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Triclosan in over the counter medicines of South China

Chong-Jing Gao · Lu-Lu Jia · Ying Guo

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Abstract Triclosan (TCS) is an endocrine disruptor which may affect endocrine function, antibiotic resistance, and thyroid hormone homeostasis. As a broad-spectrum antimicrobial agent used in medical and personal care products, TCS was frequently detected in human urine, indicating widespread human exposure to this chemical. Over-the-counter medicines (OTCs) may be a potential source of human exposure to TCS. In this study, 84 OTCs were collected from Guangzhou, South China, including medicines intended for both children and adults. We determined the concentration of TCS in OTCs and the estimated daily intakes (EDIs) of TCS by evaluating OTCs for different age groups of the Chinese population. Our results indicated over half of the evaluated medicines contained TCS and the highest concentration reached 7.825 ng/g, with a median value of 0.017 ng/g. TCS was frequently found in adult medicines (detected in 85% of samples), and the concentrations were significantly higher than those in children's medicines. TCS in OTCs may come from packaging materials, cultivated soils, or production process (Chinese patent medicines). The EDIs of TCS (estimated with 95th concentration in OTC medicines) were 0.305, 0.191, 0.287, 0.331, and 0.135 and 0.110 ng/kg-bw/day for infants, toddlers, children, teenagers, and adult females and males, respectively. Compared to other potential sources, human exposure to TCS from OTCs was limited in China—much less than TCS exposure through personal care products or indoor dust.

Keywords Triclosan · Over the counter medicines · Human exposure · South China · Children medicines

Introduction

Triclosan (TCS), or 5-Chloro-2-(2, 4-dichlorophenoxy) phenol, has been widely used as an antimicrobial agent to preserve consumer goods against bacteria, mycete, and yeast. As such, TCS has been added as a preservative in a wide range of cosmetics, personal care products (PCPs), and household cleaning products. This accounted for over 80% of total TCS usage (National Industrial Chemicals Notification and Assessment Scheme 2001), while a smaller amount of TCS (~2%) has been used in the manufacture of some plastics and textile products (Olaniyan et al. 2016). TCS has been found in multiple environmental matrices, including sludge (Armstrong et al. 2018), sediment (Pusceddu et al. 2018), and even drinking water (Li et al. 2010). Detectable levels of TCS have also been reported in human urine (Li et al. 2013), blood (Shapiro et al. 2018), and breast milk (Azzouz et al. 2016), which

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C.-J. Gao · L.-L. Jia · Y. Guo (✉)
School of Environment, and Guangdong Key Laboratory of Environmental Pollution and Health, Jinan University, Guangzhou 510632, China
e-mail: yingguo2004@jnu.edu.cn

confirmed the widespread usage and human exposure to TCS.

Although TCS has low acute toxicity (Bhargava and Leonard 1996), a growing concern is the potential of adverse effects on the human body due to long-term exposure. Available data indicated that TCS will cause both skin and eye irritation in animal studies (Bhargava and Leonard 1996). In addition, exposure to TCS may disrupt hormone cycles and cause muscle weakness in animals, though the relevance of this to humans is uncertain (Dann and Hontela 2011). In 2016, The Food and Drug Administration of the United States (US FDA) banned the use of TCS in consumer products, such as over-the-counter antibacterial hand soaps and body washes. However, TCS is still used in many other products, including children's toys, pharmaceuticals, and detergents without any label required.

TCS has been reportedly discharged into the environment, and finally will enter human body through dermal absorption (Witorsch 2014), inhalation (Liao et al. 2012), and ingestion (Lu et al. 2017) pathways. Previous biomonitoring studies indicated that humans were widely exposed to TCS in many countries based on its prevalence in human urine (Goodman et al. 2018). Considering the implications of long-term exposure, it is imperative to explore all potential sources of human exposure. Several studies have demonstrated that the usage of cosmetics (Liu and Wu 2012), other PCPs (Liao and Kannan 2014), and indoor dust (Ao et al. 2017) also contributed directly to human exposure to TCS. As a broad-spectrum anti-fungicide, TCS is also used in many pharmaceuticals, which means over-the-counter medicines (OTCs) may be an important source of exposure to TCS.

However, no research has been executed regarding the occurrence of TCS in OTCs. To accurately estimate human exposure of TCS from OTCs, it is of vital importance to evaluate the level of contamination in this form of medicine. In the present study, a total of 84 OTCs, including children and adult medicines, were collected in Guangzhou, South China. Here we attempt to determine whether OTCs are an overlooked route of human exposure to TCS, and to compare the contribution of TCS from OTCs with other potential sources. To those ends, we determined concentrations and detection frequencies of TCS in the OTCs and estimated human daily intakes of TCS through the determined concentrations of TCS detected in OTCs.

Materials and methods

Triclosan (purity > 99%) was purchased from AccuStandard (New Haven, CT), and isotope labeled TCS ($^{13}C_6$-TCS, purity > 99%, as internal standard) was purchased from Cambridge Isotope Laboratories (Andover, MA). Ethyl acetate, methanol, and methyl tert-butyl ester (MTBE) of HPLC grade and hexane of pesticide grade were obtained from Thermo Fisher Scientific (Waltham, MA).

A total of 84 OTCs made in China, including 45 pediatric medicines, 36 adult medicines, and 3 general medicines (for all age), were collected from local drug stores distributed in Guangzhou, South China (detailed information of all medicines were listed in Table S1). Those medicines were composed of oral liquids, pills, granules, and capsules.

Sample preparation was adopted from a previous study with some modification (Guo and Kannan 2013). Briefly, sample was weighed into 12 mL glass tubes (~ 5 g production weight for oral liquid and ~ 1 g production weight for other samples), and 4 mL of HPLC grade water, 1 mL of ethyl acetate (except for oral liquid) and 25 ng of internal standard were added. After equilibration at room temperature in a dark room, until all medicines were almost melted, 3 mL of hexane and 2 mL of MTBE were added to the tube. The tube was shaken for 50 min and centrifuged for 20 min, and then the supernatant was transferred into a clean glass tube. The residue was extracted another two times. All organic extracts were combined, concentrated to near dryness with a gentle stream of nitrogen, and re-dissolved in 0.5 mL of methanol for instrumental analysis.

The quantification of TCS was conducted by an API 5500 electrospray triple quadrupole mass spectrometer (Applied Biosystems, CA) interfaced with a Shimazu LC-30 Series high-performance liquid chromatograph (Shimazu, Japan). Chromatographic separation was accomplished with a Betasil C18 column (100 mm × 2.1 i.d. and 5 μm particle size; Thermo Electron, Waltham, MA). The injection volume was 5 μL, and the column temperature was maintained at 35 °C. The mobile phase consisted of methanol (A) and HPLC grade water (B) at a flow rate of 250 μL/min for TCS. The MS/MS was operated in the electrospray negative ionization mode. Nitrogen was used as the curtain and collision gas (Table S2).

Low level of TCS was found in method blanks (< 1.16 ng/g) and was subtracted from the reported values. The recoveries of $^{13}C_6$-TCS in all samples ranged from 30.6 to 221%, with a mean recovery of 80.9%. The reporting limit of TCS was 0.1 ng/g calculated by 1.0 g medicine and the lowest concentration of calibration curves for quantification in the instrument. Statistical analysis was performed with SigmaPolt (Systat Software Inc., USA), and a value of $p < 0.05$ was considered significant difference by nonparametric test.

To estimate daily human exposure to TCS from OTCs, the following equation was applied:

$$EDI = \frac{C.M.F}{BW}$$

Where, *EDI* is the estimated daily intake (EDI) of TCS from medicines (ng/kg-bw/day), *C* is measured concentration of TCS in medicines (ng/g), *M* is amount of maximum daily consumption of medicines obtained from the introductions of every medicine (g), *F* is the absorption factor (100% was used), and *BW* is average human body weight (kg). For C, the mean value and 95th percentile value of TCS concentrations in medicines were used to represent the average and worst-case scenarios due to exposure, respectively. For *BW*, average values of 8.8, 12.6, 47.2, 53.7, and 66.2 kg were used for the body weight of infants (< 1 ys), toddlers (1–3 ys), children (4–11 ys), teenagers (12–18 ys), adult females (> 18 ys), and adult males (> 18 ys), respectively, which were obtained from the Investigation Report on Nutrition and Chronic Disease of the Chinese People (2015) from the National Health and Health Commission of People's Republic of China 2015

Results and discussion

Concentrations of TCS in OTC medicines TCS was found in 60% of the 84 total medicine samples, with concentrations ranging from below the reporting limit (ND) to 7.825 ng/g. The median, mean, and geometric mean (GM) concentrations of all samples were 0.017, 0.312, and 0.063 ng/g, respectively (Table 1 and Fig. 1).

Based on raw materials, our samples were categorized into Chinese patent medicines (CHPMs) and Western medicines. TCS was detected in 63% of CHPMs and 53% of Western medicines, with median concentrations of 0.014 and 0.025 ng/g, respectively. No significant difference in concentrations of TCS was found between CHPMs and Western medicines ($p > 0.05$). However, our results indicated that it was more common to find higher levels of TCS in Western medicines than those in CHPMs (Fig. 2), and the highest concentration of TCS was found in a Western medicine (7.825 ng/g). In addition, all samples were also categorized into adult medicines and children medicines. TCS was rarely found in children medicines (23%) but was highly detected in adult medicines (85%). Concentrations of TCS in adult medicines were significantly higher than those found in pediatric medicines ($p < 0.05$), with median values of 0.041 ng/g and ND, respectively. The highest concentration of TCS (7.825 ng/g) was found in an adult medicine. Furthermore, medicines were also categorized by form, including pills, capsules, oral liquids, and granules. TCS was found in 93% and 90% of pill

Table 1 Concentration (ng/g) and detection frequency (100%) of TCS in over-the-counter medicines from South China

	DF[a]	Maximum	Median	Mean	Geometric mean
Total (*n* = 84)	60%	7.825	0.017	0.312	0.063
Medicines for different patients[b]					
Children medicines (*n* = 38)	23%	1.359	ND	0.066	–
Adult medicines (*n* = 59)	85%	7.825	0.041	0.493	0.077
Medicines made by different materials					
CHPMs (*n* = 56)	63%	2.506	0.014	0.092	0.036
Western medicines (*n* = 28)	54%	7.825	0.025	0.706	–
Medicines with different shapes					
Pill (*n* = 15)	93%	6.384	0.063	0.743	0.117
Liquid (*n* = 15)	39%	0.059	ND	0.007	–
Granule (*n* = 44)	43%	1.359	ND	0.078	–
Capsule (*n* = 10)	90%	7.825	0.161	1.155	0.263
Medicines with different packaging materials					
Plastic (*n* = 34)	70%	7.825	0.025	0.341	–
Aluminum plastic (*n* = 40)	45%	6.384	ND	0.336	–
Glass (*n* = 10)	60%	0.135	0.002	0.019	–

[a] *DF* detection frequency, *CHPMs* Chinese patent medicines

[b] There were 3 general medicines which could be taken by both adult and children

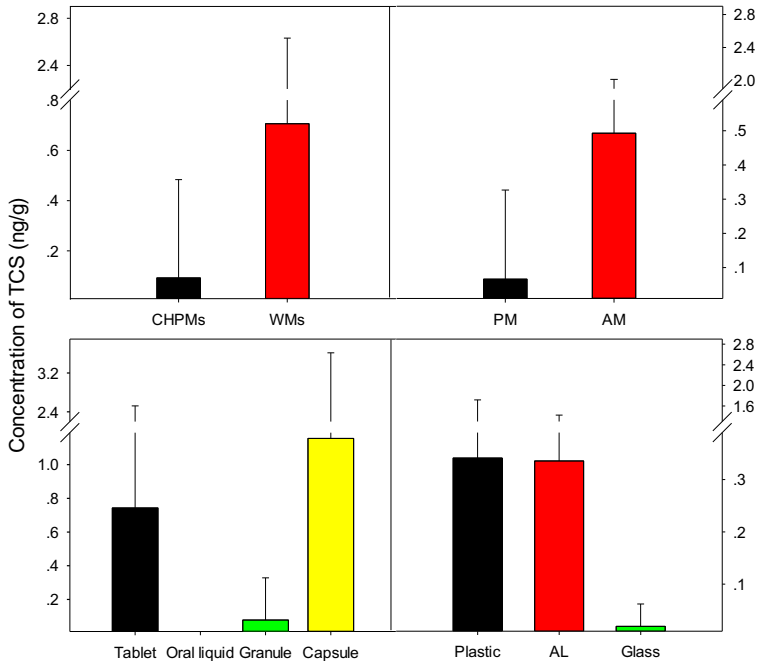

Fig. 1 Concentration distribution of TCS in different medicines from South China. (Data were presented as mean ± standard deviation. PM pediatric medicines, AM adult medicines, CHPMs Chinese patent medicines, WMs western medicines)

and capsule medicines with concentration from ND to 6.384 ng/g and ND to 7.825 ng/g, respectively. Concentrations of TCS were significantly higher in pills and capsule medicines than others ($p < 0.01$). Also, TCS was less frequently detected in oral liquid (39%) and granule medicines (43%).

Potential sources for TCS in OTC medicines There are several potential contamination sources of TCS in our medicine, and the leaching of TCS from packaging materials might be the first to consider. As indicated by the Department of Health and Aging of Australia, TCS has been used in the manufacture of plastic as an antimicrobial additive to protect the articles from deterioration. The plastic end products of this manufacturing include food storage containers, toothbrushes, and swimming pool lines (National Industrial Chemicals Notification and Assessment Scheme 2001). Several studies also reported a polymer coating containing TCS as the antimicrobial layer (Chung et al. 2003), and that TCS-based antibacterial paper had been used as packaging materials (Soares et al. 2012). In our study, we found medicines with plastic packaging materials contained significantly higher concentrations of TCS (median, 0.025 ng/g) than those with glass (0.002 ng/g) and aluminum-plastic (ND) packaging materials ($p < 0.05$). This evidence points to the theory that TCS in packaging materials may migrate into medicines.

Second, previous studies demonstrated that TCS was ubiquitous in water, even in bottled (3.5 ng/L) and drinking water (9.7 ng/L) (Durán-Álvarez et al. 2015). As it is known, bottled water and drinking water are used abundantly in some pharmaceutical processes involving CHPMs, such as drinking water in extraction processing for soaking raw materials. However, TCS may stay in extract solution since the relatively low temperature of pharmaceutical processing (< 100 °C) is lower than the boiling point of TCS (120 °C). Thus, we may speculate that the process of producing these medicines may concentrate TCS from contaminated water and make the differences of TCS in concentrations in different medicines.

Third, the raw materials used in CHPMs came from herbal plants, which may contain bioaccumulated TCS derived from cultivation soil (Prosser et al. 2014). Prosser et al. reported that crops cultivated in TCS contaminated biosolids could concentrate TCS into the roots and shoot tissues of three different plant species in the laboratory (Prosser et al. 2014).

Occurrence of TCS in environment in China Studies on TCS occurrence in the environment are limited in China.

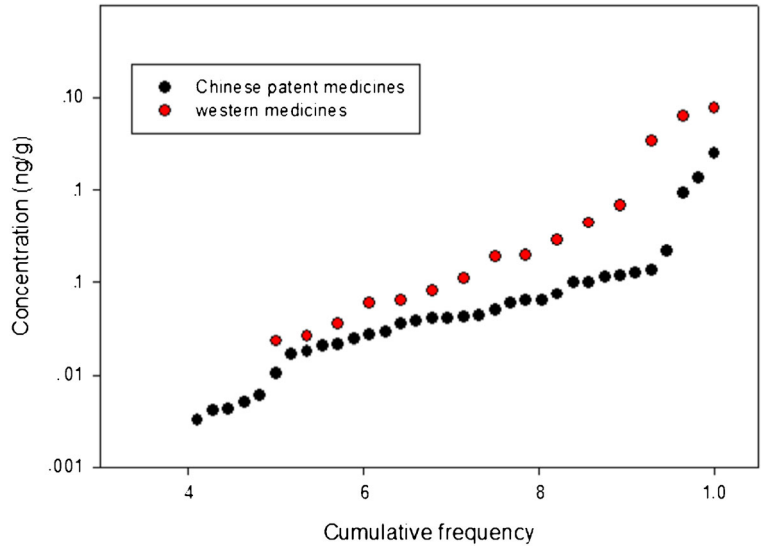

Fig. 2 Cumulative frequency distribution of TCS concentration in CHPMs and western medicines

TCS is added in many consumer products because of its antimicrobial activity, particularly in PCPs, cosmetics, and cleaning products. A previous study demonstrated that 37% of PCPs collected in China contained TCS, with a concentration range of ND to 53.9 ng/g. The levels of TCS in PCPs from China (median, 0.354 ng/g) were much lower than those from the USA (6.06 ng/g) (Liao and Kannan 2014). Due to the wide usage of PCPs and other consumer products, TCS is discharged into the environment continuously. For example, TCS was found in 96% of 110 indoor dust samples collected from Shanghai, China, and the concentration range was from ND to 1626 ng/g, with a median value of 260 ng/g (Ao et al. 2017). To add another comparison, TCS concentrations in indoor dust from China were much lower than those from Canada ($n = 63$; median, 378 ng/g; range 82–4090 ng/g) (Fan et al. 2010). In addition, TCS may remain in the aqueous phase and then be released into a receiving body of water, as it is hard to completely prevent contamination from influents of wastewater treatment plants (Montaseri and Forbes 2016). This is an important source of TCS in the aquatic environment (Zhang et al. 2013). TCS was found in 86% of bottled drinking water collected from Guangzhou, China, with a range of 0.6 to 9.7 ng/L. It was also found in all in tap water from six drinking water plants in Guangzhou in June, but was not found in those plants in December, similar to the data of the USA (Li et al. 2013). Furthermore, because it is stable to hydrolysis and rarely volatilized significantly, TCS will stay in water and finally may accumulate into sediment (Zhang et al. 2013). It was reported that the median concentration of TCS was 16.2 ng/L in water, and 58.8 ng/g in sediment in Zhujiang river, the Pearl River Delta, South China (Zhao et al. 2010). Another study in the same research area also reported TCS was detected in water (9.25 ng/g) and sediment (8.06 ng/g) collected from Dongjiang river (Zhao et al. 2010).

Human exposure to TCS from OTC medicines As TCS can be absorbed through mucous membranes of the oral cavity or gastrointestinal tract, the consumption of OTCs may be a significant pathway for human exposure to TCS. The daily exposure to TCS from OTCs was estimated by the maximum daily recommended dosage of OTCs. As shown in Table 2, all EDIs of TCS from OTCs for populations of different ages were 2~3 magnitudes lower than the biological safety dosage given by Canada Food and drugs regulations (0.03% (*w*/*w*)). Also, the EDIs of infants, kids, or teenagers were much lower than adults when estimated with median concentration of TCS in medicines. However, the 95th percentile values for infant, toddlers, children, and teenager (0.305, 0.191, 0.287, and 0.331 ng/kg-bw/day, respectively) were higher than the values for adult men and women (0.110 and 0.135 ng/kg-bw/day). In addition, the highest exposure dose among all age groups was found in infants (2.526 ng/kg-bw/day). Therefore, although lower detection frequency and median concentration of TCS was found in children medicines, due to lower body weight and higher intake frequencies compared with adult medicines, high daily intake of TCS

Table 2 Daily exposure of TCS from taking OTC medicines from South China (ng/kg-bw/day)

	Infants	Toddlers	Children	Teenagers	Adult females	Adult males
Mean	0.108	0.089	0.067	0.057	0.020	0.016
Median	–[a]	–	–	–	0.005	0.004
95th	0.305	0.191	0.287	0.331	0.135	0.110
Max	2.526	1.564	1.274	0.863	0.219	0.177

[a] Median concentration of TCS was ND

could still be observed in children through ingestion of pharmaceuticals.

As TCS was frequently detected in human samples, such as urine (Li et al. 2013), blood (Azzouz et al. 2016) and breast milk (Allmyr et al. 2006), many potential exposure routes were suggested in previous studies, such as PCPs (Witorsch 2014), indoor dust (Canosa et al. 2007), and bottled water (Olaniyan et al. 2016) (Li et al. 2010). The EDIs of TCS from PCPs (Liao and Kannan 2014), swimming pool water (Lu et al. 2017) and indoor dust (Ao et al. 2017) in China were 67.8, 8.4×10^{-9}, and 0.11 ng/kg-bw/day, respectively (Table 3). Obviously, exposure doses from OTCs were much lower than those from PCPs or indoor dust, but much higher than that from water of swimming pool for Chinese population. However, medicine is different from indoor dust and PCPs in that they are only taken when people need them, so humans are exposed to TCS through medicine in a short time frame suggested by prescriptions. Therefore, compared with other potential sources, human exposure of TCS from OTCs was limited, and much lower than that of PCPs or indoor dust.

With an average annual growth rate of 15% of sales, China has become the world's second largest pharmaceutical market (China Nonprescription Medicines Association 2018). In 2015, the total sales of pharmaceuticals in China reached 1168 billion RMB, and the sales of OTCs reached 174 billion RMB. With the growing demand for OTCs, people become more easily and frequently exposed to TCS through the consumption of pharmaceuticals. In the present study, due to the lack of degradation parameters and the limited sample size, we only considered the exposure risk of TCS during the limited consumption time of OTCs, while the exposure risk during a longer period is still unknown.

Table 3 Occurrence and daily exposure of TCS from potential sources in different countries

Exposure source	Country	Detection frequency and concentration of TCS (ng/g)				Daily exposure dose (ng/kg-bw/day unless indicated)
		DF (%)	Median	Mean	Max	
PCPs						
	China	36.8	0.354	–	53.9	67.8 (GM)/5020 (95th)
	USA	72.8	6.06	–[a]	29.5	15.9 (GM)/2570 (95th)
Indoor dust						
	Belgium	100	484	220	1828	4 and 35 ng/day for adults and toddlers, respectively
	Canada	100	–	378	4090	–
	China	96	363	360	1626	0.11 and 0.65 for adults and toddlers, respectively
	Spain	100	934	880	2444	–
Swimming pool						
	China	100	0.046[b]	0.05	0.096	3.8×10^{-9} (ingestion)/4.6×10^{-9}(dermal)/8.4×10^{-9} (all)

[a] – data not available

[b] The unit is ng/L

Conclusions

In this study, an endocrine disruptor, TCS, was determined in OTCs collected from South China. Our results indicated that TCS was found in over half of the 84 OTCs samples, with a median concentration of 0.041 ng/g. TCS existed in most adult medicines (85%). TCS in OTCs may come from their packaging materials, cultivation soils of herb materials, or pharmaceutical production process. Our results indicated that OTCs are a potential albeit limited source of human exposure to TCS. In addition, since TCS was widely found in OTCs, outdated OTCs may contribute to elevated concentrations of TCS in the environment without proper disposal.

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