



Article

Urinary Concentrations of Triclosan, Benzophenone-3, and Bisphenol A in Taiwanese Children and Adolescents

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Received: 9 October 2017; Accepted: 5 December 2017; Published: 10 December 2017

Abstract: The purpose of this study was to determine the levels of urinary triclosan (TCS), benzophenone-3 (BP-3), and bisphenol A (BPA) in 52 children and 71 adolescents. The effects of age and sex on the levels of urinary TCS, BP-3, and BPA were explored, respectively. Results demonstrated the overall detection rates of urinary TCS, BP-3, and BPA were 18.7%, 8.1%, and 49.6%, respectively. The females had higher TCS concentrations than males ($p = 0.051$). The detection rate of urinary BP-3 in females (12.3%) was higher than that in males (0%) ($p = 0.015$). Moreover, the detection rate of urinary BP-3 in adolescents (14.1%) was higher than that in children (0%) ($p = 0.005$). For children, no urinary BP-3 was found. There were no differences in detection rates and concentrations of urinary TCS, BP-3, and BPA between males and females, respectively. For adolescents, urinary BP-3 was only found in the females. Urinary TCS levels in females were higher than those in males ($p = 0.047$). The present study showed that urinary TCS concentrations in females were significantly higher than those in males, respectively. In addition, BP-3 was only detected in urine samples of female adolescents. Sex and age were the important factors influencing urinary TCS and BP-3 concentrations.

Keywords: triclosan; benzophenone-3; bisphenol A; children; adolescents

1. Introduction

Humans are potentially exposed to several phenolic chemicals in commonly used products that may cause endocrine-disrupting effects. For example, triclosan (2,4,4'-trichloro-2'-hydroxy-diphenyl ether, (TCS) has been added to soaps, toothpastes, mouthwashes, deodorants, and cosmetics as a preservative and antiseptic agent [1,2]. Bisphenol A (2,2-bis(4-hydroxyphenyl)propane, (BPA) has been used in the production of polycarbonate plastics and epoxy resins. It can be found in water bottles, baby bottles, plastic dinnerware, dental sealants, and the linings of canned food containers [3–5]. Additionally, some thermal paper and polyvinyl chloride plastics can also contain low levels of bisphenol A [6,7]. Benzophenone-3 (2-hydroxy-4-methoxybenzophenone, (BP-3) is a common ingredient in sun-blocking agents and is commercially synthesized as a sunscreen for use in lotions, conditioners, and cosmetics [8,9].

Oral and dermal absorption are the major routes of exposure of these phenolic chemicals. Due to their extensive use, the general population is potentially at risk of exposure to TCS, BPA, and BP-3, which result in their wide distribution in human blood, breast milk, and urine, respectively [10–20]. TCS, BPA, and BP-3 have also been detected in a variety of environmental substances, including water, wastewater, river, and sediment [21–29].

TCS, BPA, and BP-3 have been reported for endocrine-disruption potentials in a number of in vitro and in vivo studies. In general, BPA and TCS are considered to have a weak estrogenic effect [30,31], whereas BP-3 was determined to be slightly estrogenic or to have anti-androgenic properties [32–34].

Several studies by Howdeshell et al. (1999) indicated that BPA altered the postnatal growth rate and reproductive function in female mice [35–38]. Low doses of BPA possibly altered the development of the fetal prostate and mammary gland, and decreased efficiency of sperm production in mice [39–42]. TCS was likewise found to inhibit thyroid hormone-associated gene expression and postembryonic anuran development in tadpoles [43]. Kumar et al. [44] reported that the levels of serum luteinizing hormone, follicle stimulating hormone, pregnenolone, and testosterone were significantly diminished in TCS treated rats.

Coronado et al. [45] have suggested that reproduction and hatching success were reduced in BP-3 treated Japanese medaka, respectively (*Oryzias latipes*). Vitellogenin induction (*Oncorhynchus mykiss*) was observed in BP-3 treated male Japanese medaka and rainbow trout, respectively. Low levels of BP-3 also inhibited steroidogenesis and affected hormonal pathways in male zebrafish [46].

These phenols are also suspected to affect development in humans. Philippat et al. [47] indicated that urinary concentrations of BP-3 were positively associated with weight and head circumference at birth. Urinary BPA concentrations were positively associated with head circumference. Wolff et al. [48] reported that higher maternal BP-3 concentrations were associated with a similar decrease in birth weight among girls but with greater birth weight in boys. Koeppe et al. [49] observed a positive association between urinary TCS concentrations and serum total triiodothyronine (T3) levels in adolescents. Tsutsumi [50] reported that serum BPA concentrations were significantly higher in normal men and in women with polycystic ovary syndrome than in normal women, respectively. No hormonal changes were observed in 32 healthy volunteers during four days of application of 10% BP-3 lotion [51].

However, there are likely to be some important variables (e.g., age and sex) that determine whether people contact TCS, BP-3, and BPA-containing products. Relative children and males, adolescents, and females have more chances to use personal care products such as toothpastes, lotions, and cosmetics. The purpose of this study is to explore the effects of age and sex on the levels of urinary TCS, BP-3, and BPA. The elementary school children and college students were selected as our study subjects.

2. Materials and Methods

2.1. Chemicals and Reagents

Triclosan (TCS), bisphenol A (BPA), and benzophenone-3 (BP-3) standards were all purchased from Sigma-Aldrich (St. Louis, MO, USA). HPLC-grade methanol and water, analytical-grade ammonium acetate, formic acid, and β -Glucuronidase/arylsulfatase were provided by Merck (Darmstadt, Germany). $^{13}\text{C}_{12}$ -triclosan was obtained from Cambridge Isotope Laboratories Inc. (Andover, MA, USA).

2.2. Sample Collection

Morning urine samples were obtained from 52 elementary school children of 11–13 years old and 71 college students of 19–21 years old in southern Taiwan, respectively. Written informed consent was obtained from each subject and their parents. The study procedures were approved by the Institutional Review Board of I-Shou University (ISU-IRB-98-03).

2.3. Sample Preparation

The pretreated and analyzed methods of urine samples were referred to in the procedures, as reported by Ye et al. [52]. At first, urine samples were thawed at room temperature and vortex mixed. Next, a 100 μL aliquot of urine was transferred into a tube and spiked with 50 μL of $^{13}\text{C}_{12}$ -Triclosan. After gentle mixing, samples were added to 50 μL of glucuronidase/sulfatase solution (in a 0.5-M

ammonium acetate buffer, pH 5.0), and the hydrolysis was allowed to proceed at 37 °C overnight. After the incubation, the deconjugated urine sample was diluted with 800 µL to 0.1 M formic acid and centrifuged. The aliquot was injected into the on-line solid phase extraction (SPE) coupled to high-performance liquid chromatography (HPLC)–tandem mass spectrometry (MS/MS).

2.4. On-Line SPE-HPLC-MS/MS

The on-line SPE-HPLC-MS/MS system was constructed from several Agilent 1290 modules coupled to a triple quadrupole Agilent 6430 mass spectrometry (Agilent Technologies, Santa Clara, CA, USA). The mass spectrometer was equipped with an electrospray ionization (ESI) interface. The SPE column was a LiChrosphere RP-18 ADS (25 × 4 mm, 25 µm particle size, 60-Å pore size, Merck KGaA), and the HPLC column was ZORBAX Eclipse XDB-C18 (150 × 4.6 mm; Agilent). Methanol and water were used as mobile phases. The procedure for extracting the analytes from the urine involved concurrent SPE and HPLC-MS/MS cycles (Table 1).

The Agilent 6430 mass spectrometry was used in negative mode with the following settings: source gas (nitrogen) with a flow rate of 10 L/min at a temperature of 350 °C, nebulizer pressure of 50 psi, and capillary voltage of 4 kV. The mass spectrometer operating parameters were optimized using Agilent Optimizer Software for the analytes (Table 2). The limit of detection (LOD) of the analyte was calculated based on the standard deviation (SD) of seven replicates of the spiked sample. The LOD was defined as 3 times the SD. Recoveries for TCS, BPA, and BP-3 were $104 \pm 5\%$ (mean \pm SD), $104 \pm 6\%$, and $101 \pm 5\%$, respectively. The LOD of TCS in the present study was 1.56 µg/L, which was similar to those reported at 2.27–2.3 µg/L in some studies [10,12], but substantially higher than those used in other studies at 0.9 ng/L–0.06 µg/L [53–55]. The LOD of BP-3 in the present study was 0.16 µg/L, which was similar to those reported at 0.07–0.34 µg/L in some studies [12,18,54]. The LOD of BPA in the present study was 0.16 µg/L, which was similar to those reported at 0.05–0.4 µg/L in some studies [12–14,54–57], but substantially lower than that used in a study at 0.5 ng/L [53].

2.5. Statistical Analysis

The data were analyzed using the SPSS 18.0 statistical package. To demonstrate the distribution and disparity of urinary TCS, BPA, and BP-3 levels, only positive samples > LOD were used to calculate the geometric means and ranges, respectively. Mann-Whitney U test was used to evaluate the differences of urinary TCS, BPA, and BP-3 levels between males and females, and between children and adolescents, respectively. Chi-square/Fisher's exact test was used to compare the differences of detection rates of urinary TCS, BPA, and BP-3 between males and females, and between children and adolescents, respectively. The *p*-value of <0.05 was considered statistically significant for these tests.

Table 1. Concurrent solid phase extraction (SPE) clean up and high-performance liquid chromatography–tandem mass (HPLC/MS/MS) analysis time line.

Period		1	2	3	4	5	6	
Time (min)		0	0.1–2	2–7	7.01–11	11–17	17–18.5	18.5–23.5
SPE of Sample N + 1		Start	Analyte Transfer and dilution	Regenerate SPE column	Equilibrate SPE column	Sample loading	SPE Column Wash	Stop Pump1
	Autosampler Valve	1–2	1–2	6–1	6–1	1–2	6–1	1–2
	Pump1	mL/min MeOH%	0 20	0.25 100	1.5 100	1.0 20	1.0 20	0 20
HPLC of Sample N			Analyte Transfer	HPLC separation and MS/MS acquisition			Equilibrate Pump2	
	Ten-port valve	10–1	1–2	10–1			10–1	
	Pump2	mL/min	0.5	0.5	0.75 HPLC gradient elution			0.5
		MeOH%	50	50	Time (min) MeOH%	2 50	8 100	17 100

Table 2. MS/MS transitions and parameter for analytes and internal standard.

Analytes	Precursorion (m/z)	Production (m/z)	Fragmentor (V)	Collision Energy (eV)
Triclosan	288	142	20	32
Bisphenol A	227	212	100	24
Benzophenone-3	227	211	120	20
¹³ C ₁₂ -Triclosan	299	148	35	36

3. Results

The overall detection rates of TCS, BP-3, and BPA were 18.7%, 8.1%, and 49.6%, respectively. Concentrations of TCS, BP-3, and BPA ranged from <1.56 to 198.66 µg/L, <0.16 to 97.02 µg/L, and <0.16 to 190.10 µg/L, respectively. The geometric mean (GM) concentrations of urinary TCS, BP-3, and BPA in the detected samples were 59.52 µg/L (n = 23), 19.72 µg/L (n = 10), and 5.81 µg/L (n = 61), respectively. The present study observed that the females had higher TCS concentrations than the males ($p = 0.051$). The detection rate of urinary BP-3 in females (12.3%) is higher than that in males (0%) ($p = 0.015$). In addition, the detection rate of urinary BP-3 in the college students (14.1%) is also higher than that in elementary school students (0%) ($p = 0.005$) (Table 3).

For elementary school children, the detection rates of TCS and BPA were 13.5% and 48.1%, respectively. No urinary BP-3 was found. Concentrations of TCS and BPA ranged from <1.56 to 149.64 µg/L, and <0.16 to 22.27 µg/L, respectively. The GM concentrations of urinary TCS and BPA in the detected samples were 61.09 µg/L and 5.61 µg/L, respectively. No differences of levels and detection rates of urinary TCS, BP-3, and BPA between males and females were found, respectively.

For college students, the detection rates of TCS, BP-3, and BPA were 22.5%, 14.1%, and 50.7%, respectively. Urinary BP-3 was only found in the females. Concentrations of TCS, BP-3, and BPA ranged from <1.56 to 198.66 µg/L, <0.16 to 97.02 µg/L, and <0.16 to 190.10 µg/L, respectively. The GM concentrations of urinary TCS, BP-3, and BPA in the detected samples were 58.84 µg/L, 19.72 µg/L, and 5.95 µg/L, respectively. Urinary TCS levels in the females were higher than those in the males for college students ($p = 0.047$).

Table 3. Detection rates (%) and concentrations (µg/L) of urinary TCS, BP-3, and BPA in primary school children and college students by sex and age subgroups.

Elementary School Children (n _{male} = 30, n _{female} = 22)		College Students (n _{male} = 12, n _{female} = 59)		Total (n _{male} = 42, n _{female} = 81)		
	Detection rate	GM (range)	Detection rate	GM (range)	Detection rate	GM (range)
Male						
TCS	13.3	55.49 (28.38–127.28) *	41.7	42.98 (36.98–54.18)	21.4	48.15 (28.38–127.28)
BP-3	0	-	0	-	0	-
BPA	50.0	5.84 (2.86–12.72)	41.7	6.24 (2.86–15.18)	47.6%	5.94 (2.86–15.18)
Female						
TCS	13.6	69.43 (47.30–149.64)	18.6	67.87 (30.96–198.66)	17.3	68.21 (30.96–198.66)
BP-3	0	-	16.9	19.72 (7.14–97.02)	12.3	19.72 (7.14–97.02)
BPA	45.5	5.29 (2.55–22.27)	52.5	5.91 (1.94–190.10)	50.6	5.75 (1.94–190.10)
Overall						
TCS	13.5	61.09 (28.38–149.64)	22.5	58.84 (30.96–198.66)	18.7	59.52 (28.38–198.66)
BP-3	0	-	14.1	19.72 (7.14–97.02)	8.1	19.72 (7.14–97.02)
BPA	48.1	5.61 (2.55–22.27)	50.7	5.95 (1.94–190.10)	49.6	5.81 (1.94–190.10)

* only positive samples > limit of detection (LOD) were used to calculate the geometric mean and range.

4. Discussion

In the present study, the detection rates for TCS (18.7%) and BP-3 (8.1%) were lower than those available in the literature (74.6–100% and 85.3–98.4%) [10,12,53–55]. The detection rate of BPA in the present study was 49.6%, which was similar to that reported at 50% in a study [57], but substantially lower than those used in other studies at 77.5–100% [12–14,54–56]. In this work, we studied human

exposure to TCS, BP-3, and BPA in Taiwan, a country where, to our knowledge, there had been consumer education program caution against TCS, BP-3, and BPA and restrictions on their use over the last decade by Consumers' foundation. Consumers' foundation reported that TCS, BP-3, and BPA were suspected of affecting human hormones. People who were concerned about TCS, BP-3, and BPA exposures from personal care products could have avoided using products that contained or released TCS, BP-3, and BPA. This was one of the reasons why the detection rate for TCS, BP-3, and BPA in the present study is almost lower than those available in the literature. Our previous study [23] also showed that TCS concentrations in surface water samples of Rivers in Taiwan ($<LOQ$ (limit of quantitation)–31.1 ng/L) were lower than the ranges reported for rivers in China (Zhujiang River 90.2–478 ng/L) [58] and US streams ($<LOQ$ –2.3 $\mu\text{g/L}$) [21]. In addition, the other reasons for lower detection rates included LOD, sample size, and different cultural and living habits.

For urinary TCS of the detected samples, the present study observed that the females in the college had significantly higher TCS concentrations than the males ($p = 0.047$). This observation was consistent with the findings of Li et al. [53] that the concentrations of urinary TCS in females were higher than those in males. The females might pay more attention to their appearances and have better hygiene habits. For example, daily applying cosmetics and brushing teeth twice a day were likely to increase chances to contact TCS-containing products.

The medians of TCS of 52 children of 11–13 years old ($<1.56 \mu\text{g/L}$) and 71 adolescents of 19–21 years old ($<1.56 \mu\text{g/L}$) in the present study were lower than those for 314 children of 6–11 years old (5.9 $\mu\text{g/L}$) and 715 adolescents of 12–19 years old (10.2 $\mu\text{g/L}$) in the study of Calafat et al. [10], respectively. Other studies showed the geometric mean (GM) of TCS for 90 girls aged 6–9 years old (10.9 $\mu\text{g/L}$) [12], 20 Belgian adolescents (1.71 $\mu\text{g/L}$) [59], 95 primary school students aged 7–12 years old (7.52 $\mu\text{g/L}$), 64 college students aged 18–24 years old (3.03 $\mu\text{g/L}$) [53], and 129 children and adolescents aged 6–21 years old (1.45 $\mu\text{g/L}$) [54], respectively.

For urinary BP-3 of the detected samples, the present study observed that the females had a significantly higher detection rate of urinary BP-3 than the males ($p = 0.015$). No urinary BP-3 was found in the males. This was consistent with the findings of Calafat et al. [18] that the concentrations of urinary BP-3 in females were higher than those in males. The females might have had higher frequencies to apply BP-3 lotion to avoid sun exposure. In addition, the present study also observed that no urinary BP-3 was detected in elementary school children. This showed that the frequencies of sunscreen application in adolescents were higher than those in children.

The medians of BP-3 of 52 children of 11–13 years old ($<0.156 \mu\text{g/L}$) and 71 adolescents of 19–21 years old ($<0.156 \mu\text{g/L}$) in the present study were lower than those for 314 children aged 6–11 years old (17.2 $\mu\text{g/L}$) and 715 adolescents aged 12–19 years old (20.0 $\mu\text{g/L}$) in the study of Calafat et al. [18], respectively. Other studies showed the GM concentration of BP-3 for 90 girls aged 6–9 years old (19.7 $\mu\text{g/L}$) [12] and 129 children and adolescents aged 6–21 years old (1.41 $\mu\text{g/L}$) [54], respectively.

The sex-related differences of urinary BPA from the findings of previous studies were not all consistent. He et al. [57] reported that urinary BPA concentrations in females were significantly lower than those in males in 952 Chinese populations. On the contrary, urinary BPA levels were found to be significantly higher in females than in males for 2517 U.S. populations [14]. In the present study, there was no significant difference in urinary BPA levels between females and males, which was consistent with the finding in a particular study [60]. Kim et al. [60] reported urinary BPA levels were similar in 15 men and 15 women that were aged about 43 years old.

The medians of BPA of 52 children of 11–13 years old ($<0.156 \mu\text{g/L}$) and 71 adolescents of 19–21 years old ($<0.156 \mu\text{g/L}$) in the present study were similar to those for 922 Chinese population ($<LOD \mu\text{g/L}$) in the study of He et al. [57], but lower than those for 314 children aged 6–11 years old (3.7 $\mu\text{g/L}$) and 715 adolescents aged 12–19 years old (4.2 $\mu\text{g/L}$) in the study of Calafat et al. [14], 394 U.S. population (adults) (1.28 $\mu\text{g/L}$) in the study of Calafat et al. [13], and 1870 Korean population aged 18–69 years old (2.07 $\mu\text{g/L}$) in the study of Kim et al. [55], respectively. Other studies showed the

GM concentration of BPA for 90 girls aged 6–9 years old (2.0 µg/L) [12], 20 Belgian adolescents (1.25 µg/L) [59], 95 primary school students aged 7–12 years old (4.10 µg/L), and 64 college students aged 18–24 years old (3.03 µg/L) [53], and 129 children and adolescents aged 6–21 years old (1.37 µg/L) [54], respectively.

5. Conclusions

The present study showed that urinary TCS and BP-3 concentrations in females were significantly higher than those in males, respectively. In addition, urinary BP-3 was only detected in the female samples of college students. Sex and age were the important factors influencing urinary TCS and BP-3 concentrations, respectively. Further studies are needed to assess the effects of these phenol chemicals on human health, especially for more highly exposed groups.

Acknowledgments: The authors thank the National Science Council, Taiwan (Contract No. 99-2314-B-214-001) for financially supporting this study.

Author Contributions: Fu-Kuei Chang conceived and designed the experiments; Fu-Kuei Chang and Jentaie Shiea performed the experiments; Fu-Kuei Chang and Hsin-Jen Tsai analyzed the data; Fu-Kuei Chang and Jentaie Shiea contributed reagents/materials/analysis tools; Fu-Kuei Chang wrote the paper.

Conflicts of Interest: The authors declare no conflicts of interest.

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