominium/townhouse/shop house/other	296 (82.7)
Detached house	62 (17.3)

n: number of participants in subgroup; BMI: body mass index.

3.2. Bisphenol exposure

In the children's urine, BPA (79.6%) was most frequently detected, followed by BPF (31.0%), and BPS (16.8%). Of all participants, 286 children provide two morning voids, and the detection rates for urinary bisphenols in each individual significant varied between two samples (Table 2).

Table 2. Bisphenol detection rate in urine, N (%).

	Detection rate from first urine samples (N = 358)	**Participant who provides both urine samples (N= 286)**		***p***
		Detection rate from first urine samples	**Detection rate from second urine samples**	
BPA	285 (79.6)	235 (82.2)	173 (60.5)	<0.001
BPF	111 (31.0)	98 (34.3)	60 (21.0)	<0.001
BPS	60 (16.8)	49 (17.1)	14 (4.9)	<0.001

The p-value was evaluated using McNemar's test.

Among the three analytes, BPA had the highest concentration (mean GM 1.41 ng/mL), followed by BPS (mean GM 0.014 ng/mL), and BPF (mean GM 0.013 ng/mL) (Table 3). The ratio (95% CI) of BPF/BPA was 0.009 (0.008-0.010), and BPS/BPA ratio, 0.010 (0.009-0.012).

Table 3. Values of urine bisphenol levels of Thai children and adolescents.

Analyte	**10th**	**25th**	**50th**	**75th**	**90th**	**GM**	**95%CI of GM**
Unadjusted urine bisphenol concentration (ng/mL)							
BPA							
1st sample	0.5	0.607	1.384	2.766	4.924	1.487*	1.348-1.641
2nd sample	0.5	0.5	0.7005	1.425	2.61	0.934*	0.847 - 1.03
Mean	0.5085	0.754	1.296	2.124	3.7475	1.414	1.278 - 1.565
BPF							
1st sample	0.01	0.01	0.01	0.012	0.022	0.013	0.012-0.013
2nd sample	0.01	0.01	0.01	0.01	0.019	0.012	0.011 - 0.012
Mean	0.01	0.01	0.01	0.014	0.022	0.013	0.012 - 0.014
BPS							
1st sample	0.01	0.01	0.01	0.01	0.067	0.015*	0.013-0.017
2nd sample	0.01	0.01	0.01	0.01	0.01	0.011*	0.01 - 0.012
Mean	0.01	0.01	0.01	0.01	0.0485	0.014	0.013 - 0.016
Cr-adjusted urine bisphenol concentration (mcg/g Cr)							
BPA							
1st sample	0.560	0.927	1.446	2.446	4.920	1.586*	1.442-1.744
2nd sample	0.426	0.634	0.950	1.662	2.886	1.094*	0.985 - 1.215
Mean	0.591	0.864	1.291	1.958	3.138	1.389	1.262 - 1.529
BPF							
1st sample	0.005	0.007	0.011	0.021	0.047	0.014	0.012-0.015
2nd sample	0.006	0.008	0.012	0.019	0.042	0.014	0.013 - 0.015
Mean	0.006	0.008	0.012	0.017	0.030	0.013	0.012 - 0.014
BPS							
1st sample	0.005	0.007	0.010	0.023	0.104	0.016	0.014-0.018
2nd sample	0.005	0.007	0.010	0.018	0.044	0.013	0.012 - 0.015
Mean	0.006	0.007	0.011	0.018	0.048	0.014	0.013 - 0.016
SG-adjusted urine bisphenol concentration (ng/mL)							
BPA							
1st sample	0.5	0.607	1.384	2.766	4.924	1.487*	1.348-1.641
2nd sample	0.500	0.500	0.691	1.425	2.610	0.932*	0.845 - 1.029

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Mean	0.601	0.829	1.348	2.176	3.729	1.471	1.331 - 1.625
BPF							
1st sample	0.01	0.01	0.01	0.012	0.022	0.013	0.012-0.013
2nd sample	0.010	0.010	0.010	0.010	0.019	0.012	0.011 - 0.013
Mean	0.008	0.009	0.012	0.016	0.026	0.014	0.013 - 0.015
BPS							
1st sample	0.01	0.01	0.01	0.01	0.067	0.015*	0.013-0.017
2nd sample	0.010	0.010	0.010	0.010	0.010	0.011*	0.01 - 0.012
Mean	0.008	0.009	0.011	0.016	0.044	0.015	0.013 - 0.017
Bisphenol concentration ratio							
BPF/BPA							
1st sample	0.002	0.004	0.010	0.020	0.033	0.010	0.009-0.012
2nd sample	0.004	0.008	0.017	0.020	0.020	0.013	0.011 - 0.014
Mean	0.003	0.006	0.010	0.016	0.021	0.009	0.008 - 0.01
BPS/BPA							
1st sample	0.002	0.004	0.009	0.020	0.020	0.009	0.008-0.010
2nd sample	0.004	0.007	0.015	0.020	0.020	0.012	0.011 - 0.014
Mean	0.003	0.005	0.010	0.019	0.040	0.01	0.009 - 0.012

GM: geometric mean; CI: confidence interval. *$p < 0.001$ from pair t-test

The estimated daily intake (EDI) derived from Cr-adjusted urine bisphenol concentrations was highest for BPA, with the 95th percentile value of 0.114 mcg/kg/day, followed by BPS with 0.003 mcg/kg/day, and BPF with 0.001 mcg/kg/day. None of these EDIs exceeded tolerable daily intake (TDI) values established by the European Food Safety Authority (EFSA) in 2015 (Table 4).

Table 4. Estimated daily intake of bisphenol (mcg/kg/day).

Analyte	25th	50th	75th	95th	Regulation (TDI)	N (%) >TDI
BPA	0.0125	0.0213	0.0448	0.1136	4	0 (0%)
BPF	0.0001	0.0002	0.0004	0.0011	N/A	N/A
BPS	0.0002	0.0003	0.0006	0.0034	4.4	0 (0%)

TDI: tolerable daily intake (mcg/kg/day), an estimate of the amount of a contaminant which can be consumed over a lifetime without presenting an appreciable risk to health, value provided by EFSA in 2015. N/A: not available.

3.3. Factors correlated with urinary bisphenol concentrations

Urinary BPA, BPF, and BPS concentrations were not different by obesity status and gender (Table 5). Mean urine BPA, BPF, and BPS concentrations were higher among younger children (aged < 10 years), compared to the older age group (age ≥ 10 years) ($p < 0.05$). Specifically, GMs were 1.31 times higher for BPA, 1.46 times higher for BPF, and 1.62 times higher for BPS (table 6). Children who consumed food from plastic containers for a minimum of two days each week exhibited notably elevated bisphenol levels in comparison to those with limited exposure. Specifically, the GMs were 1.44 (1.16 - 1.78) times higher for BPF and 1.40 (1.05 - 1.87) times higher for BPS. However, when subjected to a multivariate analysis, none of the exposure variables maintained their significance.

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Table 5. Comparison of GM of Cr-adjusted urine bisphenol concentration between different groups of participants.

Parameter	Group	BPA	*p*	BPF	*p*	BPS	*p*
Obesity	Not Obese (N=226)	1.341 (1.223-1.471)	0.165	0.013 (0.012-0.015)	0.107	0.046 (0.011-0.081)	0.727
	Obese (N=60)	1.584 (1.171-2.143)		0.011 (0.01-0.013)		0.059 (-0.013-0.13)	
Sex	Boy (N=153)	1.41 (1.209-1.643)	0.748	0.013 (0.011-0.014)	0.391	0.052 (0.001-0.102)	0.842
	Girl (N=133)	1.366 (1.225-1.523)		0.013 (0.012-0.015)		0.045 (0.012-0.078)	
Age	Age < 10 years (N=182)	1.533 (1.347-1.745)	**0.007**	0.015 (0.013-0.017)	**<0.001**	0.046 (0.021-0.071)	**<0.001**
	Age ≥ 10 years (N=104)	1.169 (1.026-1.332)		0.01 (0.009-0.012)		0.053 (-0.021-0.127)	

P-value was evaluated by two sample independent t-test.

Table 6. Factors associated with urinary concentration of bisphenols.

Variable	Cr-adjusted BPA		Cr-adjusted BPF		Cr-adjusted BPS		BPF/BPA ratio		BPS/BPA ratio	
	GMR (95%CI)	*p**	GMR (95%CI)	*p**	GMR (95%CI)	*p**	GMR (95%CI)	*p**	GMR (95%CI)	*p**
Age < 10 vs ≥ 10 years	1.31 (1.07-1.6)	**0.007**	1.46 (1.22-1.73)	**0.000**	1.62 (1.26-2.08)	**0.000**	1.12 (0.87-1.42)	0.391	1.23 (0.91-1.67)	0.167
Obese/yes	1.19 (0.93-1.49)	0.165	0.84 (0.68-1.04)	0.107	0.95 (0.7-1.28)	0.727	0.71 (0.54-0.94)	0.018	0.8 (0.57-1.14)	0.223
Food with plastic container[a]	1.2 (0.99-1.45)	0.061	1.44 (1.16-1.78)	**0.001**	1.40 (1.05-1.87)	**0.024**	1.2 (0.96-1.5)	0.108	1.17 (0.87-1.58)	0.31
Water in plastic bottle[a]	1.14 (0.92-1.42)	0.229	1.06 (0.87-1.28)	0.564	1.11 (0.84-1.46)	0.456	0.93 (0.71-1.21)	0.576	0.97 (0.7-1.35)	0.867
Toothpaste[b]	1.09 (0.85-1.4)	0.485	1.13 (0.9-1.4)	0.306	1.09 (0.79-1.51)	0.578	1.03 (0.76-1.39)	0.850	1 (0.69-1.46)	0.995
Soap[b]	1.11 (0.86-1.43)	0.434	1.06 (0.84-1.34)	0.641	1.03 (0.74-1.42)	0.872	0.95 (0.7-1.3)	0.767	0.92 (0.63-1.36)	0.702
Face / body lotion[b]	1 (0.83-1.22)	0.977	1.02 (0.85-1.21)	0.866	0.9 (0.7-1.15)	0.418	1.01 (0.8-1.28)	0.919	0.9 (0.68-1.21)	0.481
Dishwashing liquid[b]	1.03 (0.84-1.25)	0.768	1.05 (0.89-1.26)	0.551	0.84 (0.66-1.07)	0.168	1.02 (0.81-1.3)	0.840	0.82 (0.61-1.09)	0.173

GMR: Geometric mean ratio. [a]≥ 2 days per week vs ≤ Once a month. [b]Yes, within 24 hr vs > 24 hr. *p < 0.05 indicate statistical significance from univariate analysis.

4. Discussion

In 2015, Thailand introduced regulations concerning the safety of milk bottles and containers used for feeding infants and young children with the goal of reducing health concerns stemming from BPA exposure. However, the current study indicates a higher BPA detection rate in urine samples from Thai children (79.6%) compared to a previous study conducted 6 years ago (during 2013-2014), which reported a BPA detection rate of 75.3%[16]. These findings suggest that despite the regulations on BPA usage in Thailand, current BPA exposure remains prevalent among Thai children and adolescents. Moreover, the detection rates of BPF and BPS in our study are notably lower than that of BPA. The considerably low concentration ratios of BPF/BPA and BPS/BPA in each urine sample indicate that urine BPF and BPS concentrations are significantly lower than that of BPA, suggesting that children are primarily exposed to BPA in comparison to BPF and BPS. There is a lack of previous data available for comparing BPF and BPS levels in Thai children.

Compared to worldwide studies, the detection frequencies of urinary bisphenols vary depending on the specific type of bisphenol, countries, and age groups of the subjects. For example, our study found that the BPA, BPF, and BPS were detected at 79.6, 31.0, and 16.8%, which lower than data from the U.S. National Health and Nutrition Examination Survey (NHANES) conducted in 2013-2014, which showed BPA, BPS, and BPF detection rate of 95.7%, 89.4%, and 66.5% of randomly selected urine samples in adults and children [12]. Similarly, a study conducted in European adolescents as part of the Flemish Environment and Health Study (FLEHS IV, 2016-2020) found detection frequencies of over 50% for BPA and its alternatives, BPF, and BPS [17]. In several Asian countries, including China, India, Korea, Kuwait, Malaysia, and Vietnam, a human biomonitoring study reported varying detection frequencies for urinary BPS, ranging from 42 to 91%[12]. We conducted a global review of urinary BPA, BPF, and BPS profiles in children and adolescents with a summary of the detection rates of bisphenols reported in recent international studies (Table 7). It is important to note that the methods used for bisphenol measurement, the detection limits, and the period of study time, may vary between these studies, potentially impacting the observed bisphenol detection rates.

The analysis of two sets of urine samples collected with a one-week interval revealed significant variation in the detection rate and the urinary concentration of BPA and BPS, which can be attributed to variations in exposure to bisphenol analogs in children over time. Since bisphenols have a short half-life of approximately 7 hours and are eliminated in urine within 1-2 days after exposure [9,10], changes in exposure patterns could contribute to the observed differences in detection rates and levels between the two urine samples. In this study, we measure the urine BP concentration twice, and used the average concentration so that the data will better reflect true exposure levels to BP among Thai children.

The GM of urinary BPA concentration in our study (1.41 ng/mL) surpassed the levels observed in most studies concerning BPA exposure in children across Belgium, Japan, China, and the US from 2013 to 2018, which reported average urinary BPA concentrations ranging from 0.25 to 1.05 ng/mL[12,17–19]. However, studies conducted in Brazil and the EU from 2011 to 2013 [20,21] displayed higher urinary BPA levels than our study, with a range of 1.66 to 1.96 ng/mL (Table 7). Furthermore, the urinary BPF and BPS concentrations in our current study (0.013 ng/mL and 0.014 ng/mL, respectively) are notably lower than those reported in nearly all previous studies from other countries, which documented BPF levels ranging from 0.07 to 0.32 ng/mL and BPS levels ranging from 0.03 to 0.29 ng/mL. It is important to note that comparisons between these different studies were primarily based on medians for uncorrected concentrations.

Table 7. Comparison between the frequency of bisphenol exposure (Detection rate, %>LOQ) and the average unadjusted-urinary bisphenol concentration in current and previous studies in children and adolescents.

Country	Year	N	Age (years)	Method	Detection rate (%)			Concentration (ng/mL)		
					BPA	BPF	BPS	BPA	BPF	BPS
Current study	2019-2020	358	6-13	LC-MS/MS	79.6	31	16.8	1.41	0.013	0.014
belgium[17]	2017-2018	423	14-15	GC-MS/MS	86	97	83	1.05	0.14	0.12
Japan[19]	2012-2017	396	7	GC-MS/MS	89	83	78	0.89	0.07	0.11
China[18]	2015	283	3-11	HPLC-MS/MS	93	12	89	0.25	0.19	0.03
US[12]	2013-2014	868	6-19	HPLC–MS/MS	95.7	66.5	89.4	1.25	0.32	0.29
Thailand [16]	2013-2014	376	3-18	LCMS	75.3	-	-	0.68	-	-
Brazil[21]	2012-2013	300	6-14	LC-MS/MS	98	9	23	1.66	<LOQ	<LOQ
EU[a][20]	2011-2012	653	5-12	LC-MS/MS	91.1	-	-	1.96	-	-

[a]Belgium, Denmark, Luxembourg, Slovenia, Spain and Sweden.

The variability in bisphenol concentration observed across different studies can be attributed to several factors. Different regions and populations might have varying exposure levels due to differences in consumption patterns, dietary habits, and environmental conditions. Time period and regulation changes resulted in shifts in manufacturing practices and consumer behavior. Studies

 doi:10.20944/preprints202308.1924.v1

conducted in different years might capture different phases of exposure. Moreover, variations in laboratory methods, detection limits, and instrumentation used for bisphenol analysis can lead to differing measurements between studies. Differences in the timing of urine sample collection, time of day, and number of samples collected per individual can also influence the measured concentrations.

When comparing the EDI of BPA and BPS in Thai children and adolescents with the TDI established by the EFSA in 2015, it was found that the EDI values for BPA is about 35-fold below the corresponding TDI values of 4 mcg/kg of body weight per day, and the EDI values for BPS is around 1200-fold below the TDI. This suggests that there might be no immediate health concerns for this population. However, it is important to note that the TDI represents an estimate of the amount of a contaminant that can be consumed over a lifetime without posing an appreciable risk to health. Since this study provides cross-sectional data and the EDI may not reflect the overall lifetime exposure to bisphenols, it is crucial to continue monitoring bisphenol exposure and conducting further research on the potential health effects of BPA, BPF, and BPS in children. Moreover, note that there are currently no TDI values available for BPF. Therefore, as the EFSA periodically reassesses the toxicity of bisphenols and may lower the TDI threshold, continuous biomonitoring of BPA, BPF, and BPS exposure, along with additional research on their health effects in children, is necessary. Considering the most recent scientific insights regarding the potential risks associated with BPA exposure to human health, the specialists at EFSA have introduced a new TDI standard of 0.2 ng/kg/day (equivalent to 0.0002 mcg/kg/day) in April 2023. This new threshold is notably lower by approximately 20,000-fold compared to the previous level. Because of this updated criterion, all participants in our study exceeded the new TDI threshold.

We observed that several factors were associated with urine bisphenol concentrations. The first factor is age. Specifically, younger age of less than 10 years old was associated with 1.3 to 1.6-fold higher urine bisphenol analogs concentrations compared to older children. This reflects a high exposure to bisphenols in young children, which is similar to the findings of the NHANES study conducted in 2013-2014[12], where children under 11 years of age had significantly higher median levels of urinary BPA compared to adolescents (GM 1.34 mcg/L vs GM 1.14 mcg/L). Tait S et al. from Italy also demonstrated that urinary BPA concentrations were higher in children aged 4–6 years compared to those aged 7–10 years and 11–14 years [11]. A human biomonitoring study from Australia consistently showed that urine BPA levels in young children were significantly greater than in adults[22]. These previous findings support the hypothesis that children may consume more of these chemicals than adults due to higher amount of bisphenol-contaminated food consumption and through probable differences in how these compounds are absorbed, distributed, metabolized, and excreted between children and adult [12]. Regarding gender, our results indicated that there was no distinction between the GM of Cr-adjusted urine bisphenol concentration in boys and girls. This aligns with outcomes from several studies focused on bisphenol exposure in children [11,12,22], suggesting that children in both genders have comparable levels of bisphenol exposure.

Apart from age, other environment and behavioral risk factors associated with high bisphenol exposure were the consumption of food stored in plastic containers. The consumption of food stored in plastic containers at least 2 days per week seem to increase the risk of having high Cr-adjusted urinary bisphenol levels, implying that these chemical substances are a potential source of bisphenol exposure in Thai children. Bisphenols are commonly used in the production of certain food packaging materials. When food or beverages contact with these packages, there is a possibility of bisphenol leaching into the food or drink, especially under certain conditions such as high temperatures or acidic environments[23].

The novelty of this research lies in its focus on Thai children, a previously underrepresented demographic. Comprehensive characterization of their exposure to BPA and its analogs has been lacking. Moreover, the collection of the double morning voids, which accounts for the fluctuating nature of exposure levels over time, further elevates the uniqueness of this study. However, it is important to acknowledge the limitations. Firstly, this study was conducted in a single center, which may affect the generalizability of the findings to the general population. Additionally, the EDI of

bisphenols was derived from 2 single spot urine samples, rather than 24-hour urine collection, which could introduce some measurement uncertainty.

In conclusion, this comprehensive study on urine bisphenol metabolites provides insights into the predominant exposure of BPA, followed by BPF and BPS, among Thai children and adolescents during the period of 2019-2020, with BPA showing the highest concentration in urine. Importantly, the EDI of BPA and BPS in this population was found to be below regulatory limits. The study also identified significant associations between Cr-adjusted urine concentrations of BPA, BPF, and BPS and younger age, indicating age-related differences in bisphenol exposure. Additionally, higher urine BPF and BPS concentrations were associated with frequent exposure to food stored in plastic containers. Future research focusses on investigating the potential health effects of exposure to BPF and BPS on children is needed.

Author Contributions: Conceptualization, V.S. and N.N.; methodology, V.S.; software, N.N.; validation, V.S., N.N. and K.C.; formal analysis, N.N.; investigation, T.T., N.N. and S.A.; resources, C.B.; data curation, N.N.; writing—original draft preparation, N.N.; writing—review and editing, V.S. and K.C.; visualization, N.N.; supervision, V.S.; project administration, N.N.; funding acquisition, N.N. and T.T. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Ratchadapisek Sompot Fund of Chulalongkorn University, Bangkok, Thailand. (RCU_H_64_006_30)

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki, and approved by the Institutional Review Board of the Faculty of Medicine, Chulalongkorn University (IRB No. 237/64).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study

Data Availability Statement: The data presented in this study are available on request from the corresponding author. The data are not publicly available due to ethical issue.

Acknowledgments: We would like to thank Miss Jiratchaya Sophonphan, participants, pediatric residents, and pediatric endocrinology fellows at Department of Pediatrics, Faculty of Medicine, Chulalongkorn University and King Chulalongkorn Memorial Hospital, Bangkok, Thailand for helping and participating in the study.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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