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Urinary levels of triclosan and triclocarban in several Asian countries, Greece and the USA: Association with oxidative stress

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ABSTRACT

Triclosan (TCS) and Triclocarban (TCC) are widely used as antimicrobial preservatives in personal care products (PCPs). Because of their potential for endocrine disrupting effects, human exposure to these chemicals is a concern. Biomonitoring studies of human exposure to TCS and TCC have shown widespread exposure of populations in western European countries and the USA. However, exposure to TCC and TCS by populations in Asian countries is less well known. In this study, concentrations of TCS and TCC were determined in human urine collected from seven Asian countries (China, India, Korea, Kuwait, Japan, Saudi Arabia, and Vietnam), and Greece and the USA. A total of 430 urine samples were analyzed for TCS and TCC, of which 355 (83%) and 82 (19%), respectively, contained measurable levels of these chemicals. The overall geometric mean [GM] concentrations of TCS and TCC, were 1.36 and 0.03 ng/mL, respectively. The highest mean concentration of TCS was found in urine from China (100 ng/mL) and the lowest concentration was found in urine from Vietnam (2.34 ng/mL). We also analyzed urinary 8-OHdG, a marker of oxidative stress, to elucidate the association with TCS and TCC levels for samples from Saudi Arabia (n = 130) and a positive correlation between Ln-transformed TCC levels and 8-OHdG was found, although this was not statistically significant. This is the first study to report urinary levels of TCS and TCC in several Asian countries, especially for Vietnam, Kuwait, and Japan.

1. Introduction

Endocrine-disrupting chemicals (EDCs) are man-made or natural compounds that can interfere with hormone synthesis and normal physiological functions of reproductive organs. Environmental EDCs comprise of a wide range of chemical classes, including organochlorine pesticides, organotins (e.g., tributyltin), brominated flame retardants (e.g., polybrominated diphenyl ethers), perfluorinated compounds (e.g., perfluorooctanoic acid and perfluorooctane sulfonate), environmental phenols (e.g., alkylphenols, bisphenols, parabens, and triclosan), and phthalates (Casals-Casas and Desvergne, 2011). Concerns over human exposure to environmental EDCs have increased due to their high production and usage in a wide range of consumer products (Casals-Casas and Desvergne, 2011).

Triclosan (TCS), also known as 5-chloro-2-(2,4-dichlorophenoxy) phenol, is a broad-spectrum phenolic biocide with activity against bacteria and fungi. This chemical and its related congener triclocarban (TCC) are added in many personal care products (e.g. soaps, toothpastes, deodorants, detergents and disinfection solutions) (Yusa et al., 2012; Liao and Kannan, 2014). Although TCS is added to products to reduce microbial infections, no other benefits have yet been established to support its widespread use in other personal care and household cleaning products. TCS is present in more than 75% of human urine samples collected from the populations in the USA, Greece, China, and Belgium (Calafat et al., 2008; Pirard et al., 2012; Asimakopoulos et al., 2014; Engel et al., 2014). TCS is considered more toxic than many other disinfectants (Brausch and Rand, 2011), and possesses estrogenic activity (Foran et al., 2000; Ishibashi et al., 2004). In experimental animal models, TCS has been reported to be an endocrine disruptor (Christen et al., 2010) and to affect thyroid hormone levels (Stoker et al., 2010;

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Sankoda et al., 2011; Zorrilla et al., 2009), as well as estrogen (Kumar et al., 2009; Jung et al., 2012) and testosterone (Kumar et al., 2009) levels. There is evidence to suggest that TCS is genotoxic in certain types of organisms and/or cell types (Dann and Hontela, 2011). In 2010, TCS was removed from the list of provisional additives for use in plastic food-contact materials in European Union (EU) countries (Dann and Hontela, 2011). Human exposure to TCS has been linked to the development of asthma in children, cancer risk (Dinwiddie et al., 2014), and obesity (Lankester et al., 2013). Similar to that reported for TCS, exposure to TCC was associated with methemoglobinemia in humans, and this compound was reported to impair mammalian reproduction (Johnson et al., 1963; Nolen and Dierckman, 1979; Chen et al., 2013).

Human exposure to TCS and TCC has been widely studied in many European countries and North America. Despite the widespread use of these estrogenic chemicals in personal care products, little is known on exposure levels in Asian countries. In this study, 430 urine samples collected from seven Asian countries, encompassing China, India, Korea, Kuwait, Japan, Saudi Arabia, and Vietnam, were analyzed to determine the levels of TCS and TCC. The concentrations measured in Asian countries were compared with levels found in samples collected from Greece and the USA. Association between urinary TCS and TCC levels with 8-hydroxy-2′-deoxyguanosine (8-OHdG), a biomarker of oxidative stress, was also examined in this study.

2. Materials and methods

2.1. Standards and regents

Analytical standards of TCS (purity: ≥97%) and TCC (99%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). $^{13}C_{12}$-TCS (99%) and $^{13}C_6$-TCC (99%) were obtained from Cambridge Isotope Laboratories, Inc. (Andover, MA, USA). Analytical standard of 8-OHdG (≥98%) was purchased from Sigma-Aldrich (St. Louis, MO, USA) and the internal standard $^{15}N_5-8$-OHdG was purchased from Cambridge Isotope Laboratories (Andover, MA, USA). β–Glucuronidase from *Helix pomatia* (145700 units/mL β–glucuronidase and 887 units/mL sulfatase) was purchased from Sigma-Aldrich (St. Louis, MO, USA). The stock solutions of target analytes and internal standards were prepared at 1 mg/mL in methanol and stored at −20 °C. Methanol (HPLC grade) and ethyl acetate (ACS grade) used in the experiments were purchased from Mallinckrodt Baker (Phillipsburg, NJ, USA). Milli-Q water was purified by an ultrapure water system (Barnstead International, Dubuque, IA, USA).

2.2. Sample collection and analysis

Details regarding the collection of urine samples have been provided in our previous publications (Guo et al., 2011, 2013; Liao et al., 2012; Asimakopoulos et al., 2013, 2016). Briefly, samples from China (number of samples; females/males: n = 47; 27/20 and 1 unknown gender), India (n = 41; 21/17; 3 unknown), Japan (n = 36; 8/28), Korea (26; 7/6; 13 unknown), Kuwait (n = 40; 13/27), and Vietnam (n = 19; 9/10) were collected during 2010–2012 except for Korea, which were collected during 2006–2007. The samples from China, India, Japan, Korea, Kuwait, and Vietnam originated from the cities of Guangzhou/Shanghai/Qiqihar, Mettupalayam/Chennai, Matsuyama/Kumamoto/Tokyo, Seoul/Busan/Yeosu, Al-Asma/Al-Jahra governorates, and Hanoi, respectively. The average age of donors for these countries ranged from 30 to 51 years. The urine samples from Jeddah, Saudi Arabia, were collected at two occasions; one from 30 individuals in 2014 (diabetic patients), and the other from 130 individuals from the general population in 2015. Of the 130 samples collected in 2015, detailed analysis on demographic factors that affect TCS and TCC levels and their association with oxidative stress were performed. Age and gender information were available for 67 individuals (31 males and 36 females; 63 unknown) and the ages of donors ranged from 1 to 87 years with a median value of 35 years. Urine samples from Greece (n = 30; 15/15) were from Athens collected in 2012 (Asimakopoulos et al., 2013), and samples from the USA were from Albany, New York (n = 31; 10/21) collected in 2011. Spot urine samples were collected in polypropylene tubes from healthy volunteers. Creatinine correction was only applied for the second set of samples from Saudi Arabia (n = 130). All samples were stored at −20 °C until analysis. The study was approved by the Institutional Review Board of New York State Department of Health.

Urine samples were analyzed for TCS and TCC by enzymatic deconjugation and liquid-liquid extraction (LLE), following the analytical method described elsewhere (Smarr et al., 2017) with slight modifications. Briefly, 0.5 mL of urine sample was transferred into a 15-mL polypropylene (PP) tube, and 2.5 ng each of $^{13}C_{12}$-TCS and $^{13}C_6$-TCC were spiked into samples. After vortex mixing, urine sample was buffered with 300 μL of 1 M ammonium acetate that contained 100 unit/mL of *β*-glucuronidase. The samples were incubated at 37 °C overnight for deconjugation. After incubation, 3 mL of ethyl acetate was added to each sample, and the sample was shaken for 60 min. The sample was separated by centrifugation and the upper layer was pipet transferred to a new 15-mL PP tube. The extraction was repeated three times. Then 1 mL of Milli-Q water was added, and the sample was shaken for 30 min, centrifuged, and concentrated under a gentle stream of nitrogen to near dryness and dissolved in 0.5 mL of methanol for UPLC-MS/MS analysis.

2.3. Instrumental analysis

The UPLC-MS/MS parameters were similar to those reported in our previous study (Smarr et al., 2017) with slight modifications. In brief, the chromatographic separation was carried out using a Waters Acquity I-class UPLC (Waters, Milford, MA, USA). Identification and quantification of target analytes were performed with an API-5500 electrospray triple quadrupole mass spectrometer (ESI − (-)-MS/MS; AB SCIEX, Framingham, MA, USA). A Pinnacle DB AQ C18 (2.1 × 50 mm, 1.9 μm; Restek Corporation, Bellefonte, PA, USA) column serially connected to a SecurityGuard ULTRA (UHPLC C18, 2.1 mm ID; Phenomenex Inc., Torrance, CA, USA) was used. The target compounds were analyzed by multiple reaction monitoring (MRM). Other details of the instrumental parameters are described in our previous publications cited as above.

2.4. Urinary 8-hydroxy-2′-deoxyguanosine and creatinine analysis

8-OHdG and creatinine levels were measured by following the method described elsewhere (Zhang et al., 2016). Briefly, for 8-OHdG analysis, 0.1 mL of urine was diluted 5-fold with Milli-Q water, and 20 ng of $^{15}N_5-8$-OHdG was added before instrumental analysis by UPLC-MS/MS. For creatinine analysis, 5 μL of urine was diluted 32-fold and 800 ng of creatinine-d_3 was added before instrumental analysis by UPLC-MS/MS.

2.5. Quality assurance/Quality control (QA/QC) and data analysis

For each batch of 20 samples analyzed, one procedural blank was analyzed simultaneously. Trace levels of TCS and TCC were found in procedural blanks (below the limit of quantification) and these levels were subtracted from the measured concentrations in urine samples. The respective limits of detection (LOD) of TCS and TCC were 0.04 and 0.01 ng/mL. A calibration check standard was injected after every 20 samples as a check for drift in instrumental sensitivity. Matrix spike sample and duplicate samples were analyzed for the calculation of recoveries and repeatability. Average recoveries of TCS and TCC standards spiked into randomly selected sample were 100% and 102%, respectively. Duplicate analysis of randomly selected samples yielded a relative standard deviation of < 7%.

Statistical analyses were performed with statistics software package