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4-Nonylphenol, bisphenol-A and triclosan levels in human urine of children and students in China, and the effects of drinking these bottled materials on the levels

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ABSTRACT

4-Nonylphenol (4-NP), bisphenol-A (BPA) and triclosan (TCS) are three industrial chemicals used widely in daily products. This study investigated 4-NP, BPA and TCS levels in urine samples of 287 children and students aged from 3 to 24 years old in Guangzhou, China. Total (free and conjugated) amounts of 4-NP, BPA and TCS in the urine samples were detected using gas chromatography–mass spectrometry with negative chemical ionization. The detection rates of 4-NP, BPA and TCS were 100%, 100% and 93% respectively, given the detection limits of 3.8, 0.5 and 0.9 ng/L respectively. Data for 4-NP, BPA and TCS were presented in both creatinine-adjusted (microgram per gram creatinine) and unadjusted (microgram per liter) urinary concentrations. The geometric mean (GM) concentrations of urinary 4-NP, BPA and TCS were 15.92 μg/g creatinine (17.40 μg/L), 2.75 μg/g creatinine (3.00 μg/L) and 3.55 μg/g creatinine (3.77 μg/L) respectively. Multiple regression models considering age, gender, preferred drinking bottle and log-transformed creatinine were used to calculate the adjusted least square geometric mean (LSGM). Among these subjects, the females had higher LSGM concentrations of 4-NP, BPA and TCS than the males; and the only statistically significant difference was found for the LSGM concentrations of triclosan ($p = 0.031$). Participants who reported to use ceramic cups more frequently had significantly lower LSGM concentrations of BPA than those who used plastic cups ($p = 0.037$).

Meanwhile, a three-week test of using polycarbonate bottles and ceramic cups to drink bottled water and boiled tap-water was carried out among 12 graduate students of 25 years old. The GM concentrations of urinary BPA at the end of the first week after using ceramic cups to drink bottled water were 7.16 μg/g creatinine, then decreased significantly to 3.49 μg/g creatinine after the second week of using ceramic cups to drink boiled tap-water ($p < 0.05$), and finally increased to 4.15 μg/g creatinine after the third week of using polycarbonate bottles in drinking boiled tap-water. The results indicate that in daily life the use of polycarbonate bottles or drinking of bottled water is likely to increase the ingestion of BPA, resulting in an increase in urinary BPA levels.

1. Introduction

4-Nonylphenol (4-NP), bisphenol-A (BPA) and triclosan (TCS) are industrial chemicals widely used in various daily products, which results in exposure to humans of these compounds in daily life (Kang et al., 2006; Singer et al., 2002; Soares et al., 2008). 4-NP is widely used in domestic products such as surfactants and food packaging films (Guenther et al., 2002; Uchiyama et al., 2008; Ying et al., 2002). BPA is used in polycarbonates (PC) and epoxy resin products (e.g. baby bottles and food containers) (Biles et al., 1997; Olea et al., 1996; Vandenberg et al., 2007). And TCS is used as an antimicrobial in some personal care products (e.g. toothpaste, cosmetics, skin care creams and lotions, soaps and dental products) (Jones et al., 2000; McAvoy et al., 2002; Tsai et al., 2008). These chemicals are known as endocrine disrupting compounds that may cause adverse effects on wildlife as well as human beings (Crain et al., 2007; Hunt et al., 2003; Yang et al., 2008; Ying, 2006).

In the last decade, studies have shown that humans are exposed to 4-NP, BPA and TCS through various routes: air, soil, sediment, water, food, drink and even skin contact (Benotti et al., 2009; Guenther et al., 2002; Kang et al., 2006; Loyo-Rosales et al., 2004). Meanwhile, 4-NP, BPA and TCS have already been detected in human urine, blood and breast milk of some countries (Ademollo et al., 2008; Allmyr et al., 2006, 2008; Calafat et al., 2008a,b; Inoue et al., 2000, 2003; Vandenberg et al., 2007); and BPA was even found in amniotic fluid, follicular fluid, placental tissue, semen, umbilical cord blood, fetal serum and adipose tissues (Vandenberg et al., 2007; Vandenberg et al., 2010).

It is an increasing concern that 4-NP and BPA could pose potential risks to human reproductive function (Bredhult et al., 2007; Tsutsumi, 2005). Some studies suggest that the increased cancers (Keri et al.,

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2007), human health abnormalities (cardiovascular disease and diabetes) (Lang et al., 2008) and externalizing behaviors in 2-year-old children (especially among female children) can be associated with BPA exposure (Braun et al., 2009). Although in literature there are no clear evidence that suggests that TCS has adverse effects on humans (Dayan, 2007; Sullivan et al., 2003), the disruption of the thyroid hormone system raises concern on the potential endocrine disrupting effects of TCS and possible adverse effect on humans (Crofton et al., 2007; Zorrilla et al., 2009).

Owing to their potential negative impacts on human health, it is important to establish comprehensive worldwide knowledge on human exposures to these contaminants. Since urine is considered to be the most appropriate matrix for biomonitoring 4-NP, BPA and TCS (Dekant and Völkel, 2008), urinary levels of these compounds have been studied in several countries, such as the U.S.A (Calafat et al., 2005, 2008a,b), Germany (Becker et al., 2009), Japan (Inoue et al., 2003; Matsumoto et al., 2003) and Korea (Kim et al., 2003; Yang et al., 2003). To our knowledge, there are few investigations into urinary 4-NP, BPA and TCS under the age of 25, especially for 4-NP and TCS. Little information on urinary levels of these compounds is available in China, except for a recent study on adults (He et al., 2009), which investigated BPA levels in blood and urine samples of 953 people from central and east China.

The objectives of this study were to examine the levels of 4-NP, BPA and TCS simultaneously in urine samples of children and students in southern China. All samples were collected from people without occupational exposure in Guangzhou. Since BPA were reported to be released from polycarbonate packaging materials (Le et al., 2008; Li et al., 2010; Vandenberg et al., 2007), we designed a three-week test to evaluate the difference of using ceramic cups and polycarbonate bottles that influence the urinary levels of 4-NP, BPA and TCS.

2. Experimental

2.1. Standards and reagents

Chemical standards 4-nonylphenol (4-NP), bisphenol-A (BPA), and triclosan (TCS) were obtained from Dr. Ehrenstorfer GmbH (Germany) or Supelco (USA), whereas 4-*n*-nonylphenol (4-*n*-NP), [$^2H_{16}$] BPA (BPA-d_{16}), and ^{13}C-labeled triclosan (^{13}C-TCS) used as internal standards were obtained from Dr. Ehrenstorfer GmbH (Germany), Supelco (USA), and Cambridge Isotope Laboratories Incorporation (Massachusetts, USA), respectively. Detailed information about these standards is listed in Table S1 (Supplementary Information). The derivatization reagent pentafluorobenzoyl chloride (PFBOCl, purity >99%) was obtained from Aldrich. Methanol, *n*-hexane, toluene, ethyl acetate, dichloromethane (DCM) and pyridine (HPLC-grade) were purchased from Merck Corporation (Shanghai, China). β-Glucuronidase (111,000 U/mL glucuronidase and 1079 U/mL sulfatase activity) and anhydrous creatinine were obtained from Sigma-Aldrich (USA). Supelclean ENVI-18 solid phase extraction cartridges (500 mg, 3 mL) were purchased from Supelco Corporation. To avoid the contamination of 4-NP, BPA and TCS, no plastics were allowed to be used in the experiment, and all glassware was baked for 4 h at 400 °C before using.

2.2. Sample collection and preparation

Morning urine samples of humans were obtained from 287 children and students aged from 3 to 24, including kindergarten children, primary school pupils, secondary school students and college undergraduates. Urine samples were obtained with approval of the school/university and their parents. All procedures were carried out under the permission of law. Meanwhile, basic personal information was collected, including gender, age, and preferred daily drinking water bottle.

In order to analyze the influence of using ceramic cups and polycarbonate plastic cups, a three-week test on human urinary levels of the three compounds was carried out. Twelve volunteers (6 females and 6 males, aged at 25 years old) from Guangzhou Institute Geochemistry, Chinese Academy of Sciences, participated in the test. We have also obtained approval from the institution and donors themselves. Taking into account any possible interference, these participants were chosen because they have not been occupationally exposed to the target compounds. In the first week, the volunteers were asked to drink a designated brand of bottled water using the same provided ceramic cups. Urine samples were collected in the morning on the eighth day. In the second week, they still used the ceramic cups, but drank boiled tap-water with provided electric kettle made of stainless steel. After the volunteers donated their urine samples on the morning of the fifteenth day, polycarbonate plastic cups were given to them for use in the last week to drink boiled tap-water. To minimize container contamination, clean glass bottles were used to collect urine samples. All the biological samples were stored at −18 °C prior to use. Determination of creatinine concentrations and hydrolyzation of urine samples are given in Supplementary Information.

2.3. Solid phase extraction

Before solid phase extraction (SPE), 100 μL each of 1 mg/L of 4-*n*-NP, BPA-d_{16} and ^{13}C-TCS were spiked into each sample as internal standards. The eluates were concentrated to dryness under a gentle stream of nitrogen, and then redissolved in methanol to a final volume of 1 mL. Each final extract was then filtered through a 0.22 μm membrane filter into a 2 mL amber glass vial and kept at −18 °C until analysis. Derivatization of each extract was carried out based on the previously reported methods (Boitsov et al., 2004; Zhao et al., 2009). Please refer to Supplementary Information for the detailed derivatization procedure. The final extract was re-dissolved in 100 μL of *n*-hexane, and then transferred to a 2 mL amber glass vial with a 250 μL flat-bottomed insert, which was ready for analysis by gas chromatography–negative chemical ionization–mass spectrometry (GC–NCI–MS).

2.4. Instrumental analysis

GC–MS analysis of the derivatized samples was performed using an Agilent 6890N gas chromatograph connected to an Agilent 5975B MSD mass spectrometer with a chemical ionization (CI) source (Agilent, USA). Selected ion monitoring (SIM) mode was used for the quantitative analysis of these compounds (Table S1, Supplementary Information). Detailed instrumental conditions are given in Supplementary Information. The method limits of detection (LOD) for 4-NP, BPA and TCS were 3.8 ng/L, 0.9 ng/L and 0.5 ng/L, while the method limits of quantification (LOQ) were 12.8 ng/L, 3.0 ng/L and 1.8 ng/L, respectively. The recoveries for the target analytes from urine samples were found more than 93% (Table S2, Supplementary Information).

2.5. Statistical analysis

For data analysis, SAS (version 9.1, the Glm procedure) and SPSS (version 13.0, the UNIANOVA procedure and the FREQUENCIES procedure) were used. To determine whether there was a difference between age, sex or preferred drinking bottle in the same demographic group, we selected multiple regression models and all possible two-way interactions to calculate the adjusted least square geometric mean (LSGM). The concentrations of creatinine, 4-NP, BPA and TCS were log-transformed due to the skewed distribution. We replaced those levels below LOD by using the LOD divided by the square root of 2, as recommended by Centers for Disease Control and Prevention (CDC, 2006). Variables included were age (categorized into 3 to 6, 7 to 12, 13 to 17, and 18 to 24 years-old age groups),

gender (categorized as males and females) and preferred bottle materials (categorized as plastic, plastic and glass, plastic and ceramic, and ceramic). Age was self-reported in years at the previous birthday and was categorized according to their education background (kindergarten, primary school, secondary school and college). To reach the final model, we eliminated nonsignificant interactions one by one by examining the β coefficient changes. If the β coefficient changed by ≥10%, then the removed interaction or variable was retained. The *p*-value was set at 0.05.

In a similar demographic group, urinary concentrations for 4-NP, BPA and TCS were adjusted to a creatinine concentration (micrograms per gram creatinine) in order to avoid the different urinary volume which may lead to variable dilutions among collected urine samples (Barr et al., 2005). Also, unadjusted creatinine concentrations (microgram per liter) were calculated in the multiple regression model recommended by Barr et al. (2005), in order to allow the statistics to be independent of effects of urinary creatinine concentration when analyzing different demographic groups.

3. Results

3.1. 4-NP, BPA and TCS levels in urine samples

Among the 287 eligible urine samples, 55% of the donors were males and 45% were females (Table 1). The geometric mean (GM), 10th, 25th, 50th, 75th, 90th and 95th percentiles for both creatinine-adjusted (micrograms per gram creatinine) and unadjusted (microgram per liter) urinary concentrations are given in Tables 3–5 (Supplementary Information) (Fig. 1). The detection rates of 4-NP, BPA and TCS were 100%, 100% and 93%, respectively. Concentrations of 4-NP, BPA and TCS ranged from 1.01 to 446.39, 0.41 to 198.05 and not detected (ND) to 558.25 μg/g creatinine respectively. The GM concentrations of urinary 4-NP, BPA and TCS were 15.92 μg/g creatinine (17.40 μg/L), 2.75 μg/g creatinine (3.00 μg/L) and 3.55 μg/g creatinine (3.77 μg/L) respectively.

In the multiple regression model for 4-NP, BPA and TCS, we included age group, sex, bottle materials and creatinine concentrations as independent variables, and also included all possible two-way interaction terms between those variables. For 4-NP, no variables or interactions between any of the covariates were statistically significant (all $p>0.05$), except for the age group. Thus, the final model only included age ($p<0.001$), as shown in Tables 2 and 3 that the adjusted LSGM concentrations declined significantly both from 3–6 years old to 7–12 years old ($p=0.003$) and from 7–12 years old to 13–17 years old ($p<0.001$), then increased significantly to 18–24 years old ($p<0.001$).

The final model for BPA included log-transformed creatinine ($p=0.017$), age ($p<0.001$) and age–sex ($p=0.018$) (Table 3). The LSGM concentrations of BPA for those using plastic bottles were higher than those using ceramic cups ($p=0.037$); and also those who reported using both plastic bottles and ceramic cups frequently had a higher LSGM levels than those only using ceramic cups ($p=0.026$). The LSGM concentrations of BPA decreased with age from 7 to 24 years. The LSGM concentrations for the 3–6 age group were drastically lower than those for the 7–12 age group ($p<0.001$) and the 13–17 age group ($p=0.024$). However, the LSGM concentration difference between the youngest 3–6 years and the relatively oldest 18–24 years category was not statistically significant ($p=0.979$).

The final model for TCS included log-transformed creatinine ($p=0.001$), age ($p=0.001$), sex ($p=0.023$) and age–sex ($p=0.032$) (Table 3). Different bottle materials reflected no

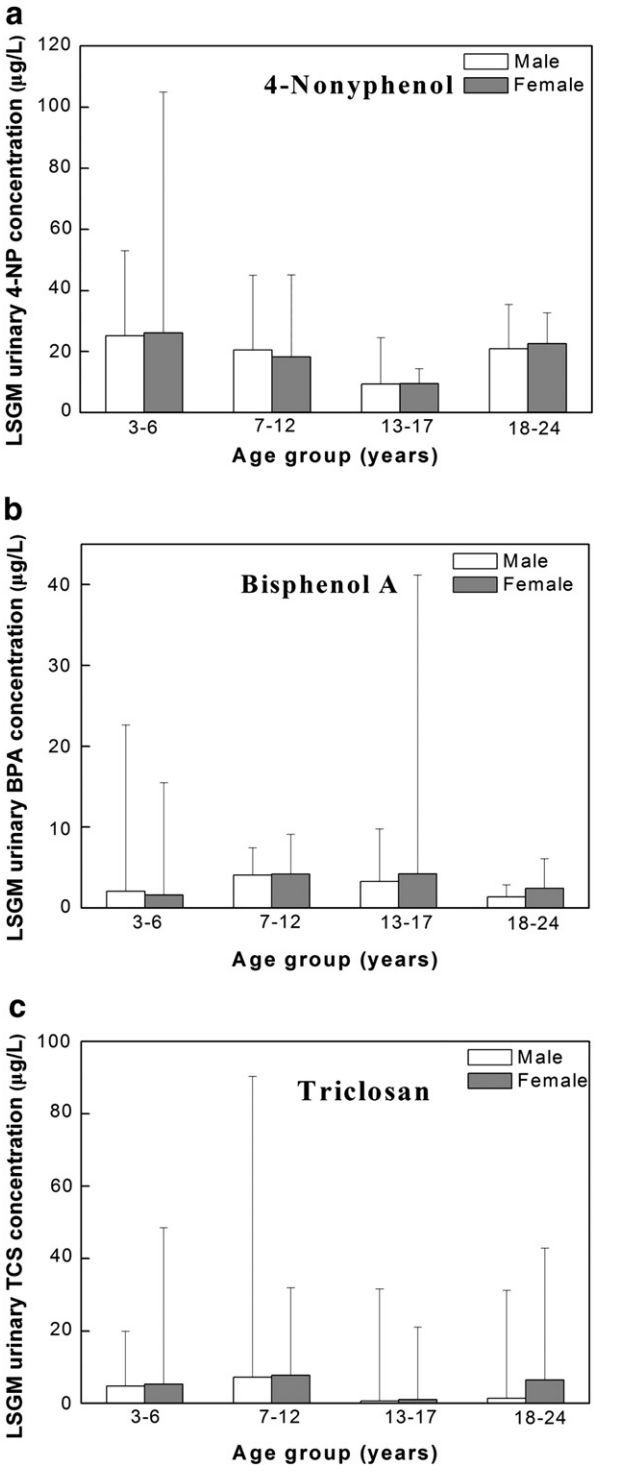

Fig. 1. Least square geometric mean (LSGM) urinary concentrations of target compounds by age and sex. Error bars indicate 95% confidence intervals. (a) 4-nonylphenol (4-NP), (b) bisphenol-A (BPA), and (c) triclosan (TCS).

Table 1
Characteristics of 287 urine donators from Guangzhou, China.

Age (years)	Education	Number			Percentage (%)
		Male	Female	Total	
3–6	Kindergarten	30	26	56	20
7–12	Primary school	51	44	95	33
13–17	Secondary school	39	33	72	25
18–24	College	39	25	64	22

Table 2
Adjusted least square geometric mean (LSGM) concentrations (95% confidence intervals) of urinary 4-nonylphenol (4-NP), bisphenol A (BPA) and triclosan (TCS) (μg/L) for 287 Chinese population.

Variable	LSGM (95% CI)		
	4-NP	TCS	BPA
Sex			
Male	18.58 (16.83–20.51)	2.57 (1.79–3.69)	2.40 (2.00–2.89)
Female	18.62 (16.48–21.04)	4.49 (2.88–6.98)	2.79 (2.22–3.48)
Age (years)			
3–6	25.59 (22.23–29.38)	5.04 (3.03–8.36)	1.80 (1.39–2.33)
7–12	19.32 (17.14–21.73)	7.52 (4.88–11.56)	4.10 (3.30–5.11)
13–17	9.42 (6.95–12.76)	0.81 (0.27–2.45)	3.71 (2.11–6.49)
18–24	21.73 (18.58–25.35)	3.03 (1.72–5.33)	1.79 (1.35–2.39)
Drinking habit (bottle material)			
Plastic			2.99 (2.62–3.40)
Plastic and glass			2.21 (1.36–3.62)
Plastic and ceramic			3.18 (2.62–3.85)
Ceramic			2.00 (1.41–2.85)

statistically significant influence for the LSGM urinary TCS levels (all $p>0.05$). Females had a statistically higher LSGM concentration than the males ($p=0.031$).

3.2. Three-week test of using ceramic and PC plastic cups

In the three-week test 12 graduate students were asked to use ceramic and PC plastic cups to drink bottled water and boiled tap-water. The concentrations of BPA in dosed bottled water and tap-water are 82.4 ng/L and 28.6 ng/L, respectively (Li et al., 2010). After the first week's test of drinking bottled water with a ceramic cup, the GM concentrations of urinary 4-NP, BPA and TCS were 25.96, 1.11 and 7.16 μg/g creatinine respectively (Table 4). After the second week's test of drinking boiled tap-water using ceramic cup, the GM concentrations of urinary 4-NP, BPA and TCS decreased to 21.28, 1.02 and 3.49 μg/g creatinine respectively. And in the third week's test of drinking boiled tap-water by using PC plastic cups, the GM concentrations for 4-NP, BPA and TCS increased to 23.04, 1.57 and 4.15 μg/g creatinine respectively. The UNIANOVA process was used to measure the bottle material effects on urinary 4-NP, BPA and TCS. Results showed that the concentrations of urinary 4-NP and TCS had no significant change ($p>0.05$) after the three-week test, but did have a significantly higher urinary BPA concentration after using PC plastic cups or bottles ($p<0.05$).

Table 3
Observed statistical significant values for differences between adjusted least square geometric mean (LSGM) concentrations of 4-nonylphenol (4-NP), bisphenol A (BPA) and triclosan (TCS) for various demographic groups in China.

Difference	p-Value*		
	4-NP	BPA	TCS
Females vs. males	0.966	0.266	0.031
3–6 vs. 7–12[a]	0.003	<0.001	0.238
3–6 vs. 13–17	<0.001	0.024	0.004
3–6 vs. 18–24	0.140	0.979	0.208
7–12 vs. 13–17	<0.001	0.740	<0.001
7–12 vs. 18–24	0.239	<0.001	0.012
13–17 vs. 18–24	<0.001	0.023	0.035
Plastic vs. plastic and glass[b]	0.634	0.244	0.516
Plastic vs. plastic and ceramic	0.757	0.615	0.632
Plastic vs. ceramic	0.088	0.037	0.691
Plastic and glass vs. plastic and ceramic	0.748	0.182	0.680
Plastic and glass vs. ceramic	0.146	0.748	0.430
Plastic and ceramic vs. ceramic	0.077	0.026	0.519

*Considering of all possible two-way interactions.
The final model for 4-NP included age ($p<0.001$).
The final model for BPA included creatinine (log-transformed) ($p=0.017$), age ($p<0.001$) and age–sex ($p=0.018$).
The final model included creatinine (log-transformed) ($p=0.001$), age ($p=0.001$), sex ($p=0.023$) and age–sex ($p=0.032$).
[a] 3–6, 7–12, 13–17 and 18–24 are age groups categorized according to donators' educational background (kindergarten, primary school, secondary school and college).
[b] Plastic, plastic and glass, plastic and ceramic and ceramic are donators' preferred material for drinking container.

Table 4
Geometric mean concentrations (μg/g creatinine) of 4-nonylphenol (4-NP), bisphenol A (BPA) and triclosan (TCS) in human urine of 12 volunteers aged 25-year-old during three-week test.

Compounds	Geometric mean concentrations		
	Week 1[a]	Week 2[b]	Week 3[c]
4-NP	25.96 ± 44.92 A*	21.28 ± 27.32 A	23.04 ± 20.76 A
TCS	1.11 ± 3.20 A	1.02 ± 127.07 A	1.57 ± 40.22 A
BPA	7.16 ± 5.25 B	3.49 ± 1.96 A	4.15 ± 3.99 AB

[a] Week 1: after one week drinking bottled water with ceramic cup.
[b] Week 2: after the second week drinking boiled tap water with ceramic cup.
[c] Week 3: after the third week drinking boiled tap water with polycarbonate bottle.
* UNIANOVA analysis of 4-NP, TCS and BPA concentrations by week (Duncan), with different letters indicating significant difference in concentration ($p<0.05$).

4. Discussion

The results of urinary samples in the present study showed that young people in Guangzhou, China have daily exposure of the three endocrine disrupting chemicals: 4-NP, BPA and TCS. The detection rates of 4-NP, BPA and TCS were 100%, 100% and 93%, respectively (Tables S3–S5). Previous studies suggested that urinary concentrations of these compounds could be used to assess daily exposure, although detected NP in urine might only account for a small percentage of ingested amounts (Dekant and Völkel, 2008; Müller et al., 1998). Since GM concentrations of the target compounds were not significantly different between morning and evening collection (Calafat et al., 2008a,b), in order to eliminate variation related to time of day, we collected morning urine samples. For inter-person variability, a single urine sample can still be used to characterize long-term exposure to these compounds (Mahalingaiah et al., 2008; Teitelbaum et al., 2008).

For urinary 4-NP, previous studies reported lower detection rates ranging from 40% to 70% (Calafat et al., 2005; Inoue et al., 2003; Mao et al., 2004). The result from Inoue et al. (2003) and Mao et al. (2004) might not reflect limited exposure to 4-NP as the sample number used in their studies were small. In fact, Calafat et al. (2005) also pointed out that human exposure to 4-NP is likely to be underestimated due to its complicated metabolic mechanism (Müller et al., 1998) and that the detected NP concentration only accounts for a small percentage of the NP exposure.

The results of urinary BPA from the present study were not all consistent with the reported data. The present study showed that the LSGM concentrations of urinary BPA in children (7–12 years old) (4.10 μg/L) and adolescents (13–17 years old) (3.71 μg/L) were significantly higher than those in adult (18–24 years old) (1.79 μg/L) (Table 2), which is consistent with the findings in a previous study (Calafat et al., 2008a). Unexpectedly, children from kindergarten (3–6 years old) had a lower level of LSGM urinary BPA (1.80 μg/L) than the former two categories (Table 2), despite the significant effect of age ($p<0.001$) on the LSGM of urinary BPA in the multiple regression models (Table 3). In fact, previous studies also reported a decreasing trend of urinary BPA levels both in 599 German children that aged from 3 to 14 years old (Becker et al., 2009) and in 2517 U.S. populations that aged from 6 years to above 60 years old (Calafat et al., 2008a). Yet, the data of the 3–6 age group seemed abnormal; it may be less appropriate to categorize samples according to their age group

because the major route of human exposure to BPA was through food and drink ingestion (Kang et al., 2006; Vandenberg et al., 2007). Therefore, when we categorized the samples based on their drinking habit, it became much clearer that those who used plastic containers in daily life had a higher LSGM of urinary BPA. Even though the present study had lower BPA levels for the 3–6 age group (Table 2), the reported higher exposure in 54 premature infants (28.6 μg/L) (Calafat et al., 2009) and conclusions of a recent review that fetuses and children are particularly vulnerable to BPA exposure (Vandenberg et al., 2010) remind us that BPA is still a concern.

In terms of gender effect on urinary BPA levels, there have been contradictory reports. Some studies found that the total BPA concentrations were similar in females and males (Becker et al., 2009; Kim et al., 2003). Others found females had significantly lower urinary BPA concentrations than males in 952 Chinese populations (He et al., 2009). On the contrary, BPA levels were found significantly higher in females than in males for 2517 U.S. populations (Calafat et al., 2008a). In the present study, there is no significant difference of urinary BPA levels between females and males. The variation in BPA may well be associated with the selected objects, their lifestyle and metabolism conditions.

For urinary TCS, the present study found a decreasing tendency with age in creatinine-adjusted urinary TCS GM concentrations in the 7–24 age groups (Table S5). It is different to the findings of Calafat et al. (2008b) that the concentrations of urinary TCS (≥6 years old) in the 20–29 age groups were the highest. Since oral route (by ingesting toothpaste or other oral health care products) and dermal route (by contact with soap, skin cleanser, shower gel or other skin-care products) are recognized as two major routes of human exposure to TCS (Moss et al., 2000; Sandborgh-Englund et al., 2006), kindergarten children and pupils (<12 years old) might use more personal care products under the care of their parents, thus having higher exposure to TCS. The present study also observed that the females had a significant higher LSGM concentration of TCS than the males ($p = 0.031$) (Table 3). This difference might reflect different hygiene habits between the females and males.

The detected concentration range for TCS (ND-681.38 μg/L) in the present study (Table S5) is similar to those available in the literature: TCS detected in previous studies of 90 girls of 6–9 years old (Wolff et al., 2007), 2517 U.S. population (Calafat et al., 2008b) and 20 Belgian (Geens et al., 2009) ranged from <1.6–956.0 μg/L, <2.4–3790 μg/L and 0.18–672 μg/L, respectively. The geometric mean of TCS of 90 children of 7–12 years old (6.91 μg/L) in the present study (Table S5) is lower than that for 90 girls of 6–9 years old (10.9 μg/L) in the study of Wolff et al. (2007) and 314 U.S. children of 6–11 years old (8.2 μg/L) in the study of Calafat et al. (2008b). This may be attributed to the different lifestyles or personal hygiene habits between China and other developed countries.

In the three-week test on the influence of drinking bottles, ceramic cups were used instead of stainless steel cups as used in Carwile et al. (2009), since most Chinese people used ceramic cups more often. However, our results are consistent with those of Carwile et al. (2009). Urinary BPA had a statistically significant decrease ($p<0.05$) when replacing plastic bottled water (7.16 μg/g creatinine) with boiled tap-water (3.49 μg/g creatinine) after the first two weeks, and increased to 4.15 μg/g creatinine after using PC drinking bottle in the third week (Table 4). Since BPA was reported to be released from polycarbonate bottles (Le et al., 2008; Li et al., 2010; Vandenberg et al., 2007), these changed levels of urinary BPA may indicate that drinking plastic bottled water or using PC bottles would probably increase the ingestion of BPA. In the study of Carwile et al. (2009), they just assessed the impact of using PC bottles on cold beverages, but did not count for heating factors on PC bottles. The released BPA from PC bottles increased with water temperature, especially at the high temperature (e.g. 100 °C) (Li et al., 2010), thus the test conditions used in the present study were closer to the situations in real life. This could be the reason that the GM concentrations of urinary BPA in the present study (3.49–7.16 μg/g creatinine) are higher than those of Carwile et al. (2009) (1.2–2.0 μg/g creatinine). Therefore, drinking bottle materials and habits could affect BPA levels in urine.

5. Conclusion

Detection of 4-NP, BPA and TCS in children and students from Guangzhou, China suggests a wide human exposure to these chemicals. This study provided the first investigation into the exposure of children under 6 years old to the three chemicals in China, which can be helpful in health risk assessment in a Chinese population. Hygiene habits and drinking bottles could influence the exposure levels of these chemicals. The females had significantly higher LSGM concentrations of TCS than the males possibly due to different hygiene habits. Drinking water using PC bottles or plastic bottled water could significantly increase the exposure levels of BPA due to leaching of BPA from plastic and PC bottles; since young children prefer to use plastic bottles or drink bottled beverages according to the collected information, the young children are therefore more vulnerable to BPA. It is recommended that children should avoid using polycarbonate containers to drink water or beverages in order to reduce the exposure of chemicals.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at doi:10.1016/j.envint.2011.03.026.

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