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Parabens and triclosan in shellfish from Shenzhen coastal waters: Bioindication of pollution and human health risks☆

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ABSTRACT

This work aimed to determine the concentrations of parabens and triclosan (TCS) in shellfish from coastal waters of Shenzhen, South China. A method of isotope dilution with high performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) was used to determine TCS and five paraben analogues, including methyl paraben (MeP), ethyl paraben (EtP), propyl paraben (PrP), butyl paraben (BuP), and benzyl paraben (BeP), in 186 shellfish samples covering eight species. Concentrations of parabens and TCS were 0.13–25.5 ng/g wet weight (ww) and <LOQ–6.51 ng/g ww, respectively, indicating their ubiquitous contamination in Shenzhen coastal waters. MeP was the most predominant paraben, followed by EtP and PrP. These three analogues accounted for more than 95% of the total concentrations of parabens. The "high" estimated daily intakes of parabens and TCS with the 95th percentage concentrations were estimated to be 2.15–26.1 and 0.41–10.3 ng/kg bw/day, respectively, much lower than the acceptable dietary intakes of parabens (1×10^7 ng/kg bw/day) and TCS (200 ng/kg bw/day), indicating no significant human health risks from shellfish consumption in the studied region. To our knowledge, this is the first report on the occurrences of parabens and TCS in shellfish products from Shenzhen coastal waters.

1. Introduction

Parabens are a group of esters formed by *p*-hydroxy benzoic acid. They have been widely used in cosmetics, medicine, and food because of their wide antimicrobial spectrum, stability, and cheap costs (Soni et al., 2005). Depending on the length of the ester chain, parabens are divided into short chain parabens, including methyl paraben (MeP) and ethyl paraben (EtP), and long chain parabens, including propyl paraben (PrP), butyl paraben (BuP), and benzyl paraben (BeP) (Haman et al., 2015). The antimicrobial activities of parabens increase with the increasing chain length, which however decreases its solubility in water (Núñez et al., 2008; Soni et al., 2005). As the preservative ability of a paraben is determined by the amount of the chemical dissolved in water, parabens with the lower esters, such as MeP and PrP, are subjected to broadest applications in commercial products (Soni et al., 2005).

Parabens had been considered ideal preservatives with environmental safety. However, Darbre et al. (2004) revealed that parabens may be associated with breast cancer, raising broad attentions on their toxicity. Studies have shown that exposure to parabens may change or disrupt endocrine systems and cause harmful consequences on human health (Tavares et al., 2009). Both *in vitro* and *in vivo* studies have shown that parabens and their metabolites possess estrogenic activities, which may lead to male infertility and other diseases (Darbre et al., 2004; Liao et al., 2013a; Tavares et al., 2009). Oral exposure to PrP and BuP have been reported to cause adverse effects on testosterone levels and sperm production in young male rats (Oishi, 2001, 2002).

Triclosan (TCS), as another broad-spectrum antimicrobial agent, has been widely used in toothpaste, textiles, cosmetics, and other household products, with annual global consumption of over 600

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tons (Chen et al., 2018; Lu et al., 2018; Ramaswamy et al., 2011; Zhao et al., 2010). It may be continuously released into aquatic environments via sewage discharges, because normal wastewater treatment plants could not completely remove TCS (Lindström et al., 2002; Yost et al., 2017). Because of its relatively high octanol-water partition coefficient ($Log K_{OW} = 4.76$), its unionized form can readily pass through phospholipid membranes and bioaccumulate in organisms (Fair et al., 2009; Orvos et al., 2002). A number of reports have demonstrated its toxicity to aquatic organisms, particularly in fish, crustaceans, and microalgae (Adolfsson-Erici et al., 2002; Orvos et al., 2002; Wilson et al., 2003). TCS is also associated with cancer development. It can enhance hepatocyte proliferation and production of reactive oxygen species (ROS), thus acting as liver tumor promoter in mouse liver (Yueh et al., 2015). TCS exposure can reduce the production of estrogens and androgens in mice and affect the normal function of placenta and implantation of embryo (Feng et al., 2016; Kumar et al., 2009; Lankester et al., 2013). Moreover, environmental transformation may turn it into chlorinated dibenzo-p-dioxins or methyl-triclosan (MTCS) which is either more toxic or more stable than TCS (Weatherly and Gosse, 2017).

There are also many reports on the toxicity of parabens and TCS to aquatic organisms. EtP, PrP, and BuP were found can cause estrogenic responses after intraperitoneal injections in rainbow trout, inducing vitellogenin (VTG) concentration in the male fish (Pedersen et al., 2010). PrP also was found to induce increases in plasma VTG concentration and up-regulate the expression of VTG and choriogenin (CHG) relevant genes in male medaka (Inui et al., 2003). Ciniglia et al. (2005) found C. *ehrenbergii* cells survival rate greatly reduced with the TCS concentration of 0.5–1.0 mg/L after 48 h exposure. Foran et al. (2000) studied the reproduction of fish and found that low concentration of TCS had impact on the body shape changes of offspring.

Parabens and TCS have received considerable attentions because of their toxicity and extensive exposure. As a result, Denmark has banned the use of PrP and BuP, their isoforms and salts in children's cosmetic products in March 2011 (Lilienblum, 2013). TCS was banned in soap products (liquid, gel, foam, and bar) by the Food and Drug Administration (FDA) beginning in September 2016 (Kux, 2016) and in all human hygiene biocidal products by the European Union (EU) starting from January 2017 (Juncker, 2016). However, no relevant regulations have been established for parabens and TCS in China. The available Chinese reports on these chemicals mainly focused on food, cosmetics, and urine (Guo et al., 2017; Liao et al., 2013a; Liao and Kannan, 2014). Seafood contamination and associated human exposure risks via consumption have not been well studied in China.

Shenzhen is located at the frontier of the Pearl River Delta, one of the most developed areas in China. It has a coastline of 282 km and hosts multiple aquaculture farms, which produce more than 16000 tons of shellfish a year. Human activities may produce potential contamination to local aquaculture, which may consequently affect human health via seafood consumption. Therefore, the present study aimed to: (1) investigate offshore parabens and TCS pollution by measuring their concentrations and distribution characteristics in shellfish collected from Shenzhen aquaculture farms; and (2) estimate human health risks via shellfish consumption.

2. Materials and methods

2.1. Chemicals and reagents

Reference standards of MeP, EtP, PrP, BeP, BuP, and TCS were obtained from Dr. Ehrenstorfer (Augsburg, Germany). The deuterated internal standards of d_4-MeP, d_4-EtP, d_4-PrP, d_4-BeP, d_4-BuP, and d_3-TCS were purchased from CDN Isotopes (Quebec, Canada). Methanol, n-hexane, and acetonitrile were bought from Merck (Darmstadt, Germany). Water used in the present study was prepared by a Millipore water purification system (Millipore Co., Ltd., Billerica, MA, USA). Sodium sulfate (Na_2SO_4) and solid phase extraction (SPE) cartridges (Poly-Sery HLB, 500 mg/6 mL) were purchased from Fisher Scientific (Houston, TX, USA) and CNW Technologies GmbH (Dsseldorf, Germany), respectively.

2.2. Sample collection and preparation

A total of 186 shellfish samples from eight species (Buccinidae n = 11, Veneridae n = 25, Haliotidae n = 26, Mytilidae n = 13, Pectinidae n = 32, Ostreidae n = 47, Corbiculidae n = 14, and Pteriidae n = 18) were collected from Yang Meikeng waters (YM), Dongshan Pearl Island waters (DP), Shenzhen Dapeng New Area Nan'ao Street skew Bay Waters (SD), and Shenzhen Longkeyuan Aquaculture Farm (SL) during the period of August 2014 to December 2016 (Fig. S1). There were approximately 40 km as the crow flies from Hong Kong across a bay. The species and number of samples from the four sites are shown in Table S1. Shellfish samples were rinsed and transported to analytical lab. The edible part of shellfish was lyophilized and the water content was measured. The ground powder of each sample was stored at −20 °C.

2.3. Sample treatment protocols

A 1.0 g dried shellfish sample and 1 g Na_2SO_4 were mixed, spiked with internal standards (d_4-MeP, d_4-EtP, d_4-PrP, d_4-BeP, d_4-BuP, and d_3-TCS), and then extracted with 8 mL acetonitrile with ultra sonication at room temperature for 15 min. The mixture was centrifuged at 6000 rpm for 5 min, and the supernatant was transferred to a 15 mL centrifuge tube. The extraction was repeated two more times (4 mL acetonitrile each), and the supernatants were combined and concentrated to approximately 2 mL under a gentle nitrogen stream. The extract was added with 2 mL of n-hexane and vigorously shaken for 1 min (Ye et al., 2013). After the n-hexane layer was removed, the resulting extract was purified by a HLB cartridge (pre-activation with 15 mL methanol and 10 mL water) with 10 mL of methanol elution. The eluent was evaporated to near dryness and then reconstituted with 0.5 mL methanol and filtered through a 0.22 μm filter.

2.4. Instrumental analysis

Determination of parabens and TCS in shellfish was performed on a 20A high performance liquid chromatography (HPLC; Shimadzu, Japan) coupled with a Q-Trap 5500 mass spectrometer (MS/MS; Applied Biosystems, Foster City, CA, USA). Each target analyte was quantified by its own deuterated internal standard. The analytes were separated on an Atlantis C18 column (2.1 mm × 150 mm, 5 μm, Waters, Ireland). The mobile phase was a binary mixture of methanol and water. The gradient elution program was set as follows: 0–1.5 min, 5% methanol; 1.5–2.5 min, 5%–10% methanol; 2.5–4 min, 10%–50% methanol; 4–8 min, 50%–95% methanol; 8–12.5 min, 95% methanol; 12.5–16 min, 95%–5% methanol; and 16–18 min, 5% methanol. The flow rate was 0.3 mL/min and the column temperature at 40 °C. A 10 μL sample was injected. Electrospray ionization was operated in negative mode. Parabens and TCS were quantified in multiple reactions monitoring mode with a dwell time of 50 ms.

2.5. Quality assurance and quality control

All glassware was thoroughly cleaned and baked at 450 °C for 4 h prior to use. Working standards of parabens and TCS were prepared in methanol and stored at −20 °C. Nine-point standard calibration curves were used for the quantification of corresponding analytes over a concentration range of 0.05–50.0 μg/L, with the correlation coefficients (R^2) above 0.99 for all curves. Analyte recoveries at three spiking levels (0.1, 5.0, and 20.0 μg/L) ranged from 63.9% to 85%. To investigate the stability of instrumental responses, a calibration standard with a moderate concentration was analyzed after every 10 samples, and the relative standard deviations (RSDs) of responses were less than 10%. Method blanks (Na_2SO_4) were analyzed with every batch of 20 samples, where trace MeP was detected. Blank contamination was subtracted to report MeP concentrations in shellfish samples. The limits of detection (LOD) and limits of quantification (LOQ) ranged from 0.9 to 22.9 pg/g and from 3.2 to 76.5 pg/g for the target substances (MeP, EtP, PrP, BeP, BuP, and TCS), which calculated on the basis of the concentrations of standards with three and ten times of the signal-to-noise ratios, respectively.

2.6. Calculations and statistical analysis

To assess human exposure via shellfish consumption, the estimated daily intake (EDI) was calculated as follows:

$$\mathrm{EDI} = \frac{C_s \times CR}{BW} \quad (1)$$

where EDI (ng/kg bw/day) is the estimated daily intake of a chemical; C_s (ng/g) is the measured concentration of a chemical in shellfish; CR (g/day) is the shellfish consumption rate per day (117.5 and 94.6 g/day for urban and rural residents, respectively); BW (kg) is the body weight of an adult (63.9 and 54.0 kg for urban male and female, and 69.4 and 55.1 kg for rural male and female, respectively). These reference values were taken from Shenzhen Center for Disease Control and Prevention (SGPPH, 2008).

A hazard quotient (HQ) was used to assess the human health risks of exposure to contaminants through a single pathway:

$$\mathrm{HQ} = \frac{EDI}{ADI} \quad (2)$$

where EDI is the estimated daily intake of a chemical; ADI (ng/kg bw/day) is the acceptable daily intake which refers to a daily dose to human without producing known adverse effects on health. In the present study, an ADI value of 1×10^7 ng/kg bw/day was used for ∑parabens (FAO/WHO JECFA, 1974), and 200 ng/kg bw/day for TCS (Murray et al., 2010). A HQ value greater than unit indicates a health risk via oral ingestion, whereas no risk exists if HQ < 1.

Statistical analysis was carried out using SPSS 13.0. A half LOQ was assigned to a measurement below LOQ for statistical analysis. The relationships among the concentrations of individual parabens and TCS were tested using Pearson's correlations (two tailed). One-way analysis of variance or the Mann–Whitney *U* test was used to determine differences between groups. The statistical significance level was set at $p < 0.05$. The sum concentration of five targeted parabens was denoted as $\Sigma_5$parabens. Concentrations of parabens and TCS were reported on a wet weight (ww) basis unless otherwise specified.

3. Results and discussion

3.1. Concentrations of parabens and TCS

The detection frequencies and concentrations of parabens and TCS in the collected shellfish samples are summarized in Table 1. MeP, EtP, PrP, and TCS were detected in more than 98% of the samples, while more than 50% of the samples contained measurable BuP and BeP. The concentrations of $\Sigma_5$parabens ranged from 0.129×10^3 to 2.55×10^4 pg/g in all species, with the highest detected in Pteriidae and the lowest in Corbiculidae (Table 1). Our concentrations of MeP (1.29×10^2–2.48×10^4 pg/g) were consistent with the data of 0.4×10^3–1.0×10^3 pg/g in clam tissues collected from Antarctic coastal environment and in bivalve mollusk gathered from Florida (Emnet et al., 2015; Xue et al., 2017). However, the present concentration of PrP (<LOQ–406 pg/g) was an order of magnitude lower than that of from Antarctic coastal (0.4×10^3–1.9×10^3 pg/g), and Florida (<2.01×10^3 pg/g). The present median concentration of MeP (905 pg/g), EtP (40.9 pg/g), PrP (20.6 pg/g), BuP (<LOD pg/g), and BeP (<LOD pg/g) were comparable with reports on MeP (336 pg/g), EtP (9 pg/g), PrP (42 pg/g), BuP (5 pg/g), and BeP (5 pg/g) in fish and shellfish from the United States (Liao et al., 2013b). The present mean concentration of MeP (1.78×10^3 pg/g), EtP (62.1 pg/g), PrP (65.6 pg/g) in shellfish (Table S2) were lower than those in Portugal (MeP: 4.5×10^3 pg/g, EtP: 0.3×10^3 pg/g, and PrP: 0.9×10^3 pg/g) and Italy (MeP: 11.3×10^3 pg/g, EtP: 0.3×10^3 pg/g, and PrP: 2.8×10^3 pg/g) (ÁlvarezMuñoz et al., 2015). They also were comparable with MeP, EtP, PrP and BuP in 3 species of shellfish collected from the northern coast of Spain with mean concentrations of <LOQ–7.0×10^3, <LOQ–3.7×10^2, and <LOQ–5.6×10^2 pg/g,respectively (Villaverde-de-Sáa et al., 2016). The present study showed that MeP was the predominated compound among the five parabens with the concentrations varied between 1.29×10^2 and 2.48×10^4 pg/g, while EtP and PrP fell in the ranges of <LOQ and several hundred pg/g, and BeP and BuP were only a few dozen. The results indicated broad contamination in Shenzhen coastal areas and aquaculture shellfish.

Concentrations of TCS ranged from <LOQ to 6.51×10^3 pg/g in different species (Table 1), with the highest in Ostreidae and the lowest in Pteriidae. There are very limited TCS data in shellfish from the literature. The bivalves collected from South Australia (Kookana et al., 2013) and Spain (ÁlvarezMuñoz et al., 2015) were containing 2.4×10^3 pg/g (mean) and 1.5×10^3 pg/g (mean) TCS, respectively, which were an order of magnitude higher than that (542 pg/g, mean) in our study (Table S2). A study on bivalves collected from the Aegean Sea, Greece showed the TCS concentrations varied from <LOD to 2.58×10^6 pg/g (dw) (Gatidou et al., 2010). Concentrations of TCS in our samples were overall one to four orders of magnitude lower than those reported in other aquatic systems.

3.2. Composition profiles of parabens and source analysis

The composition profiles of parabens in shellfish are shown in Fig. 1. MeP was the most abundant compound in each species, accounting for 92.1% of the $\Sigma_5$parabens, followed by PrP (3.1%), EtP (3.4%), BeP (0.7%), and BuP (0.7%). Similar trends were observed in fish and shellfish produced from the United States, with MeP constituting over 65%, followed by PrP (~20%), EtP (~5%), BeP (~4%), and BuP (~2%) (Liao et al., 2013b). Similar composition profiles were also observed in human breast tumor tissues with a sequence of MeP (~60%) > PrP (~13%) > BuP (11%) > EtP (10%) (Soni et al., 2005). These similar patterns could not be explained by bioaccumulation potency, as MeP has the lowest lipophilicity ($\mathrm{Log}K_{OW} = 1.96$) among the five parabens. Instead, it may simply be related to greater usage of MeP than other parabens. Although the antimicrobial activity of parabens is positively associated with the alkyl chain length, MeP has been reported to be the most commonly used antimicrobial in cosmetic products (Soni et al., 2005), Thus, great usage consequently results in elevated levels in aquatic systems and organisms

Table 1
Concentrations (pg/g ww) of parabens and TCS in shellfish.

	MeP	EtP	PrP	BuP	BeP	$\Sigma_5$parabens	TCS
Buccinidae (n = 11)							
Median	4.76×10^2	<LOQ	6.92	<LOQ	<LOQ	4.83×10^2	53.4
95th Percentile	3.26×10^3	88.6	177	26.7	43.1	3.59×10^3	2.39×10^3
Range	1.29×10^2–5.54×10^3	<LOQ–143	4.29–268	<LOQ–53.3	<LOQ–61.1	134–6.06×10^3	<LOQ–4.44×10^3
DF (%)	100	100	100	63.6	100	100	100
Veneridae (n = 25)							
Median	1.95×10^3	68.3	79.5	<LOQ	1.64	2.10×10^3	1.95×10^2
95th Percentile	8.27×10^3	205	167	70.2	56.7	8.76×10^3	1.01×10^3
Range	2.56×10^2–9.64×10^3	0.69–239	9.61–205	<LOQ–102	<LOQ–75.3	2.67×10^2–1.03×10^3	<LOQ–1.35×10^3
DF (%)	100	100	100	72	88	100	96
Haliotidae (n = 26)							
Median	7.25×10^2	8.78	11.1	<LOQ	<LOQ	7.46×10^2	4.47×10^2
95th Percentile	3.65×10^3	106	123	1.60	78.9	3.96×10^3	1.32×10^3
Range	1.66×10^2–4.11×10^3	<LOQ–188	<LOQ–143	<LOQ–14.5	<LOQ–81.3	1.66×10^2–4.53×10^3	<LOQ–1.53×10^3
DF (%)	100	100	100	76.9	88.4	100	100
Mytilidae (n = 13)							
Median	1.01×10^3	75.1	49.0	<LOQ	<LOQ	1.13×10^3	1.46×10^2
95th Percentile	2.64×10^3	187	264	123	37.9	3.25×10^3	4.54×10^2
Range	4.47×10^2–2.70×10^3	<LOQ–204	5.27–343	<LOQ–220	<LOQ–42.0	4.52×10^2–3.51×10^3	<LOQ–5.41×10^2
DF (%)	100	100	100	69.2	84.6	100	100
Pectinidae (n = 32)							
Median	5.15×10^2	21.8	28.0	<LOQ	<LOQ	5.65×10^2	3.58×10^2
95th Percentile	2.00×10^3	105	66.2	68.1	36.2	2.27×10^3	1.06×10^3
Range	2.39×10^2–2.43×10^3	<LOQ–220	<LOQ–268	<LOQ–151	<LOQ–54.2	2.39×10^2–3.12×10^3	35.7–1.53×10^3
DF (%)	100	100	100	50	81.5	100	100
Ostreidae (n = 47)							
Median	1.06×10^3	73.4	73.7	<LOQ	<LOQ	1.21×10^3	4.21×10^2
95th Percentile	7.96×10^3	218	275	104	43.2	8.59×10^3	4.75×10^3
Range	2.70×10^2–1.66×10^4	<LOQ–293	<LOQ–406	<LOQ–359	<LOQ–96.7	2.70×10^2–1.77×104	<LOQ–6.51×10^3
DF (%)	100	97.8	100	76.5	78.7	100	97.8
Corbiculidae (n = 14)							
Median	6.80×10^2	12.6	6.10	<LOQ	<LOQ	6.99×10^2	1.88×10^2
95th Percentile	1.34×10^3	50.0	102	14.6	70.6	1.58×10^3	6.70×10^2
Range	2.95×10^2–1.35×10^3	<LOQ–72.0	<LOQ–148	<LOQ–32.7	<LOQ–74.3	2.95×10^2–1.68×10^3	<LOQ–1.04×10^3
DF (%)	100	100	100	71.4	57.1	100	100
Pteriidae (n = 18)							
Median	1.57×10^3	75.3	78.6	<LOQ	16.8	1.74×10^3	72.8
95th Percentile	1.14×10^4	205	190	77.2	83.6	1.20×10^4	2.58×10^2
Range	3.05×10^2–2.48×10^4	4.98–330	4.45–219	<LOQ–82.8	<LOQ–97.1	3.14×10^2–2.54×10^3	<LOQ–2.70×10^2
DF (%)	100	100	100	77.8	77.7	100	100
All samples (n = 186)							
Median	9.05×10^2	40.9	20.6	<LOQ	<LOQ	9.66×10^2	2.61×10^2
95th Percentile	6.47×10^3	259	200	75.6	67.7	7.07×10^3	1.48×10^3
Range	1.29×10^2–2.48×10^4	<LOQ–330	<LOQ–406	<LOQ–359	<LOQ–97.1	1.29×10^2–2.56×10^4	<LOQ–6.5×10^3
DF (%)	100	99.5	100	69.9	77.9	100	98.9

DF: detection frequency; LOQ: limits of quantification.

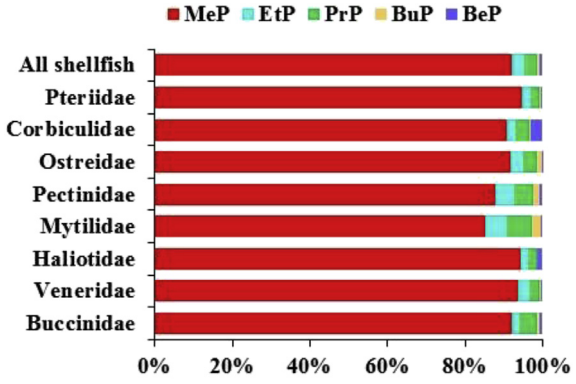

Fig. 1. Composition profiles of parabens in shellfish from Shenzhen coastal waters.

compared with other parabens (Ramaswamy et al., 2011).

To further analyze contamination characteristics and possible sources, the correlations among paraben compounds and TCS were determined and their correlation coefficients are shown in Table 2. The correlation coefficients among the six compounds ranged from

Table 2
The correlation coefficients between individual parabens and TCS in shellfish from Shenzhen coastal waters (n = 186).

	MeP	EtP	PrP	BuP	BeP	TCS
MeP	1					
EtP	0.561**	1				
PrP	0.242**	0.396**	1			
BuP	−0.016	0.192**	0.296**	1		
BeP	0.043	0.07	0.094	0.104	1	
TCS	0.295**	0.224**	0.006	0.001	0.012	1

**$p < 0.01$ (two tails).

0.001 to 0.561 using Pearson correlation analysis. There were significant correlations among MeP, EtP, and PrP as well as among EtP, PrP, and BuP, while no significant correlations were observed among MeP, BuP, and BeP (Table 2). Here, given that BeP did not correlate with any other four parabens, we believe that it may have different sources from other parabens (Table 2).

Similar to our results, other studies also reported significant correlations between selected parabens in different species or locations. For example, Liao et al. (2013a) measured parabens in 282 types of foodstuffs collected from nine cities in China, and found a significant correlation between MeP and PrP ($r = 0.49$, $p < 0.01$) and between EtP and PrP ($r = 0.81$, $p < 0.01$). The relationships between BuP and MeP or PrP were also significant ($p < 0.01$), although the correlation coefficients were smaller (i.e., $r = 0.25$ and 0.16, respectively). Strong positive correlation between MeP and PrP ($r = 0.316$, $p < 0.01$) was also reported in commercial pharmaceuticals from China, while no significant correlation was found between MeP and EtP or between PrP and EtP (Ma et al., 2016). Additionally, a positive correlation between MeP and PrP was reported in urine of Chinese teenagers (Ma et al., 2013). It was reported that MeP and PrP are usually used in combination in pharmaceuticals, foodstuffs, and personal care products (Ma et al., 2016). All the results suggested that MeP and PrP could have the same or similar sources.

In addition, we also found that TCS was significantly correlated with MeP ($r = 0.295$, $p < 0.01$) or EtP ($r = 0.224$, $p < 0.01$), but not with any of the other three parabens. TCS might have different sources from PrP, BeP, and BuP or they possess different bioaccumulation potencies.

3.3. Estimated daily intake of parabens and TCS and health risk assessment

In the present study, the mean and 95th percentile concentrations of parabens and TCS in shellfish were used to estimate a "typical" and "high" human daily intake. Additionally, we assumed the chemical absorption rates in human gastrointestinal tract as 100% because of a lack of bioavailability data (fraction of a chemical entering into circulation in human body accounted for the total ingested ones) or bioaccessibility data (fraction of the chemical released from its matrix into the gastrointestinal digestion solution) for parabens and TCS. The EDIs of parabens and TCS via shellfish consumption by adults of different genders in urban and rural areas of Shenzhen were calculated and shown in Table 3.

The "typical" and "high" EDIs of $\Sigma_5$parabens were estimated to be 3.55 and 13.0 ng/kg bw/day for urban adult males, and 4.20 and 15.4 ng/kg bw/day for urban adult females, respectively. For rural residents, the "typical" and "high" EDIs of $\Sigma_5$parabens were 2.63 and 9.64 ng/kg bw/day for rural adult males, and 3.31 and 12.14 ng/kg bw/day for females, respectively. Greater EDIs for urban than rural residents are also obtained for TCS (Table 3). The EDIs were slightly higher for females than males from both urban and rural regions, which is likely because of lower body weights for females. Additionally, urban resident exposure higher levels of parabens and TCS than rural ones. By shellfish species, the EDIs of parabens and TCS had the highest contribution from Pteriidae, followed by Veneridae, Ostreidae, Haliotidae, Buccinidae, Mytilidae, Pectinidae, and Corbiculidae (Table 3).

As there is a lack of reports on the exposure assessment of parabens and TCS in marine crustaceans, we compare our data with other aquatic food to evaluate the exposure scenarios in the present study (Table S3). The EDIs of $\Sigma_5$parabens and TCS for male and female populations in our study were higher than those reported from fish and seafood consumption (Liao et al., 2013a), but are approximately two orders of magnitude lower than those (2.0×10^3 and 100 ng/kg bw/day, respectively) reported in Philippines, because of greater contamination in fish from the latter study (Ramaswamy et al., 2011). Greater human exposure to TCS (165 ng/kg bw/day) was also reported in the River Gomti basin of India (Nag et al., 2018).

To further understand human exposure risks from shellfish consumption, we calculated the HQ values using the high EDI data. The HQs for male and female residents from both urban and rural regions ranged from 1.0×10^{-7} to 0.05 (Fig. 2), indicating there is no significant human health risk from shellfish consumption with respect to parabens and TCS in the Shenzhen coastal regions. However, cautions should still be taken given the possible co-existence of many other highly toxic contaminants, such as organochlorine pesticides and polycyclic aromatic hydrocarbons, in seafood (Wang et al., 2012; Yu et al., 2012a).

3.4. Uncertainties of human exposure and risk assessment

Uncertainties exist during the estimation of human daily intake

Table 3
Estimated daily intakes of parabens and TCS via shellfish consumption (ng/kg bw/day).

		Urban				Rural			
		Male		Female		Male		Female	
		$\Sigma_5$parabens	TCS	$\Sigma_5$parabens	TCS	$\Sigma_5$parabens	TCS	$\Sigma_5$parabens	TCS
Buccinidae (n = 11)	Typical	1.80	0.92	2.13	1.09	1.33	0.80	1.68	0.86
	High	6.60	4.40	7.81	5.20	4.89	3.81	6.16	4.10
Veneridae (n = 25)	Typical	6.03	0.71	7.13	0.84	4.47	0.61	5.63	0.66
	High	16.1	1.86	19.1	2.21	12.0	1.61	15.1	1.74
Haliotidae (n = 26)	Typical	2.47	1.05	2.92	1.24	1.83	0.91	2.30	0.98
	High	7.29	2.43	8.62	2.88	5.40	2.11	6.81	2.27
Mytilidae (n = 13)	Typical	3.12	0.32	3.69	0.38	2.31	0.28	2.91	0.30
	High	5.98	0.83	7.08	0.99	4.43	0.72	5.58	0.78
Pectinidae (n = 32)	Typical	1.63	0.83	1.93	0.98	1.21	0.72	1.52	0.78
	High	4.17	1.95	4.94	2.31	3.09	1.69	3.89	1.82
Ostreidae (n = 47)	Typical	5.00	2.14	5.92	2.53	3.71	1.85	4.67	2.00
	High	15.8	8.73	18.7	10.3	11.7	7.56	14.8	8.15
Corbiculidae (n = 14)	Typical	1.61	0.54	1.91	0.64	1.19	0.47	1.51	0.51
	High	2.90	1.23	3.43	1.46	2.15	1.07	2.71	1.15
Pteriidae (n = 18)	Typical	8.04	0.19	9.51	0.22	5.96	0.16	7.50	0.17
	High	22.1	0.47	26.1	0.56	16.4	0.41	20.6	0.44
All samples (n = 186)	Typical	3.55	1.00	4.20	1.18	2.63	0.86	3.31	0.93
	High	13.0	2.73	15.4	3.23	9.64	2.36	12.1	2.55

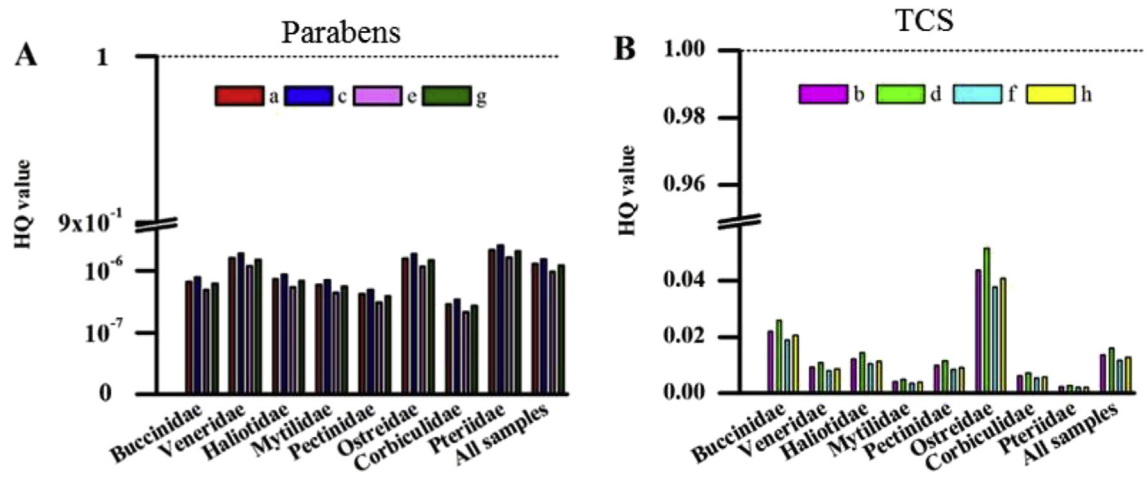

Fig. 2. HQ values of parabens and TCS for males and females in urban and rural regions (a and b: urban male; c and d: urban female; e and f: rural male, g and h: rural female).

and risk assessment. In the present study, because of a lack of the absorption rates for parabens and TCS from oral ingestion, an absorption factor of 100% was used. Many studies indicated that the particle-bound or matrix-bound chemicals could not be absorbed, and only the free forms can be transported through intestinal cells and as bioaccessible fractions (Cui et al., 2016; Li et al., 2016; Yu et al., 2012b). Therefore, the present results could overestimate human exposure because parabens and TCS in shellfish are most likely not completely absorbed in the gastrointestinal tract. In addition, the EDIs in the present study were determined using 95th percentile concentrations of parabens and TSC, which was a high marginal risk assessment and may overestimate the average exposure risks.

Concentrations of lipophilic contaminants such as parabens and TCS in shellfish could be reduced by cooking practices (USEPA, 2009). The extent of reduction depends on the methods of cooking. There are no cooking loss data for parabens and TCS, while USEPA (2000) summarizes the loss of hydrophobic organic contaminant from cooking can range from 0% to 80% with an average reduction of 30%. However, cooking loss was not considered in the present study, which may further overestimate human exposure risks.

Additionally, the risk following parabens and TCS exposure could also originate from their transformation products, such as *p*-hydroxy benzoic acid glycerine, glucuronic acid, and TCS-glucuronide, (Cowan-Ellsberry and Robison, 2009; Provencher et al., 2014; Wu et al., 2017). Inclusion of these degradation products can give a full picture of exposure, but has rarely been considered in previous studies. Much more studies are warranted.

4. Conclusion

Measurable levels of five parabens and TCS were determined in a variety of shellfish from Shenzhen coastal waters, which demonstrated the ubiquitous presence of these estrogenic chemicals in the studied region. The highest concentrations $\Sigma_5$parabens were detected in Pteriidae and the lowest in Corbiculidae. The significant correlations between MeP and PrP, or TCS indicate that they may share the same or similar exposure sources. Pteriidae contribute the highest EDIs of parabens and TCS in our collected shellfish species. Adult females from the studied region had slightly greater exposure to parabens and TCS than males from both urban and rural regions, while urban residents were subjected to greater exposure than rural residents. The HQ values of parabens and TCS were much lower than a unit, indicating no significant health risks via shellfish consumption. This is the first study on parabens and TCS in shellfish from the Pearl River Delta. Our findings constitute a baseline for further investigations of parabens and TCS contamination in the Pearl River Delta and associated human exposure risks.

Compliance with ethical standards

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2018.12.002.

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