Environmental Monitoring Strategies for Assessing Chemical Threats to Public Health

by

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ABSTRACT

Monitoring human exposure to chemicals posing public health threats is critically important for risk management and for informing regulatory actions. Chemical threats result from both environmental pollutants and elected substance use (e.g., consumption of drugs, alcohol and tobacco). Measuring chemical occurrence and concentrations in environmental matrices can help to pinpoint human exposure routes. For instance, indoor dust, a sink of indoor environmental contaminants, can serve to assess indoor air contamination and associated human exposures. Urban wastewater arriving at treatment plants contains urine and stool from the general population, the analysis of which can provide information on chemical threats in the community and ongoing harmful exposures. Analysis of sewage sludge can serve to reveal the identity and quantity of persistent organic pollutants in cities and inform estimates of toxic body burdens in local populations.

The objective of this dissertation was to investigate the occurrence and quantity of select, potentially harmful, anthropogenic chemicals in various environmental matrices and to explore the diagnostic value of analytical assays for informing public health decision-making. This dissertation (i) is the first to report spatio-temporal variations and estrogenic burdens of five parabens in sewage sludge from at the U.S. nationwide scale; (ii) represents the first China-wide survey to assess the occurrence and toxic emissions of parabens, triclosan, triclocarban, as well as triclocarban metabolites and transformation products contained in Chinese sewage sludge; (iii) documents the first use of a dispersive solid phase extraction method for indoor dust to measure dust-borne parabens, triclosan and triclocarban and estimating associated human exposures from dust ingestion; and (iv) is the first U.S. study to assess population-level alcohol and nicotine consumption in three

U.S. communities using wastewater-based epidemiology (WBE). Obtained data on baseline levels of selected emerging contaminants in sewage sludge and indoor dust can serve to inform the future monitoring needs, risk assessment, and policy making. This work showcases the utility of WBE and urban metabolism metrology via dust and sewage sludge analysis to assess human behavior (e.g., drinking and smoking) and exposure risks more rapidly, efficiently and anonymously than traditional approaches can.

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1 INTRODUCTION

Human Exposure to Chemicals and Monitoring Strategies

Humans are exposed throughout their lifetime to various chemicals, including naturally-occurring and anthropogenic toxic chemicals. Human exposure to harmful chemicals takes place via ingestion, inhalation, absorption and injection, with important exposure routes including consumption of food and drinks, inhalation of contaminated air, dermal application of personal care products (PPCPs) and injection of medications (Figure 1-1). Monitoring human exposure to potentially harmful chemicals is essential for identifying health threats, to inform public health decision-making and the regulatory process, as well as to assess the effectiveness of implemented interventions.

Conventional monitoring strategies include: (A) biomonitoring: direct measurement of chemical concentrations in human biological specimens, with the National Health and Nutrient Examination Survey (NHANES) performed by the Centers of Disease Control and Prevention (CDC) representing the most ambitious effort in the U.S. to date. The NHANES survey measures the occurrence and concentrations of select chemicals in bio-specimens of the general U.S. population with the goal of assessing the health and nutritional status of both adults and children. While biomonitoring can indicate actual levels of chemicals present in the human body at a certain time or over a period of time, it typically requires consent from individuals for collecting samples, the sampling methods are sometimes invasive (e.g., drawing blood), the process can be expensive and labor-intensive, and it does not necessarily inform on the identity of the exposure source; (B) conducting population surveys/questionnaires or to extrapolate data from sales statistics, such as through the Global Survey on Alcohol and Health conducted by the World Health

Organizations (WHO 2014a), and the State Tobacco Activities Tracking & Evaluation (STATE) System developed by the U.S. CDC (Center for Disease Control and Prevention 2015). However, surveys including interviews and questionnaires reach only a very small subsample of the target population and also require individual consent of participants, which can be time-consuming and prone to biases because of underrepresentation of subpopulations in the study cohort and misleading or dishonest responses from participants seeking to protect sensitive, personal information. Sales statistics cannot reflect stock piling, unrecorded legal home production and illicit production, importation and sales (Rehm et al. 2014); (C) indirect measurement of chemical concentrations in food, water and consumer products, and in environmental matrices (e.g., water, soil, and dust). Compared to biomonitoring, this latter approach does not require sampling of individuals directly and thus the sampling procedure is relatively easy but the data do not necessarily inform on actual uptake, metabolism and the effects of toxicants.

As discussed in the following, wastewater-based epidemiology (WBE) and urban metabolism metrology (UMM) recently have emerged as alternative means for assessing exposures and the health status of large numbers of individuals and can do so anonymously through the analysis of composited urine and stool contained in municipal wastewater as well as through the analysis of municipal sewage sludge, whether these materials have been further treated or untreated.

Selection of Environmental Matrices

In developed countries, people spend over 90% of their time indoors (Mitro et al. 2016), and chemicals used in consumer products can mobilize into indoor air and house

dust (Ginsberg and Balk 2016), leading to uptake and exposure. Humans get exposed to dust-borne chemicals through inhalation of suspended particles, dermal absorption and hand-to-mouth activity (Rager et al. 2016), especially for children who, compared to adults, spend more time on the floor and have a larger ratio of skin surface area to body weight. Therefore, indoor dust has frequently been used as a matrix for assessing indoor air quality and exposure levels to dust-borne chemicals. Measuring occurrence of chemicals in indoor dust thus can help in prioritizing chemicals and in better understanding potential human exposures and impacts. For example, the use of consumer products and indoor exposures are major contributors to human exposure to some phthalates (Koch et al. 2013, Wormuth et al. 2006). For some flame retardants, dust has also been suggested as a significant contributor to their exposure (Stapleton et al. 2012, Watkins et al. 2011).

Another matrix suitable for environmental monitoring is wastewater influent, as most of the chemicals used in PPCPs are disposed of into sewer systems that convey sewage to centralized wastewater treatment plants (WWTPs). Chemicals entering the human body via direct consumption, inhalation, dermal adsorption or ingestion, frequently are excreted through urine or feces in the form of either the parental compounds, their characteristic metabolites, or both. In urban centers of the developed world, human excreta are conveyed via sewage systems to local WWTPs. Therefore, wastewater influent is a process stream and chemical reservoir that can provide up-to-date information on chemical usage rates and temporal usage patterns of pharmaceuticals and consumer products of the whole population served by the WWTP. Raw wastewater further can serve as a complimentary data resource for epidemiology studies, risk assessment, and for assessing the effectiveness of regulatory, education and behavioral interventions. This concept of

using information contained in raw sewage often is referred to as “sewage epidemiology” or “wastewater-based epidemiology (WBE)” and was first proposed by Daughton in 2001 (Daughton 2001). This approach was expanded to consider matrices other than wastewater, such as municipal sewage sludge and dust, expanding the power of this diagnostic science focusing on human populations and leading to the larger umbrella term urban metabolism metrology (Venkatesan and Halden 2014b). Since then, this approach has been adopted for monitoring on the community level illicit drug use, smoking, and drinking behavior (Andres-Costa et al. 2016, Boogaerts et al. 2016, Gatidou et al. 2016, Gonzalez-Marino et al. 2016, Mackul'ak et al. 2015, Senta et al. 2015). Despite the uncertainties associated with this approach, it remains promising for providing objective, near real-time, cost-effective and anonymous information on chemical intake and incurred exposures, information that is useful in characterizing the health status of and latent health risks in human populations.

During wastewater treatment, removal efficiencies vary depending on chemical properties and the treatment processes and treatment durations implemented at individual WWTPs. While some of the chemicals can be easily removed, some are recalcitrant to treatment and will accumulate in sewage sludge or will be discharged contained in effluent, leading to downstream contamination of surface waters, sediments and aquatic organisms, and potential contamination of the human food supply (Venkatesan et al. 2012, Zhao et al. 2010). Sorption of contaminants to sewage sludge can lead to secondary environmental contamination as a result of sludge disposal on land, especially for sludges that are destined for application on agricultural land (biosolids). Studies have shown contamination of groundwater and surface water from land application of biosolids as well as uptake by agricultural crops of sludge-borne pollutants (Chen et al. 2014, Sherburne et al. 2016,

Verlicchi and Zambello 2015, Xia et al. 2010). Hence, it is important to monitor the environmental occurrence and temporal trends of contaminants. In addition, opportunities have been identified to utilize municipal sewage sludge as a diagnostic matrix and surrogate of human adipose tissue, to obtain information on human chemical consumption, the bioaccumulation potential of hydrophobic pollutants in aquatic and terrestrial ecosystems, and to estimate the average human body burden of toxic, persistent, and bioaccumulative, hydrophobic chemicals used and released by modern society (Qiu et al. 2015, Venkatesan and Halden 2014b).

Selection of Chemicals for Monitoring

As more and more anthropogenic chemicals are being introduced (about 7 new chemicals per day or 2000 per year in the U.S.) into consumer products, potential risks associated with these new chemicals need to be identified and managed. According to California Department of Toxic Substances Control, currently there are over 85,000 chemicals in commerce in the United States, and about 2,500 chemicals are of high production volume (HPV), meaning that they are produced or imported into the United States at a rate of more than one million pounds per year. Nearly 45 percent of the HPV chemicals lack adequate toxicological studies of their health effects on humans and other organisms; therefore, there is an increased interest in identifying contaminants of emerging concern (CECs). Diamond et al. estimated that more than 40,000 organic chemicals have been identified as CECs (Diamond et al. 2011), including certain personal care products and pharmaceutics (PPCPs), flame retardants, antibiotics, and plasticizers. Monitoring the environmental occurrence, fate and transport, and evaluating the toxicities of these

chemicals in humans and wildlife is of critical importance for risk assessment and for drafting future regulations.

Triclosan (5-chloro-2-(2,4-dichlorophenoxy) phenol; TCS), and triclocarban (3,4,4'-trichlorocarbanilide; TCC) are antimicrobial agents widely used in various personal care products such as hand soaps, kitchen detergent, toothpastes, bar soaps, and medical disinfectants with levels up to 2% and 0.3% (w/w), respectively (Dann and Hontela 2011, Halden and Paull 2005). Parabens are a group of compounds that have been extensively used as preservatives in lotions, face wash, facial creams, food, beverages, and industrial products, due to their broad spectrum of antimicrobial activity, good stability over a wide pH range, and moderate solubility (Bledzka et al. 2014). The most commonly used parabens in commercial products include methyl paraben (MePB), ethyl paraben (EtPB), propyl paraben (PrPB), butyl paraben (BuPB) and benzyl paraben (BePB). Parabens are found in more than 22,000 cosmetic products with levels up to 0.4% for individual paraben and 0.8% in combination. In pharmaceuticals, maximum paraben content rarely exceeds 1% (Haman et al. 2015). Both TCC and MePB are listed as HPV chemicals in the United States.

Recently, concerns have arisen over the potential risks posed by the above mentioned antimicrobial agents on human and animal health (Halden 2014, Soni et al. 2005). The aforementioned antimicrobial compounds are considered to represent a group of emerging endocrine disruptors that cause immune dysfunctions and affect human reproductive outcomes (Ahn et al. 2008, Boberg et al. 2010, Dann and Hontela 2011, Routledge et al. 1998, Smith et al. 2013, Tavares et al. 2009). Studies have shown toxic effects of antimicrobials toward aquatic organisms, such as algae, fish and invertebrates

(Brausch and Rand 2011, Chalew and Halden 2009, Madsen et al. 2001, Terasaki et al. 2015, Yamamoto et al. 2011). Potential links have been suggested between exposure to parabens and the etiology of breast cancer (Charles and Darbre 2013, Darbre et al. 2004, Darbre and Harvey 2014). There are also studies showing positive associations between the occurrence of antimicrobials and the detection frequency of antibiotic-resistance genes (Carey et al. 2016, Hartmann et al. 2016b). Widespread use of antimicrobial chemicals has led to ubiquitous human exposure and environmental occurrence in various diverse environmental matrices, including indoor dust, wastewater influent and effluent, surface water, and sewage sludge (Hartmann et al. 2016b, Li et al. 2015, Liao and Kannan 2013, Pycke et al. 2014b), as well as in biological matrices such as breast milk, serum, urine, cord blood and amniotic fluid (Calafat et al. 2010, Philippat et al. 2013, Pycke et al. 2015, Pycke et al. 2014a, Ye et al. 2008, Ye et al. 2016).

With inadequate evaluation on the safety of these CECs, continuous release of these chemicals into the environment will pose potential adverse effects to humans and the ecosystems humanity relies upon. Parabens, TCS and TCC have been listed in biomonitoring programs in both national (NHANES) and regional monitoring programs (Biomonitoring California) in the U.S. In addition, TCS and TCC have recently been banned by the U.S. Food and Drug Administration (FDA) for their use in over-the-counter antiseptic wash products including liquid and solid hand soaps (FDA 2016).

Apart from environmental contaminants, elected intake of alcohol and tobacco, both known to cause serious health problems, also were selected for monitoring in this dissertation.

Objective

The objective of this dissertation was to explore the diagnostic value of select environmental matrices in assessing chemical threats to public health, namely indoor dust, municipal wastewater, and municipal sewage sludge.

The specific aims were: (*i*) to investigate the spatial and temporal variations of parabens in U.S. sewage sludge and the associated estrogenic burdens; (*ii*) to measure the occurrence of a group of emerging contaminants in sewage sludge from China and to derive estimates of mass emissions and associated risks to aquatic organisms from sludge-borne contaminants; (*iii*) to measure levels of commonly used antimicrobials in indoor dust collected from different indoor environments, and to estimate human intake of these antimicrobials through dust ingestion; (*iv*) to assess the population-level consumption of both alcohol and nicotine in three small communities from different states in the U.S. using wastewater-based sewage epidemiology. Chemicals and environmental matrices addressed in this dissertation were shown on Figure 1-1, where numbers within boxes indicate corresponding chapter numbers.

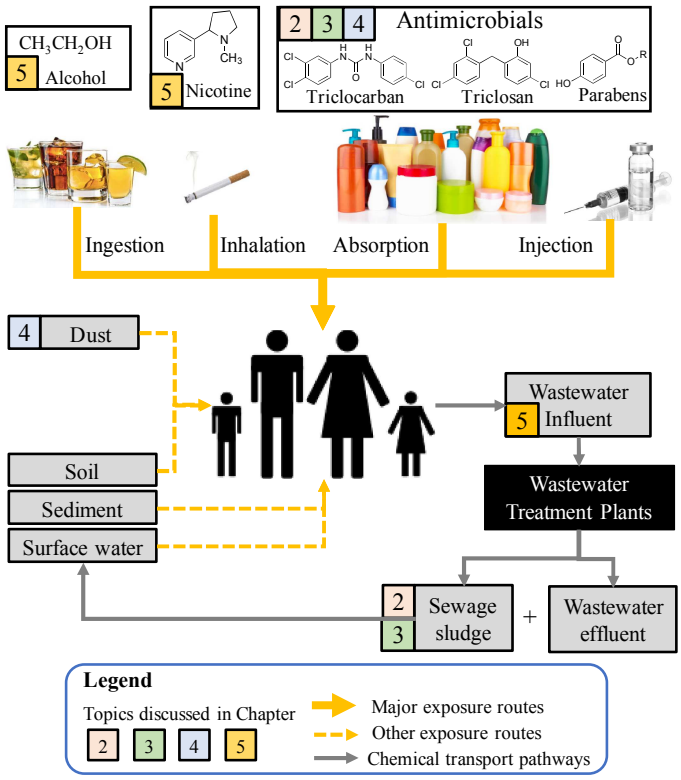

Figure 1-1. An overview of human exposure routes to chemicals and flow of chemicals in wastewater system. Grey arrow represents routes of exposure, and boxes with numbers indicate chapters addressing different chemicals in different environmental matrices.

2 OCCURRENCE, TEMPORAL VARIATION, AND ESTROGENIC BURDEN OF FIVE PARABENS IN SEWAGE SLUDGE COLLECTED ACROSS THE UNITED STATES

Abstract

Five parabens used as preservatives in pharmaceuticals and personal care products (PPCPs) were measured in sewage sludges collected at 14 U.S. wastewater treatment plants (WWTPs) located in nine states. Detected concentration ranges (ng/g, dry weight) and frequencies were as follows: methyl paraben (15.9 to 203.0; 100%), propyl paraben (0.5 to 7.7; 100%), ethyl paraben (<0.6 to 2.6; 63%), butyl paraben (<0.4 to 4.3; 42%) and benzyl paraben (<0.4 to 3.3; 26%). The estrogenicity inherent to the sum of parabens detected in sewage sludge (ranging from 10.1 to 500.1 pg/kg 17β- estradiol equivalents) was insignificant when compared to the 10^6-times higher value calculated for natural estrogens reported in the literature to occur in sewage sludge. Temporal monitoring at one WWTP provided insights into temporal and seasonal variations in paraben concentrations. This is the first report on the occurrence of five parabens in sewage sludges from across the U.S., and internationally, the first on temporal variations of paraben levels in sewage sludge. Study results will help to inform the risk assessment of sewage sludge destined for land application (biosolids).

Introduction

Parabens are a group of compounds that have been extensively used as preservatives in pharmaceutical and personal care products (PPCPs), food, beverages, and industrial products, due to their broad spectrum of antimicrobial activity, good stability over a wide pH range, and moderate solubility (Bledzka et al. 2014). Recently there has been an increase in the concern over the potential risks of parabens on human and animal health (Soni et al. 2005) . Parabens are considered to represent a group of emerging endocrine disruptors that cause immune dysfunctions and affect human reproductive outcomes (Boberg et al. 2010, Routledge et al. 1998, Smith et al. 2013, Tavares et al. 2009). Potential links have been suggested between parabens and breast cancer etiology (Charles and Darbre 2013, Darbre et al. 2004, Darbre and Harvey 2014). Furthermore, studies have shown parabens to be toxic to aquatic organisms, such as algae, fish and invertebrate (Brausch and Rand 2011, Madsen et al. 2001, Terasaki et al. 2015, Yamamoto et al. 2011). With the continuing debate and ongoing study on the safety of parabens, monitoring of parabens in the environment is important for effective assessment and management of ecological risks.

Widespread use of parabens has led to their ubiquitous presence in various environmental matrices, including wastewater, surface water, soil, sediments, sewage sludge and indoor dust (Fan et al. 2010a, Gonzalez-Marino et al. 2011, Hartmann et al. 2016c, Liao and Kannan 2013, Sun et al. 2016, Wang et al. 2012b) as well as biological matrices including human urine (Calafat et al. 2010), serum, cord blood (Pycke et al. 2015), breast tissues (Shanmugam et al. 2010), placenta (Jimenez-Diaz et al. 2011) and amniotic fluid (Philippat et al. 2013). The most commonly used parabens in commercial products

include methyl paraben (MePB), ethyl paraben (EtPB), propyl paraben (PrPB), butyl paraben (BuPB) and benzyl paraben (BePB). As parabens are mainly used in PPCPs, they are continuously released into domestic and industrial wastewater and conveyed through the sewer system to wastewater treatment plants (WWTPs) (Haman et al. 2015). Although over 90% of parabens can be effectively removed during conventional wastewater treatment in which biodegradation plays a significant role, ng/L levels of parabens are known to remain detectable in treated wastewater (Bledzka et al. 2014). One study reported the total concentration of six parabens including MePB, EtPB, PrPB, BuPB, BePB and HePB (heptyl paraben) to range from 1.08 to 7.93 ng/L in the final effluent from two WWTPs in Albany, NY, representing 1.57–8.03% of the mass loading (Wang and Kannan 2016). During conventional treatment, a fraction of the paraben load can be removed by sorption to sewage sludge. In an advanced WWTP, 91.8% of initial parabens mass loading was lost mainly due to degradation, while the contribution of sorption and output of primary and excess sludge was about 7.5% (Li et al. 2015). Since environmental monitoring studies have focused mainly on the aqueous phase (dissolved parabens), only a limited number of studies are available on the presence of parabens in sewage sludge. However, monitoring of parabens in sludge is important for both ecological and human health risk assessments (Venkatesan and Halden 2014a). Treated sewage sludge is being recycled in many countries via application on land, and current sludge disposal practices can lead to contamination of soil, groundwater and surface water in susceptible settings (Venkatesan and Halden 2013). Thus, the potential effect of such practices on soil and water from paraben contamination needs to be evaluated. There have been few studies worldwide on the occurrence of parabens in sewage sludge (Albero et al. 2012, Li et al.

2015, Viglino et al. 2011, Yu et al. 2011), and only one such study in the U.S. from two WWTPs in the Albany area of New York State (Wang and Kannan 2016). Thus, additional studies are necessary to understand the temporal and spatial variations of parabens in sewage sludge.

The goal of the present study was to address this knowledge gap by determining: 1) the variation of parabens in U.S. sewage sludge from 14 WWTPs located in nine U.S. states; 2) the temporal variations in paraben concentrations in sewage sludge over the course of a year at one WWTP; and 3) the estrogenic potency contributed by parabens in sewage sludge relative to levels of co-occurring estrogens.

Methods

Chemicals and Reagents

Methylparaben (MePB) was purchased from Aldrich (Sigma-Aldrich, St. Louis, MO), and $^{13}C_6$-MePB (99%) were obtained from Cambridge Isotope Laboratories (Andover, MA). Ethylparaben (EtPB), propylparaben (PrPB), butylparaben (BuPB), and benzylparaben (BePB) were purchased from RT Corp (Laramie, WY) (**SI, Table S 2-1**), and their deuterated standards (d_5-EtPB, d_4-PrPB, d_4-BuPB) were purchased from C/D/N Isotopes (Quebec, Canada). LC-MS-grade (99%) methanol, water, and acetic acid were obtained from Fluka and LC-MS-grade acetone was obtained from Sigma-Aldrich (St. Louis, MO). Individual stock solutions of the native and isotopically-labeled compounds were prepared in methanol. The working standards were prepared by serial dilution of stock solutions with methanol prior to use. All stock solutions were stored in glass vials with

polytetrafluoroethylene septa at -20 °C. All glassware was washed with detergent, rinsed with ultrapure water and heated at 550 ˚C for 4 hours prior to use.

Sewage Sludge Samples

Sludge samples were collected at 14 sludge-processing facilities located in nine states (Arizona, Indiana, Florida, Maryland, Montana, New York, Texas, Wisconsin, and Vermont), with an additional commercially available product (A3) from another plant purchased at a nationwide retail store. We relied on cooperation with WWTPs and U.S. Geological Survey employees to provide the samples studied, which were provided based on condition of nondisclosure of their identity and exact geographic location. Basic information about the WWTP operations is provided in Appendix, Supporting Information (SI, Table S2-2). Locations of WWTPs were named from A to N, and each sample was named using the same ID as the plant, except where multiple samples were taken, then numbers were assigned after plant ID.

The facilities sampled in this study treated a broad range of wastewater flows (<10 to >150 million liters of wastewater per day) and employed a variety of sludge treatment strategies. In the U.S., treated sewage sludge destined for application on land is categorized into Class A and Class B biosolids based on the pathogen reduction criteria described by USEPA (40 CFR Part 503). Class A biosolids contain no detectible levels of pathogens and can be sold or given away in a bag, or to be applied to land, lawn, and home gardens, whereas Class B biosolids are highly treated but may still contain low levels of pathogens (Walker et al. 1994). Samples in this study included untreated sludge, Class B biosolids with many of them being subjected to anaerobic digestion, and Class A biosolids prepared

using one or two additional treatments (dewatering, extended storage and composting) after digestion. One or two types of sludge from each site were sampled once between March and June of 2009, with additional samples collected (n = 18) in plant A between March 25, 2009 and April 7, 2010 for the temporal study. The samples were collected as discrete units, then frozen after sampling, thawed, subsampled, shipped to Arizona State University on dry ice in glass jars with polytetrafluoroethylene septa, stored at -80 °C, and homogenized prior to extraction.

Sample Preparation

Sludge samples were oven dried at 60 ˚C for 24 h, and then about 100 mg dried sewage sludge was weighed and transferred into a 15-mL polypropylene conical tube. Ten ng of $^{13}C_6$-MePB, d_5-EtPB, d_4-PrPB, and d_4-BuPB were spiked as internal standard. The sludge sample was extracted with a 5-mL solvent mixture of methanol and acetone (1:1, v/v) with 10 mM of acetic acid by placement in a sonication bath (Branson 5510) for 60 min. The resultant slurries then were centrifuged at 1500 g for 5 min (Eppendorf Centrifuge 5810 R, Hamburg, Germany), and their organic supernatant transferred into clean glass tubes. The extraction step was repeated one more time using 3 mL of the solvent mixture as above. Extracts originating from the same sample were pooled and concentrated to 0.5 mL under a gentle stream of nitrogen at 40 °C using a blow-down station (Thermo Scientific TS-18821). The remaining 0.5 mL extract was then diluted to 5 mL with 10 mM acetic acid in water and further purified by an Oasis MCX cartridge (60 mg/3 cm^3; Waters, Milford, MA). Each cartridge was conditioned with 5 mL of methanol followed by 5 mL of water. The diluted extract was loaded onto the cartridge and rinsed with 12 mL of 25%

methanol in water followed by 5 mL of water. The cartridge was dried under vacuum for 30 min and then the target analytes were eluted with 4 mL of methanol. The eluate was dried under a gentle flow of nitrogen at 40 °C and then reconstituted in 1 mL of methanol. The extract was diluted with 1:1 (v/v) MS grade water prior to injection into high performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) for analysis.

Instrumental Analysis

A Shimadzu 2100 HPLC (Shimadzu Scientific, Kyoto, Japan) coupled with ABsciex API 4000 triple quadrupole mass spectrometer (Applied Biosystems, Framingham, MA) with electrospray ionization was used for the chemical analysis. The analytes were separated on a Waters X-Bridge C_8 column (4.6 × 150 mm, 3.5 μm particle size) preceded by an equivalent guard column using a gradient LC protocol. The injection volume was 10 µL. Methanol was used as mobile phase A and water as the mobile phase B. The gradient program started at 60% A and then ramped up to 95% A over 4 min, held at 95% for 3.5 min, followed by dropping back to 60% A within 1.5 min, and maintained at 60% A for 2 min. The ESI was operated under negative mode and the source parameters were set as follows: curtain gas = 172 kPa, gas 1 = 483 kPa, gas 2 = 345 kPa, IS = -4500 eV, source temperature = 500 °C, entrance potential (EP) = -10 eV, and collision activated dissociation (CAD) gas = 83 kPa. Analytes and labeled standards were identified using their specific retention time and multiple reaction monitoring transitions as reported earlier (SI, Table S2-3) (Pycke et al. 2015).

Quality Assurance/Quality Control

All reported concentrations were determined based on a standard curve containing between 5 to 8 data points at concentrations ranging from 0.01 to 10 ng/mL, with minimum coefficients of determination $r^2 \geq 0.99$. Average recoveries for parabens were determined based on spike-recovery experiments (6 replicates). Relative recoveries, calculated by including information from performance of labeled standards from 6 matrix spike samples, ranged between 78 ± 11% for MePB to 113 ± 6% for BuPB (SI, Table S2-4). Average absolute recoveries for isotope standards spiked into all samples were between 65 to 72%.

As detection of background levels of parabens is a known issue resulting from the ubiquity of the compounds (Pycke et al. 2015, Wang and Kannan 2016), special care has been taken to avoid possible contaminations. Authors avoided using products containing parabens and wore gloves all the time when handling the samples. All extractions were performed along with method blanks (*i.e.*, procedural controls), and a pure methanol/water mixture (50/50, v/v) was injected once per 10 samples as a check for carryover of parabens from sample to sample. No parabens were found present in solvent blanks, but an average of 1.08 ng/g of MePB and 0.80 ng/g PrPB were found in four method blanks. Therefore, mean concentrations measured in method blanks were subtracted from measured concentrations found in samples.

Limit of detection (LOD), limit of quantification (LOQ), and method detection limit (MDL) were determined and are provided in supplemental Table S4. MDLs were determined following the U. S. Geological Survey (USGS) (Childress et al. 1999) and United States Environmental Protection Agency (USEPA) guidelines, and LOD and LOQ were determined according to the USEPA guideline (EPA. 1984). Method detection limits

(MDLs) for parabens in sludges ranged from 0.28 to 0.97 ng/g, which were comparable to other studies analyzing parabens in sewage sludge (Albero et al. 2012, Nieto et al. 2009, Nieto et al. 2008).

For the sewage sludge samples from different locations, based on sample mass availabilities, either duplicates or triplicates were prepared for individual sample. Precisions were expressed either by RPD (relative percentage differences) for duplicates or RSD (relative standard deviation) for triplicates. For the 18 time-series samples from the same plant A, six random samples were extracted in duplicate, and the RPDs were calculated using the following equation:

$$\mathrm{RPD}(\%) = \frac{|C_{sample} - C_{duplicate}|}{\frac{C_{sample} + C_{duplicate}}{2}} \times 100 \qquad (1)$$

Where C_{sample} and $C_{duplicate}$ are the concentrations detected in the original sample and in its duplicate, respectively. Precision expressed by averages of RSDs from triplicates and RPDs from duplicates ranged from 11% to 19%. These method performance parameters were comparable to those from previous work by others (Liao and Kannan 2013), who reported a coefficient of variance <16% for duplicate analyses.

Data Analysis

LC-MS/MS data were acquired with Analyst 1.5 software (Applied Biosystem, Foster City, CA). The concentrations of parabens in sludges were calculated using the isotope-dilution method and were reported as ng/g on dry weight (dw) basis (all concentrations reported hereafter were on dry weight basis). Concentrations were reported when the analyte peak height to background signal had a signal-to-noise ratio of >3, the

sample peak areas fell within the dynamic range of the calibration, and the calculated concentrations were above MDL. "Non-detects" failing to meet the above requirements were assigned a conservative value of MDL/√2 for statistical analysis, allowing for a worst-case scenario analysis that is conservative rather than reflective of the best possible estimate (Hornung and Reed 1990). The average contribution of five parabens in sewage sludge was determined by calculating the mean of the contribution (%) of individual paraben to the total parabens concentrations in all the samples.

Statistical analyses were performed using Microsoft Excel 2007 and R (version 3.2.2). Correlation among the concentration of individual paraben and the total parabens were analyzed using a non-parametric Spearman's rank correlation test, and the correlation coefficients were expressed using Spearman's *rho*. For comparison of sludge with different treatments, a Shapiro-Wilk test was used to test the normality of data, and a Bartlette test was performed to determine the variance between groups, then a one-way ANOVA was used for the comparison of total parabens' concentrations in sewage sludge with different treatments. A Tukey HSD test was further performed for pair-wise comparison. A one-way ANOVA test was also used to test seasonal variations of paraben concentrations. The significance levels were set at $p < 0.05$ and $p < 0.01$.

Estradiol Equivalency Quotient Calculation

The Estradiol Equivalency Quotient (EEQ) contributed by parabens in sewage sludge was calculated using equation 2:

$$EEQ_i = EEF_i \times C_i \qquad (2)$$

Where C_i is the measured concentration of an individual paraben in sludge samples (ng/g, dw), and EEF_i is the estradiol equivalency factor of an individual paraben, defined as the quotient of $EC50_{E2}/EC50_{Compound}$, namely the ratio between the half-maximal effective concentration of E_2 (17β-estradiol) and the investigated compound. Three sets of EEFs (SI, Table S2-5) from three previous studies were used to calculate the EEQs, including minimum, maximum and average EEFs. As an additive estrogenic activity of the endocrine disruptors has been proven, ∑EEQ, which represented the overall endocrine-disrupting activity of the sample, was obtained by adding up individual EEQs.

Percent EEQ contribution (EEQ_i%) of each paraben was calculated as shown below:

$$EEQ_i\% = \frac{EEQ_i}{\sum EEQ} \times 100 \quad (3)$$

Average contribution of individual paraben was determined by calculating the mean of EEQ_i% in all samples.

Results and Discussions

Parabens in U.S. Sewage Sludges

A total of 19 sludge samples from 14 WWTPs were tested for five target parabens (Figure 2-1, SI, Table S2-6), MePB and PrPB were detected in every sample (100% detection frequency), with a concentration range of 15.9-203.0 ng/g and 0.5–7.7 ng/g, respectively. EtPB was found in 12 samples (63%), ranging from 0.7–2.6 ng/g, BuPB was detected in 8 samples (42%) ranging from 0.6–4.3 ng/g, and BePB was detected in 5 samples (26%) with a concentration range of 0.6–3.3 ng/g. The average contribution of individual paraben to total parabens concentration in decreasing order was: MePB (89.4 ± 6.1%) > PrPB (5.7 ± 3.1%) > EtPB (1.8 ± 2.5%) > BePB (1.6 ± 2.7%) > BuPB (1.5 ± 2.3%).

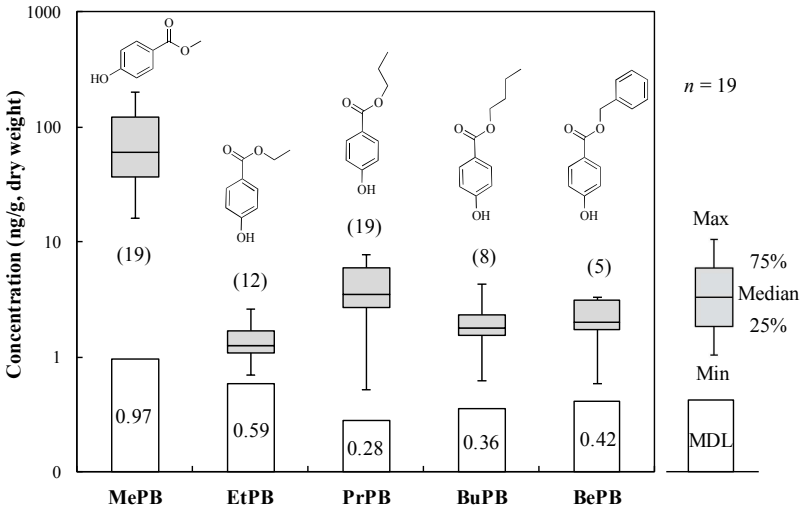

Figure 2-1. Box-and-whisker plot of paraben concentrations in 19 sewage sludge samples from 14 WWTPs located in 9 U.S. states. Numbers in parentheses indicates the number of samples yielding detectable concentrations. Bottom columns represent the compound-specific method detection limits (MDLs) with the exact value shown inside the column.

The sum of five parabens' concentration (∑PBs) for each sample is shown in Figure. 2-2, where sludges were categorized into three groups: untreated sludge (n = 3), digested sludge (n = 8) and digested sludge with additional treatments (n = 8). ∑PBs ranged from 21.2–213.2 ng/g, and the median ∑PBs concentrations for untreated, digested and digested with additional treatments sludge were 172.1, 92.3 and 45.1 ng/g, respectively. A one-way ANOVA revealed the ∑PBs concentrations for the three groups were statistically different ($p < 0.01$), a Tukey HSD test showed significant differences were found between untreated sludge with treated sludge ($p < 0.05$), and untreated sludge with treated sludge with additional treatments ($p < 0.001$), whereas no significant difference was observed between treated sludge and the ones with additional treatments ($p = 0.20$). Median concentrations of individual paraben in three types of sludge can be found in SI (Table S2-7).

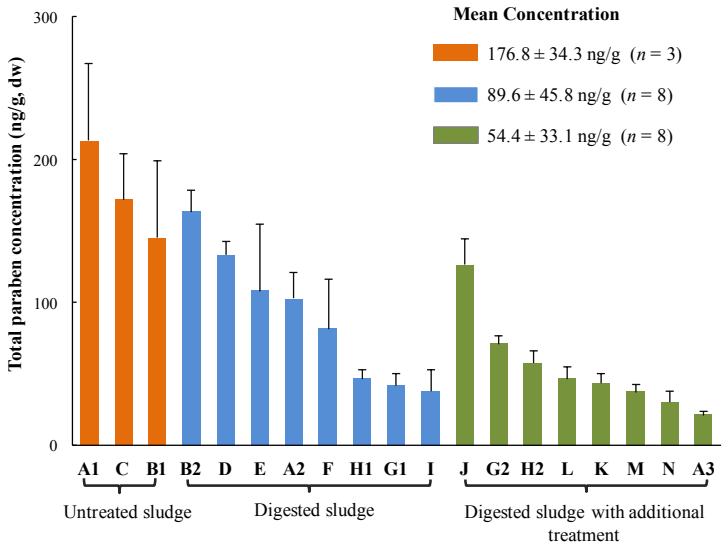

Figure 2-2. Total concentration of five parabens in untreated sludge (orange bar), digested sludge (blue bar), and digested sludge with additional treatment (green bar) from 14 different WWTPs across the U.S. Error bars indicate the sum of standard deviation for each paraben. Median ∑PBs concentration for each category of sludges was shown on top right, and the median concentrations were 172.1, 92.3 and 45.1 ng/g for untreated, digested and digested sludge with additional treatment, respectively.

In general, higher concentrations of parabens were found in untreated sewage sludges compared to those found in treated sludges, indicating that treatment may help decrease parabens in sewage sludge. This difference, however, could also be related to water content, with higher water content in untreated sludge yielding higher parabens concentrations. Different characteristics of the WWTPs including plant location, population, source of release, treatment capacity, sludge production and treatment approaches, also likely contribute to the variability in paraben concentrations. Changes in extractability or other aspects of the sample condition or method performance over the 20-month period could also contribute to the observed variability shown in Figure S2-2.

Comparison of Paraben Concentrations with Other International Studies

Table 2-1. Comparison of concentrations of parabens in sewage sludge found in this study and reported previously for different regions of the world.

Location	WWTP (#)	Sludge Type	Concentration (ng/g, dw)					Ref
			MePB	EtPB	PrPB	BuPB	BePB	
Quebec, **Canada**	2	NA	72-91	<6.4 [a]	<2.8-8	<2.9	<2.1	(Viglino et al. 2011)
Guangzhou, **China**	1	Untreated & Treated	5.7-53.5	<0.6	3.6-17.8	<0.3	\	(Yu et al. 2011)
Beijing, **China**	1	Untreated	274-517	1.14-6.80	13.0-29.9	0.62-4.57	<0.4	(Li et al. 2015)
Xiamen, **China**	3	NA	8.2-48.1	\	<0.1-6.0	\	<0.1	(Sun et al. 2016)
South Korea	40	NA	4.31-540	<0.1-2.12	<0.05-32.5	<0.05-1.36	<0.1-2.89	(Liao et al. 2013a)
Tarragona, **Spain**	1	NA	46-202	<1.75	6-10	\	<3-5	(Nieto et al. 2009)
Madrid, **Spain**	19	Treated	5.1-26.2	<1.1	5.6-44.1	<1.2	<1.0	(Albero et al. 2012)
Albany, **U. S.**	2	Untreated	24.3-87.4	1.6-12	0.36-4.64	0.36-19.0	<0.01	(Wang and Kannan 2016)
U. S.	14	Untreated & Treated	15.9-204.0	<0.6-2.6	0.5-7.7	<0.4- 4.3	<0.4-3.3	This study

"#": Number of WWTPs tested in the study; [a] Non-detects were expressed as "<MDL"; NA: not available; "\" analytes not included in the study

Data on paraben concentrations in sewage sludge are limited. Most of previous studies generally collected samples from a limited number of WWTPs with limited temporal frequency. Only two previous studies have included multiple WWTPs, one from Spain that analyzed 19 WWTPs (Albero et al. 2012), and the other investigated 40 WWTPs throughout South Korea (Liao and Kannan 2013). Unlike the current study, most previous research did not specify whether the sludge had been treated or not and what treatment approaches had been applied.

Comparisons of paraben concentrations found in sewage sludge from different locations/countries were listed in Table 2-1 (also see SI, Figure S2-1). Among all previous

studies, MePB and PrPB were the most frequently detected and most abundant parabens present in sewage sludge. MePB (15–204 ng/g) and PrPB concentrations (0.5–7.7 ng/g) found in this study were similar with the ones reported elsewhere (MePB: 4.31–540 ng/g; PrPB: <0.05–44.1 ng/g) (Albero et al. 2012, Li et al. 2015, Liao and Kannan 2013, Nieto et al. 2009, Sun et al. 2016, Viglino et al. 2011, Wang and Kannan 2016, Yu et al. 2011). Less frequently detected parabens (e.g. EtPB, BuPB and BePB) from this study were similar to those from South Korea (Liao and Kannan 2013). Overall, the levels of parabens in sludges from United States were comparable to the ones reported elsewhere.

Table 2-2. Spearman correlation matrix determined for concentrations of individual and total parabens detected in U.S. sewage sludge (n =19) in this study.

Spearman's rho	MePB	EtPB	PrPB	BuPB
EtPB	0.28			
PrPB	0.65**	0.35		
BuPB	0.29	0.33	0.13	
∑PBs	0.99**	0.31	0.70**	0.33

* Correlation is significant at the 0.05 level (2-tailed)
** Correlation is significant at the 0.01 level (2-tailed)
BePB was not included due to its low detection frequency

Varying mixtures of parabens are often used to enhance antimicrobial activities (Peck 2006); hence, significant positive correlations have been reported among individual parabens concentrations in various environmental monitoring and biomonitoring studies (Liao and Kannan 2013, Pycke et al. 2015). In this study, a non-parametric Spearman correlation was performed among concentrations of individual parabens and ∑PBs (Table 2-2). Similar with the Korea study (Liao and Kannan 2013), significant positive correlations were observed between MePB and PrPB concentrations from U.S. sludge. A correlation test also was conducted on reported MePB and PrPB concentrations in sludge from Madrid, Spain (Albero et al. 2012). However, the levels of MePB and PrPB in Spain's

sewage sludge were not significantly correlated (r = 0.11, p = 0.65). As positive correlations between parabens could be due to the co-occurrence of individual parabens in consumer product formulations (Guo and Kannan 2013), the results observed in Spain might indicate that product formulations or consumption practices of parabens in Spain differed from those in the U.S. and Korea.

Temporal Variation of Parabens' Concentration in Sludge

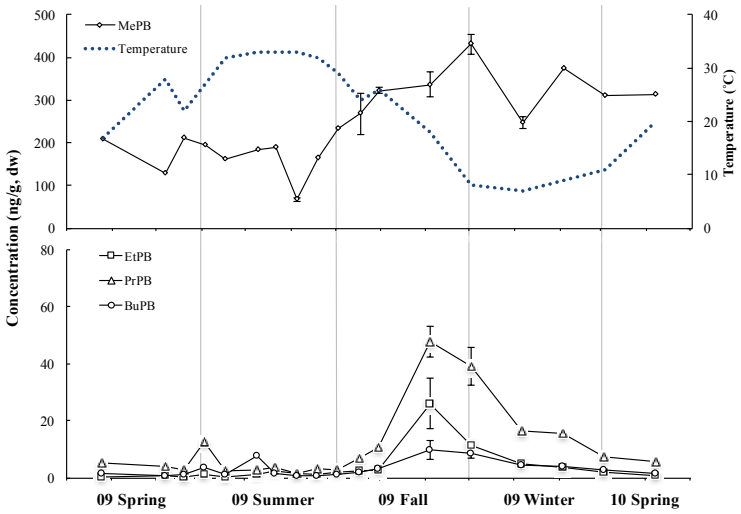

Figure 2-3. Temporal variation of parabens in sewage sludge (n = 18) from one WWTP collected during 2009–2010. Panel (A) shows the concentration of MePB and panel (B) includes concentrations for EtPB, PrPB, and BuPB. Error bars indicate minimum and maximum concentration from duplicate extractions. The plant is located in the southwestern U.S. in a humid, subtropical climate.

To evaluate temporal changes of paraben concentration in sludge, 18 sludge samples from plant A collected from March 2009 to April 2010 (Figure 2-3) were examined. Concentration ranges for individual parabens were as follows: MePB (67.2–430.9 ng/g), PrPB (1.8–47.7 ng/g), EtPB (0.4–26.1 ng/g) and BuPB (0.8–9.9 ng/g).

Concentration of ∑PBs ranged from 71.5 to 490.1 ng/g, with a median concentration of 227.5 ng/g (BePB was excluded for this analysis due to its low detection frequency and low concentration). MePB accounted for 80–98% of ∑PBs, and individual parabens were significantly positively correlated (r = 0.60–0.78, **SI, Table S2-8),** indicating common source releases for these four parabens throughout the sampling period.

All four parabens share similar temporal trends in that the concentrations were similar between 2009 spring, 2009 summer and the spring of 2010, but increased during fall and winter of 2009. One-way ANOVA tests revealed no significant differences on the concentrations of EtPB, PrPB and BuPB among different seasons, but significant differences ($p < 0.05$) were observed for MePB. This observation could be at least partially due to an increase in microbial activity in warmer seasons, resulting in increased biodegradation rates of parabens in wastewater during spring and summer seasons, translating to lower concentrations in sludge. Conversely, decreased microbial activity in colder seasons could translate to higher observed paraben concentrations in fall and winter seasons. Similar behavior has been observed previously in that significantly lower concentrations of PPCPs in sludge were observed in August samples, when compared to the ones from February, May and December with p values of 0.0001, 0.0001, and 0.002. (Sun et al. 2016). To better illustrate the correlations of temperatures and parabens concentration in sewage sludge, daily average temperatures on the sampling dates were compiled (SI, Table S2-9) and tested against paraben concentration (SI, Table S2-8). Temperature was significantly negatively correlated with the concentration of MePB (r = -0.77, $p < 0.001$), PrPB (r = -0.77, $p < 0.001$), BuPB (r = -0.55, $p < 0.05$) and the ∑PB (r = -0.78, $p < 0.001$). The significant negative correlations observed here were supporting

evidence that biodegradation rates were faster in warmer seasons than cooler seasons. However, these variations can also be caused by other factors such as the consumption volume of parabens (i.e. increased use of personal care products during fall and winter), and the operational parameters of WWTPs.

Estradiol Equivalency Quotient

Table 2-3. Calculated Estradiol Equivalency Quotient (EEQ) values of five parabens detected in U.S. sewage sludge samples collected in 2009/2010 (n = 19).

EEQ (pg/kg, dw)	MePB	EtPB	PrPB	BuPB	BePB	$\sum EEQ_{PBs}$
Minimum [a]	2.0	0.3	0.1	2.0	4.0	10.1
Maximum [b]	146.1	5.3	61.3	42.9	325.9	500.1
Average [c]	36.5	1.7	14.8	9.4	34.8	97.2
Median [d]	27.4	1.4	12.3	2.3	13.1	88.3
Contribution (%) [e]	39.3	1.9	17.3	10.5	31.0	

[a] Calculated using the minimum estradiol equivalency factor (EEF) from three studies
[b] Calculated using the maximum EEF from three studies
[c] The average EEQ for 19 sludge samples calculated using the average EEF from three studies
[d] The median EEQ for 19 sludge samples calculated using the median EEF from three studies
[e] Mean of the contribution of each individual paraben to the total EEQ in 19 samples

The minimum EEQs in Table 2-3 indicated lowest EEQs calculated using the lowest concentration of paraben in the samples and the minimum EEF. Conversely, the maximum EEQ were calculated using the maximum EEF and highest concentration detected in 19 sludge samples. The lowest EEQ contributed by individual paraben ranged from 0.1 pg/kg for PrPB to 4.0 pg/kg for BePB, and the highest ranged from 5.3 for EtPB to 325.9 pg/kg for BePB. In terms of the individual paraben's contribution to total EEQs, it is unsurprising that although MePB accounted for an average of 89.4 ± 6.1% to the total

parabens concentrations, it only contributed an average of 39.3 ± 18.5% to total EEQs, since the estrogenic potential of parabens increases with the alkyl chain length.

In general, as expected, EEQs contributed by the five parabens in U.S. sludges were minimal, with the largest ∑EEQ not exceeding 500.1 pg/kg, and a median ∑EEQ at 88.3 pg/kg, which was almost 10^6 times lower than the estrogenic activity contributed by natural estrogens present (Estrone + 17β-Estradiol + Estriol, E1 + E2 + E3) in sewage sludge samples reported at 24.8 to 63.8 μg/kg (Marti and Batista 2014, Vega-Morales et al. 2013).

Study Limitations

The sewage sludge samples in this study were collected in 2009 to 2010, and then kept at -80°C prior to extraction and analysis in 2015. The widespread use of parabens as long-term preservatives implies that the compounds are expected to be relatively stable under ambient conditions. Aqueous solutions of parabens are reported stable at pH 3-6 for up to 4 years at room temperature, but have been observed to be subject to hydrolytic attenuation at or above pH 8, with a loss rate of about 10% or more over the course of 60 days when stored at room temperature (Soni et al. 2002). Biosolids collected in the present study were not subjected to lime stabilization, thereby avoiding such degradative high-pH regimes and potentially associated significant analyte loss by hydrolysis. In addition, sludge samples were stored at -80 ˚C upon receipt to avoid potential chemical, physical and biological transformation processes. Occurrence of analytes in treated sewage sludge implies that the compounds already had to be refractory enough to withstand aerobic/anaerobic digestion and in some cases additional treatment. For the above stated reasons, notable loss of analytes during sample storage thus was not to be expected. Indeed,

repeated analysis of the same samples over a period of 20 months indicated parabens to display marked persistence in archived sludge, with no appreciable losses being evident after long-term storage at -80 ˚C (see Figure S2-2 in Supplementary Information). Since there are no reports in the literature on formation of parabens during sample storage and none such phenomena were observed in the experiments conducted here, the data presented in this work should be interpreted as conservative estimates of true concentrations that potentially could have been somewhat higher than the values determined experimentally and reported here.

In this work, potential transformation products of parabens and their hormonal potency was not explored. It is possible that transformation products of parabens were present and that these may increase the total estrogenicity imparted by paraben presence in sewage sludge.

Conclusions

Parabens were found to be ubiquitously present in the sewage sludge sampled in 14 WWTPs in 9 U.S. states, at a level of ∑PBs 21.2–213.2 ng/g dry weight. The concentrations of parabens in this study were similar to those observed in previously published studies. The estrogenic activities contributed by parabens in U.S. sludge were estimated using EEQ as an indicator, showing minimal estrogenic activities (10.1–500.1 pg/kg, dw) were contributed by parabens in U.S. sludge compared to that of biogenic estrogens. A temporal study monitoring parabens concentration over 12 months revealed seasonal variations, but generally similar concentrations on an annual scale, indicating a consistent source releasing parabens to WWTPs. This study establishes baseline

concentrations for five parabens in sewage sludge from United States, providing important information for future risk assessment and trends of environmental release of parabens.

3 NATIONWIDE RECONNAISSANCE OF FIVE PARABENS, TRICLOSAN, TRICLOCARBAN AND ITS TRANSFORMATION PRODUCTS IN SEWAGE SLUDGE FROM CHINA

Abstract

With rapid economic growth and industrialization, one of the environmental challenges China is facing is the management of sewage sludge. Inadequate treatment and improper disposal of sewage sludge can lead to secondary contaminant release, resulting in potential risks to aquatic and terrestrial organisms as well as human populations. In this study, the occurrence (detection frequency and detected concentrations in ng/g dry weight) of triclosan (99%, 7-4870), triclocarban (95%, 30-43300), 2'-hydroxy-triclocarban (94%, 20-2340), 3'-hydroxy-triclocarban (91%, 6-1250), 3,3',4,4'-tetrachlorocarbanilide (100%, 22-580) dichlorocarbanilide (94%, 38-23890), monocarbanilide (92%, 22-120), carbanilide (90%, 4-1340), and five parabens: methyl- (98%, 5-630), ethyl- (96%, 6-170), propyl- (99%, 5-27), butyl- (89%, 7-11) and benzyl-(7%, 9-12) paraben were measured in 100 sewage sludge samples collected from 21 provinces/districts across China. Contaminant profiles and concentrations differed by region, by treatment plant capacity, and by effluent treated (primarily domestic, $n = 86$; primarily industrial; $n = 14$). About 68 ± 71 metric tons of all 13 analytes combined are estimated to be sequestered in Chinese sewage sludge annually. This China-wide survey covered 3% of about 3500 sewage treatment plants total and established baseline levels of known or suspected endocrine disrupting antimicrobials in municipal sludges whose disposal in the environment is being performed with little regulatory oversight and enforcement.

Introduction

Over the past few decades, the consumption of pharmaceuticals and personal care products (PPCPs), including a number of antimicrobials known or suspected to act as endocrine disruptors, has risen steadily, leading to widespread human exposure and still increasing inputs of these chemicals into wastewater and the environment (Hopkins and Blaney 2016). A number of additives to PPCPs chemicals have been identified as emerging contaminants, because of their extensive uses, continuous inputs, frequent detections and potentials threats to the environment and human health (Andrade et al. 2015, Gasperi et al. 2014). For instance, parabens (PBs), a group of antimicrobial compounds used as preservatives in numerous PPCPs and food items (Haman et al. 2015, Liao et al. 2013b), have been associated with breast cancer etiology, sperm DNA damage, ovarian aging and reduced fertility (Boberg et al. 2010, Darbre et al. 2004, Smarr et al. 2017, Smith et al. 2013), and are known to be toxic to aquatic organisms (Yamamoto et al. 2011). Triclosan (5-chloro-2-(2,4-dichlorophenoxy) phenol; TCS) and triclocarban (3,4,4'-trichlorocarbanilide; TCC) are additional antimicrobial agents commonly used in hand soaps, bar soaps, deodorants, toothpastes, textiles, and building materials (Bledzka et al. 2014, Dann and Hontela 2011, Halden and Paull 2005). They are environmentally-persistent endocrine disruptors known or suspected to impart adverse reproductive and developmental impacts in animals and, by extension, in humans (Chen et al. 2007). TCS, TCC and their transformation products have been shown to bioaccumulate in and are toxic to aquatic organisms (Chalew and Halden 2009). They are sources of additional toxic and carcinogenic compounds including dioxins, chloroform and chlorinated anilines (Coogan and Point 2008, Halden et al. 2017). Furthermore, PBs, TCS and TCC have been associated with sensitization to food allergens (Savage et al. 2012), altered thyroid hormone levels (Koeppe et al. 2013) and the promotion of antibiotic-resistance in microbial pathogens (Carey et

al. 2016, Drury et al. 2013, Hartmann et al. 2016b). The U.S. Food and Drug Administration (FDA) has issued a final rule, effective September 2017, banning the use of TCS and TCC, along 17 other antimicrobial ingredients in many over-the-counter antiseptic personal care products (FDA 2016).

Ubiquitous human exposure to these emerging contaminants has led to their frequent detection in human urine, plasma, cord blood and breast milk (Pycke et al. 2015, Pycke et al. 2014a, Wei et al. 2017, Ye et al. 2008, Ye et al. 2016). Environmental occurrence also is widespread, with contaminated matrices including indoor dust, wastewater, sewage sludge, surface water, and sediments (Albero et al. 2012, Liao et al. 2013a, Liu et al. 2015, Sapkota et al. 2007). As these chemicals are mainly used in PPCPs, they enter wastewater system either directly in gray water after dermal application, or after passage through the human body excreted in urine and feces. The removal efficiency for these contaminants during wastewater treatment varies with their physico-chemical properties and is often incomplete, thereby creating pathways for residual contaminants to enter aquatic environment via wastewater reclamation or through disposal of sewage sludge on land (McClellan and Halden 2010). Sewage sludge is a by-product of wastewater treatment and a known sink of wastewater-borne persistent organic pollutants. Contaminants accumulated in sludge can cause secondary pollution through disposal of these materials, impacting soil, surface water, groundwater, and the food supply (Wu et al. 2010). Therefore, understanding the levels of contaminants in sludge is important to assess the risks to human health and the environment. Although over 90% of parabens are typically removed from wastewater during treatment (Li et al. 2015, Sun et al. 2016, Wang and Kannan 2016), parabens have been observed to persist and accumulate in sewage sludge to ng/g concentrations (Albero et al. 2012, Chen et al. 2017, Liao et al. 2013a, Nieto et al. 2009, Yu et al. 2011). Compared to parabens, TCS and TCC have been found to be even more recalcitrant to treatment and to remain in sludge at concentrations of up to

tens of microgram per gram (Heidler and Halden 2007, Heidler et al. 2006). TCS and TCC have been identified as top contaminants in sludge from several countries (Liu et al. 2017b, McClellan and Halden 2010, Subedi et al. 2014), and triclocarban derivatives with a higher and lower chlorine substitution number also have been observed, including dichlorocarbanilide (DCC), monocarbanilide (MCC), carbanilide (NCC), manufacturing by-products 3,3',4,4'-tetrachlorocarbanilide (3'-Cl-TCC), and two human metabolites 2'-hydroxy-triclocarban (2'-OH-TCC) and 3'-hydroxy-triclocarban (3'-OH-TCC) have also been reported to be present in U.S. sewage sludge (Pycke et al. 2014b). In contrast, comparable data on the presence of parental antimicrobials, transformation products and human metabolites are still rare or completely lacking for municipal sludge from China (Chen et al. 2014, Liu et al. 2017b, Sun et al. 2016).

In China, the need for environmental protection has grown both among the public and the government in an economic climate of rapid growth and development over the past decades (Zhang et al. 2016). With 3508 wastewater treatment plants (WWTPs) having come online in China by the year 2013, the capacity and efficiency of WWTPs have increased dramatically (Zhang et al. 2016). More than 69.5 billion metric tons of wastewater and 6.25 million metric tons of dry weight sludge were generated in China in 2013 (Yang et al. 2015). However, over 80% of the sludge in China is disposed of improperly (Feng et al. 2015). In the past 30 years, although over 700 organic contaminants have been analyzed in Chinese sludge (Meng et al. 2016), nationwide surveys of contaminating chemicals are scarce. Most previous studies were often limited to a few sampling sites from places that were more developed than the rest of the country.

Therefore, the objective of this study was to investigate the nationwide occurrence and distribution of PBs, TCS, TCC and major transformation products of the same in Chinese sewage sludge, using a QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) method for sludge

extraction followed by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) analysis, and to explore spatial variations on contaminants level, and to estimate the mass output of contaminants through sludge, as well as extrapolate mass load into WWTPs and the associated risks to aquatic organisms.

Experimental

Chemicals and Reagents

Methylparaben (MePB), triclosan (TCS), triclocarban (TCC), 1-(3-chlorophenyl)-phenylurea (MCC), and carbanilide (NCC) were purchased from Aldrich (Sigma-Aldrich, St. Louis, MO); ethylparaben (EtPB), propylparaben (PrPB), butylparaben (BuPB), and benzylparaben (BePB) were purchased from RT Corp (Laramie, WY). Dichlorocarbanilide (DCC) was obtained from Oakwood Products Inc. (West Columbia, SC). Isotopically labeled standards $^{13}C_6$-MePB, $^{13}C_6$-TCC, and $^{13}C_{12}$-TCS were obtained from Cambridge Isotope Laboratories (Andover, MA). Standards of d_5-EtPB, d_4-PrPB, and d_4-BuPB were purchased from C/D/N Isotopes (Quebec, Canada). Oxidative metabolites of TCC, 2'-hydroxy-TCC (2'-OH-TCC) and 3'-hydroxy-TCC (3'-OH-TCC) were kindly provided by Dr. Bruce Hammock (University of California, Davis) and were manufactured as previously described (Schebb et al. 2011a). The 3,3', 4,4'-tetrachlorocarbanilide congener (3'-Cl-TCC) was obtained from Dr. Ehrenstorfer, Augsburg, Germany. Their purity was verified by LC-MS/MS upon arrival in the laboratory. The chemical structures of the 10 analytes of interest are presented in SI Figure S3-1. Methanol (LC-MS-grade; 99%) water, and acetic acid were obtained from Fluka, and LC-MS-grade acetone was obtained from Sigma-Aldrich (St. Louis, MO, USA). Individual stock solutions of the native and isotopically-labeled compounds were prepared in methanol. The working standards were prepared

by serial dilution of stock solutions with methanol immediately prior to use. All stock solutions were stored in glass vials with polytetrafluoroethylene septa at –20 °C. Anhydrous magnesium sulfate ($MgSO_4$) was obtained from ACROS Organics (New Jersey, USA) and anhydrous sodium acetate (NaAc) from Dionex (Sunnyvale, CA, USA). Extraction tubes (2 mL; DiSQuE QuEChERS containing 150 mg $MgSO_4$, 50 mg PSA, and 50 mg C_{18}) were purchased from Waters Corporation (Milford, MA, USA).

Sludge Sample

A total of 100 sewage sludge samples from 86 domestic and 16 industrial WWTPs representing various regions in China were collected during 2009 to 2014, covering 21 provinces and municipalities across China (SI, Table S3-1). Grab samples were collected by staff from local WWTPs and shipped to Tongji University on ice. Upon reception, the samples were air dried and homogenized, then wrapped with aluminum foil and kept in dark at –20 ˚C. An aliquot of 5-10 g of each sample was prepared and shipped to Arizona State University for extraction of LC-MS/MS analysis.

Sample Preparation

Sewage sludge aliquots (0.1 to 0.15 g dry weight) were distributed to 15-mL polypropylene tubes and spiked with 30 µL of a methanolic solution containing isotope-labeled standards (100 ng/mL of $^{13}C_6$-MePB, d_5-EtPB, d_4-PrPB, d_4-BuPB, $^{13}C_6$-TCC, and 1 µg/mL of $^{13}C_{12}$-TCS), followed by 1 mL of MS-grade water. Tubes were vortexed for 30 s and then 1.5 mL of acetonitrile containing 0.1% acetic acid was added. Tubes were vortexed again for 30 s and placed into a sonication water bath for 60 min. After sonication, 0.4 g of anhydrous magnesium sulfate and 0.1

g of anhydrous sodium acetate were added. The slurry was vortexed for 1 min and centrifuged at 4,000 *g* for 10 min. The resultant upper organic layer was transferred into a 2-mL DiSQuE QuEChERS tubes, vortexed for 1 min and centrifuged at 21,130 rpm for 10 min. Finally, the extract was transferred into a 4-mL amber vial, and 100 µL of the extract was taken and diluted with 100 µL of MS-grade water for LC-MS/MS analysis.

LC-MS/MS Analysis

A Shimadzu 2100 HPLC (Shimadzu Scientific, Kyoto, Japan) coupled to a Applied Biosystems Sciex API 4000 triple quadrupole mass spectrometer (Applied Biosystems, Framingham, MA) with electrospray ionization (ESI) was used for chemical analysis. Samples were injected at 10 µL and analytes were separated on a Waters X-Bridge C_8 column (4.6 × 150 mm, 3.5 µm particle size) preceded by an equivalent guard column. A gradient protocol consisting of methanol (eluent A) and water (eluent B) at a flow rate of 0.5 mL/min was used. The gradient program started at 60% A and ramped up to 95% A over 4 min, was maintained at 95% for 6 min, and then reduced back to 60% A over 1 min and was held at 60% A for 2 min. The ESI was operated in negative ionization mode and the source parameters were set as follows: curtain gas = 12 psi, Gas 1 = 70 psi, Gas 2 = 50 psi, IS = -4500 eV, source temperature = 500°C, entrance potential (EP) = -10 eV, and collision induced dissociation (CID) gas = 12 psi. Additional LC and MS/MS parameters for target analytes and labeled standards are provided as Supplementary Information (SI, Table S3-2).

Quality Assurance/Control

Analytes and labeled standards were identified using their specific retention time and multiple reaction monitoring transitions. All extractions were performed along with method blanks, and a mixture consisting of acetonitrile containing 0.1% acetic acid and water (50/50, v/v) was injected every 10 samples as a check for potential carryover from sample to sample. No contamination was observed in solvent blanks for any of the analytes except for TCC. However, average TCC levels found in sludge samples were over 1000-times higher than the background level observed, and background correction therefore was inconsequential. Recoveries were obtained by spiking 60, 300 and 600 ng/g of native standards into a randomly selected sludge sample matrix and three replicates were prepared at each spiking level. Relative recoveries ranged from 83% to 128% for all analytes, indicating good recovery rates of the method for all analytes. Method detection limits (MDLs) (SI, Table S3-3), determined following the United States Geological Survey (USGS) and United States Environmental Protection Agency (USEPA) guidelines (Halden et al. 2001), ranged from 1.3 ng/g to 3.7 ng/g. Precision, expressed by relative standard deviations (RSDs) from spiking tests, varied from 1 to 30% for all compounds. In addition, 26 of the samples were prepared in duplicate, and relative percent difference (RPD, %) was calculated using the following equation:

$$RPD\ (\%) = \frac{|C_{sample} - C_{duplicate}| \times 100}{\frac{C_{sample} + C_{duplicate}}{2}} \qquad \text{(Eq. 1)}$$

Where C_{sample} and $C_{duplicate}$ represent the concentrations detected in the original sample and corresponding duplicate, respectively. Average RPDs for individual compound varied from 7% to 21%, showing good repeatability of the method.

Data Analysis

Target analytes were quantified using the isotope-dilution method by spiking isotope-labeled analogs of target compounds into field samples prior to sample processing. For BePB, d_4-BuPB was used as the internal standard for quantification, and $^{13}C_6$-TCC served as the internal standard for all transformations products of TCC. All concentrations were reported on a dry weight (dw) basis unless stated otherwise. The sum of the concentrations of all five parabens investigated were expressed as ∑PBs; and the combined total concentrations of TCC, those of its two human metabolites (2'-OH'-TCC and 3'-OH-TCC), as well as its manufacturing by-products and transformation products (3'-Cl-TCC, DCC, MCC and NCC) were expressed as ∑TCCs; the sum of all 13 compounds were represented by ∑All. For statistical analysis of concentration populations, non-detects were interpolated with a value of MDL/√2. For correlation analysis, concentration values were first log-transformed. Spearman's correlation and Kruskal-Wallis tests were performed using the software R (v 3.3.2).

Results and Discussions

Contaminant Levels in Sewage Sludge and Determinants

The 13 target compounds were detected in sewage sludges from 100 WWTPs from across China at the frequencies and concentrations shown in Figure 3-1A&B, with Table S3-4 providing additional summary statistics on the results obtained. Sludges from the 86 and 14 WWTPs treating primarily domestic and industrial effluent, respectively, showed distinct patterns of contaminant occurrence. Two-tailed *t*-tests revealed significant differences in levels of TCS, TCC, 2'-OH-TCC, 3'-Cl-TCC, DCC, MCC and MePB between the two types of sludges. Except for BePB, detection frequencies and levels of contaminants were higher in domestic than in industrial sludges. This

finding is not surprising, given that the primary use of the chemicals investigated here is in personal care products and that occurrence of monitored metabolites requires prior human exposure and excretion. Additional differences in the relative abundance of contaminants were observed between domestic and industrial sludges (SI Figure S3-2); whereas TCC and TCS were the predominant contaminants in domestic sludge samples, individual compounds were more evenly distributed in industrial sludges. This finding may be due in part to different treatment techniques performed in domestic and industrial WWTPs.

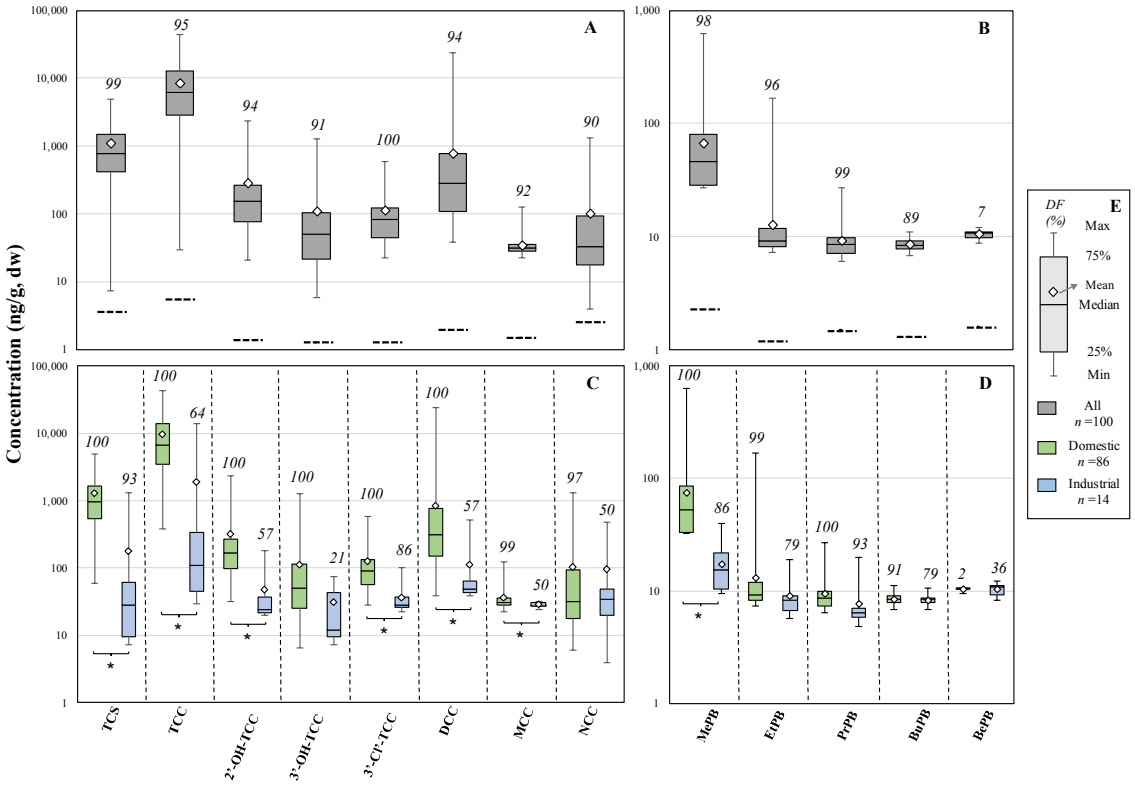

Figure 3-1. Box-and-whisker plots of detected contaminant levels (ng/g, dw) and detection frequencies (DF, %) found in sewage sludge from China. Panel A&B: levels of contaminants in all (n = 100, gray) sewage sludge samples; Panel C&D: contaminant levels in sewage sludges collected from 86 domestic (green) and 14 industrial (blue) facilities; a legend is provided in Panel E. The black dashed lines in Panels A&B represent MDLs (ng/g) for individual target compounds, and the asterisk (*) in Panels C&D indicates contaminant levels were significantly different (p<0.05) between domestic and industrial sewage sludges. Non-detected samples were not shown on the figure.

The impact of regional differences was explored separately by dividing the total of 86 domestic sludge samples into four economic regions of China: East Coast (n = 55), Central China (n = 20), Western China (n = 7) and Northeast China (n = 4). Analyte groups considered included ∑PBs (five parabens), TCS, and ∑TCCs (TCC plus its human metabolites and transformation products) (Figure S3-3A). The relative contribution of each region in percent to the geometric mean (GM) concentration of all 13 compounds considered jointly and individually is shown in Figure 3-2A. Results show little variations of paraben levels among different regions but higher contributions to TCS and ∑TCCs levels by China's Northeastern and East Coast region relative to the lesser developed Western and Central region, which could be related to higher per-capita consumption and discharge of PPCPs and in economically stronger, more developed areas. More specifically, province-specific geometric mean concentrations of ∑PBs, TCS and ∑TCCs were compared (Table S3-5 and Figure S3-4), revealing a pattern of higher sludge-borne contaminant levels along the coastal provinces compared to inland provinces.

Furthermore, sludge samples from 76 domestic WWTPs were categorized into four groups depending on treatment plant capacity in flow (F) units of millions of liters per day: small (F<10), medium (10<F<30), large (30<F<100), and super large (F>100). Contaminant concentrations (Figure S3-3B) and percent relative contributions (Figure 3-2B) were then compared, showing paraben levels to be evenly distributed, and no notable difference being observable with respect to concentrations of TCS and ∑TCCs among small, medium and large WWTPs; very large WWTPs showed elevated levels of TCS and ∑TCCs levels but observed differences were not statistically significant, as determined by Kruskal-Wallis tests (p > 0.05).

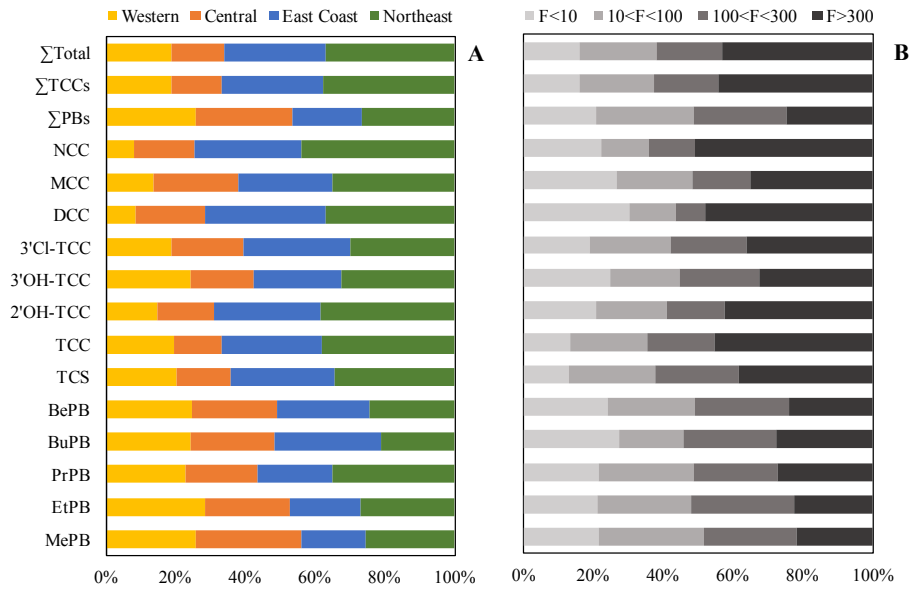

Figure 3-2. Regional contribution in percent to the total geometric mean (GM) concentration of 13 compounds (reported as a total and individually) in domestic sewage sludge from Western (n=7), Central (n=4), East Coast (n=20), and Northeast (n=55) China (Panel A); and treatment capacity of the WWTPs (Panel B). "F" is the flow volume in millions of liters per day. Sample sizes by plant size were n=4 (F<10), n=51 (10<F<100), n=17 (100<F<300), and n=6 (F>30).

Occurrence of TCS and TCC in Sewage Sludge

TCC was the most abundant contaminant among the 13 target compounds found in 100 sludge samples across China, with concentrations of up to 43,300 ng/g, and an average of 8,890 ng/g. The second most abundant compound was TCS, with the highest concentration at 4,870 ng/g and an average value of 1,100 ng/g. TCC and TCS are known co-contaminants in the environment and have been identified as top contaminants in sludge from several countries (Guerra et al. 2014, Heidler and Halden 2009, Subedi et al. 2015, Subedi et al. 2014). In this study, strong positive associations were found between sludge-borne levels of TCC and TCS (Pearson's r = 0.92, Figure 3-3A).

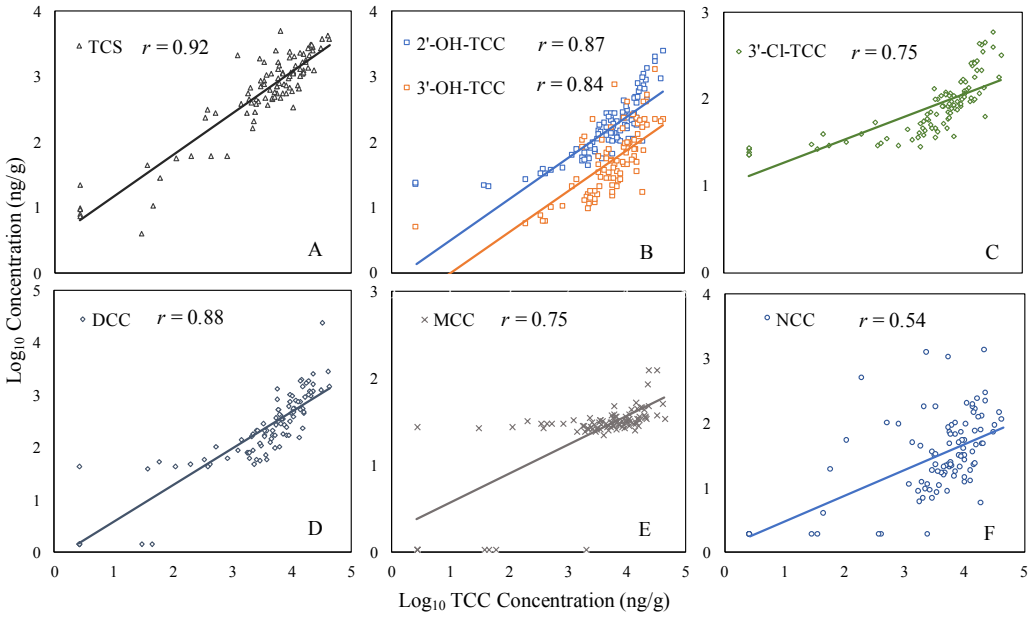

Figure 3-3. Concentrations of antimicrobials detectable in Chinese sewage sludge showed positive correlations between TCS and TCC (Panel A), human metabolites of TCC and TCC (Panel B), the manufacturing by-product 3'-Cl-TCC and TCC (Panel C), and three individual dechlorination products of TCC and TCC (Panels D, E and F).

Ubiquitous Presence of TCC Transformation Products in Chinese Sludge

Only a limited number of studies have examined the concentrations of TCC transformation products in sludge previously, and none have been conducted in China so far. In this study, two TCC human metabolites, 2-OH'-TCC and 3-OH'-TCC were found in over 90% of the samples, with concentrations ranging from 20 to 2,340 and 6 to 1,250 ng/g, respectively. Both the detection frequency and mean concentration of 2'-OH-TCC (94%, 290 ng/g) were higher than those of 3'-OH-TCC (92%, 108 ng/g), which matched finding from human biomonitoring studies (Ye et al. 2011). Strong positive correlations (Figure 3-3B) were found between 2'-OH-TCC, 3'-OH-TCC and TCC (Pearson's r = 0.87 and 0.84, respectively), suggesting human use of personal care products containing TCC as a strong source of TCC leading to human exposure and subsequent

excretion of its characteristic metabolites. Similar positive correlations between 2-OH'-TCC and TCC previously had been observed in U.S. sludge ($r = 0.84$) (Pycke et al. 2014b) and in the urine of pregnant U.S. women ($r = 0.49$) (Pycke et al. 2014a). In addition, levels of 2'-OH-TCC and 3'-OH-TCC also were positively correlated ($r = 0.86$) in sludge, mirroring results from a recent biomonitoring study in humans ($r = 0.99$) (Pycke et al. 2014a). Results reported here represent the first data on the ubiquitous occurrence of the human metabolite 3'-OH-TCC in sewage sludge of any region in the world and a strong correlation between this metabolite and TCC levels in sludge. The ratio of 2'-OH-TCC to TCC in 86 domestic sludges ranged from 0.005 to 0.100, with an average ratio of 0.032 ± 0.016. In U.S. municipal sewage sludge, similar ratios were found in the range of 0.008 to 0.045 (average 0.020 ± 0.010), whereas in biomonitoring studies a range of 0.50 to 1.10 (Schebb et al. 2011b) and 0.004 to 1.01 (Pycke et al. 2014a) was observed. Lower ratios determined for sludge could be a result of a higher removal efficiency and weaker adsorption to sludge of the more hydrophilic metabolite 2'-OH-TCC when compared to TCC and also could be caused by TCC uses in cleaning products other than personal care products (Pycke et al. 2014b).

The carbanilide, 3'-Cl-TCC, can have different origins, including representing an impurity in the manufacture of TCC (0.2% w/w) and the herbicide diuron (Sapkota et al. 2007), as well as potentially arising from the chlorination of TCC. Every sludge sample analyzed showed detectable concentrations of 3'-Cl-TCC in the range of 22 to 580 ng/g, with a mean of 113 ng/g. Concentrations of 3'-Cl-TCC and TCC were positively correlated (Pearson's $r = 0.75$, Figure 3-3C), suggesting TCC as an important source of 3'-Cl-TCC. Among all sludges examined, the ratio of 3'-Cl-TCC to TCC ranged from 0.003 to no more than 0.08 in facilities treating primarily domestic sewage and was as high as 10.40 in WWTPs treating industrial effluent. All these ratio values were higher than the established impurity ratio of 3'-Cl-TCC in TCC of 0.002, suggesting

the existence of additional sources of 3'-Cl-TCC and a potential accumulation in sludge due to stronger partitioning of the latter compound(Sapkota et al. 2007).

Peng *et al.* have recently studied two dechlorination products (DCC and NCC) of TCC in the sediment from Pearl River Estuary, which was the first time TCC dechlorination products were reported in the environment in China (Peng et al. 2017). Their study pointed to WWTP effluent as a major source and in-situ dechlorination in sediments as being negligible. Data on TCC dechlorination products in Chinese sewage sludges thus far have been lacking. In the present work, three dechlorination products of TCC were observed, with DCC being the most frequently detected (94%, detection frequency) and most abundant one (range: 38-23,890 ng/g, average: 760 ng/g), followed by MCC (92%, 22-120 ng/g, 35 ng/g) and NCC (90%, 4-1,340 ng/g, 100 ng/g). Similar to 3'-Cl-TCC, technical grade TCC also contains about 0.2% DCC as a production impurity (Heidler and Halden 2009) and ratios of DCC to TCC greater than 0.002 can thus indicate (microbially mediated) dechlorination activity (Souchier et al. 2015, Venkatesan et al. 2012). The here observed ratios varied from 0.012 to 16.154, strongly suggesting dechlorinating activity toward TCC during treatment of Chinese wastewater and sludge. The strong linear relationship found between levels of TCC and DCC (Pearson's $r = 0.88$, Figure 3-3D) indicates TCC playing an important role as a source and precursor of DCC release. The average ratio of DCC to TCC in domestic sewage sludge (0.068 ± 0.082) statistically different and much lower than values computed for industrial sludge (1.515 ± 4.225; Welch's test, $p < 0.05$) and in two industrial sludges the concentration of DCC actually was higher than that of TCC (1.043 and 16.154). At 0.012 to 0.738, the ratios of DCC to TCC found in Chinese facilities treating domestic sewage were similar to those reported for U.S. sludge (0.001 to 0.901) (Pycke et al. 2014b).

Positive correlations between TCC and its less chlorinated products MCC and NCC were also observed (Figure 3-3E and 3-3F, Pearson's r = 0.75 and 0.59, respectively). Widespread occurrence of MCC and NCC suggested complete dechlorination of TCC in the WWTP system. However, MCC and NCC levels were much lower than those of TCC and DCC, suggesting limited potential for full dechlorination of TCC from DCC over MCC to NCC. In addition, DCC and MCC were positively correlated with Pearson's r value of 0.74, but the correlation between NCC and MCC was weaker (Pearson's r = 0.53), and NCC levels were sometimes higher than those of MCC, suggesting other factors may influence conversion of MCC to NCC, or alternatively, other release sources of NCC to wastewater.

If all non-, mono-, and dechlorinated carbanilides observed in this work originated from the dechlorination of TCC, the maximum removal efficiency for TCC was no more than 52%, averaged 11 ± 8%, and thus was similar to the removal efficiencies (1-35%) reported from monitoring of U.S. WWTPs (Pycke et al. 2014b).

Parabens in Sewage Sludge

Parabens were ubiquitous in Chinese sewage sludge except for BePB (8% detection frequency, DF). Detected concentrations (range and mean) in units of ng/g were as follows: MePB (5-630, 67), EtPB (6-170, 10), PrPB (5-27, 9), BuPB (7-11, 8) and BePB (9-12, 10). Total concentrations (∑PBs) ranged from 5 to 810 ng/g, and the average was 95 ng/g. MePB contributed an average of 60 ± 17% to ∑PBs, followed by EtPB (14 ± 6%) > PrPB (12 ± 7%) > BuPB (11 ± 6%) > BePB (3 ± 5%), which was in accordance with prior studies showing MePB, EtPB and PrPB as the predominant parabens in PPCPs from China (Guo et al. 2014, Ma et al. 2016), as well as in urine samples of young Chinese adults (Ma et al. 2013). The fact that parabens often are used in

combination to improve antimicrobial activities (Liao et al. 2013a) may account for the here observed positive associations among MePB, EtPB and PrPB (Spearman's *Rho* = 0.45 – 0.49, $p < 0.001$, $n = 100$).

Comparison of TCS, TCC and ∑PBs Levels with Other Countries

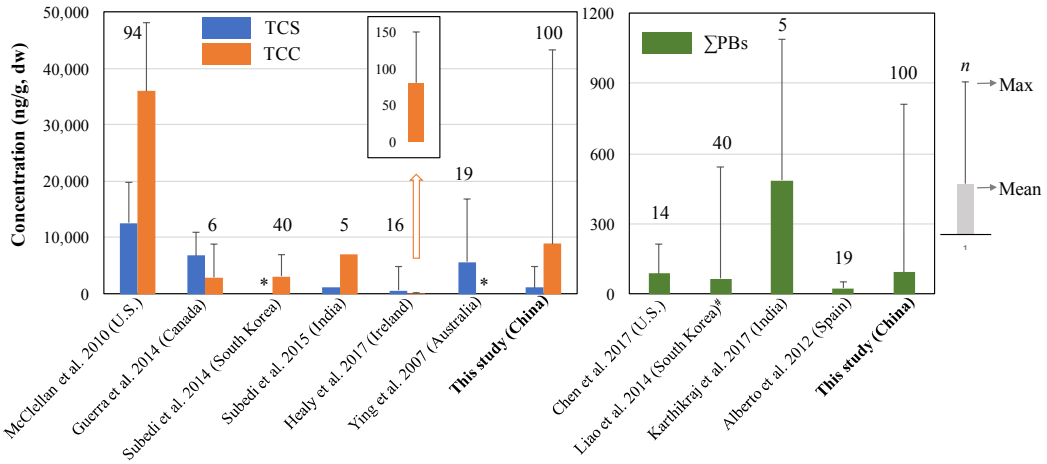

Figure 3-4. Mean and maximum concentrations of TCS, TCC and ∑PBs in sewage sludge from 100 Chinese WWTPs compared to data from other countries. Bars represent mean and whiskers maximum concentrations. Number shown on top of bars refer to the number of samples per study. Irish data were low and are depicted in the insert. *TCS was not measured in South Korea and TCC was not measured in the Australia. South Korean data show the geometric mean (**#**) of ∑PBs concentration instead of mean concentrations.

In the U.S., previous studies had demonstrated the occurrence of TCC and TCS in sewage sludge at levels of up to 48,100 and 19,500 ng/g, and at mean concentrations of 36,000 and 12,600 ng/g, respectively (McClellan and Halden 2010); these U.S. concentrations were, respectively, about 7 and 11 times higher than those found in China. However, with the U.S. ban of many antimicrobials taking effect in September 2017, concentrations of TCC and TCS are expected to decrease, with monitoring data on this outcome currently being pendent. In South Korea, an average of 3,100 ng/g TCC was reported to be present in sludge from 40 WWTPs across the

country (Subedi et al. 2014). Healy *et al*. recently reported average TCC and TCS levels in Irish sewage sludge of 610 and 80 ng/g, respectively, with this finding of lower concentrations likely being a result of the restricted uses of TCS in the EU since 2014 and the phasing out of TCC by pharmaceutical companies in the past several years (Healy et al. 2017). A comparison of mean and maximum TCC and TCS concentrations found in several other countries (Guerra et al. 2014, Subedi et al. 2015, Ying and Kookana 2007) is shown in Figure 3-4. While some countries or regions have already started restricting the use of TCC and TCS, China has not drafted any regulations yet. The high levels of TCC and TCS found in this study may provide information helpful in prioritizing contaminants in Chinese sludge and may inform future policy decisions.

Paraben levels found here were similar to those observed in previous studies in South Korea, Spain, U.S. and India (Figure 3-4**)** (Chen et al. 2017, Liao et al. 2013a, Nieto et al. 2009). The relative abundance of parabens varies among different countries, such as in South Korea (Liao et al. 2013a) and the U.S. (Chen et al. 2017), both reporting MePB representing the predominant paraben accounting for over 85% of ∑PBs, whereas in Spain and India, PrPB occurred at higher concentrations than MePB (Albero et al. 2012, Karthikraj et al. 2017). These differences likely are attributable to different preferences in the formulations of parabens into PPCPs.

Mass Emissions of Antimicrobials in Sewage Sludge

The annual mass of analytes sequestered in sludge per year in China was estimated using here determined concentration in conjunction with sludge production estimates from 2013 (6.25 million metric tons of dry sludge) and further normalized these values to the per-capita level (Table 3-1). Based on the average concentrations detected, the annual mass of all 13 compounds combined sequestered in Chinese sewage sludge is about 68.4 ± 70.6 metric tons per year (t/y),

with 400 t/y representing a worst-case scenario, where maximum measured concentration of 13 chemicals were assumed to present in 6.25 million dry sludge. Mean mass emissions of TCC and TCS were estimated to be 52.8 ± 53.7 and 6.8 ± 6.2 t/y, contributing 77% and 10% to the sum of all 13 compounds, respectively. Zhu *et al.* estimated the total usage of TCC and TCS in the Chinese market at 74 and 179 t/y in 2012 (Zhu et al. 2016), with the mass estimate obtained here accounting for 71% of all TCC and 4% of all TCS used in China annually. Heidler *et al.* previously had reported persistence of 76 ± 30% of TCC and 50 ± 19% of TCS during conventional wastewater treatment in the U.S. (Heidler and Halden 2007, Heidler et al. 2006). Since TCC is mainly used in bar soaps (Halden 2014), most of which would be released into wastewater by washing hands or taking showers, our estimated percentage of TCC retained in sludge was similar to the results from a mass balance study performed in WWTPs. In contrast, TCS is known to be added to a large variety of consumer products, including hand soaps and toothpaste but also other products such as fabric, toys, paints, household products and medical devices; therefore, a much smaller portion of TCS is expected to be discharged into wastewater, potentially accounting for the discrepancy between the low mass of 4% of the total chemical production accounted for in Chinese sewage sludge and the known persistence of TCS during wastewater treatment. In addition to these commonly monitored contaminants, mass emissions of TCC transformation products and metabolites through sludge were estimated for the first time for China. The sum of these transformation products was estimated to not exceed 18.4 t/y, with average concentrations translating into a value of 8.2 t/y. Both values are still higher than the annual inventory of TCS in Chinese sewage sludge, suggesting a necessity to include these compounds in conventional monitoring and risks assessments for sludge disposal.

Considering 80% of the sludge in China was disposed by improper dumping (Yang et al. 2015), annual average mass release of 13 contaminants into environment through improper sludge disposal was estimated to be 54.7 metric tons, with 41.6 tons of TCC and 5.4 tons of TCS, respectively. When sludge is dumped near rivers, contaminants could potentially enter aqueous phase and pose risks to aquatic organisms. Liu *et al*. estimated the predicted no effect concentrations (PNECs) of TCC and TCS in aqueous phase to be 661 and 26 ng/L, respectively (Liu et al. 2015). Assuming all TCC and TCS retained in sludge could enter aqueous phase, 6.4×10^{11} and 2.1×10^{12} cubic meters of water would be needed per year to dilute TCC and TCS concentrations below their PNECs, which were approximately 0.6 and 2 times the amount of flow coming to the sea by way of Yangtze River basin in 2012 (CWRC 2012).

Table 3-1. Estimated mass emission (t/y) and per capita emission (μg/d/capita) of 13 compounds through sludge.

Compound	Mass emission (t/y)			Per capita emission (*μ*g/d/person)		
	Range	Median	Mean	Range	Median	Mean
MePB	0.01-3.93	0.29	0.41	0.02-7.98	0.58	0.84
EtPB	0.01-1.05	0.06	0.08	0.01-2.14	0.12	0.15
PrPB	0.01-0.17	0.05	0.06	0.01-0.35	0.11	0.12
BuPB	0.01-0.07	0.05	0.05	0.01-0.14	0.10	0.10
BePB	0.01-0.08	0.01	0.01	0.01-0.15	0.01	0.02
TCS	0.03-30.5	4.79	6.80	0.05-61.8	9.71	13.8
TCC	0.02-271	36.7	52.8	0.03-550	74.5	107
2'-OH-TCC	0.01-14.6	0.85	1.68	0.01-29.6	1.73	3.40
3'-OH-TCC	0.01-7.80	0.28	0.60	0.01-15.8	0.56	1.22
3'-Cl-TCC	0.14-3.63	0.52	0.70	0.28-7.37	1.05	1.43
DCC	0.01-14.9	1.49	4.47	0.02-303	3.03	9.06
MCC	0.01-0.78	0.19	0.20	0.01-1.58	0.39	0.41
NCC	0.01-8.35	0.19	0.57	0.02-17.0	0.38	1.15
∑PBs	0.06-5.09	0.48	0.60	0.12-10.3	0.97	1.23
∑TCCs	0.20-375	42.8	61.0	0.40-761	86.8	123
∑All	0.35-400	50.6	68.4	0.71-810	103	140

When normalized to the per-capita level, an average of 140 μg of the 13 compounds accumulate in sewage sludge per person per day in China. More specifically, 107 μg of TCC, 13.8 μg of TCS and 1.23 μg of PBs are sequestered in sludge per person per day, results that are similar to those of a recent study estimating 32.8, 12.6 and 0.94 μg of TCC, TCS and PBs being released through excess sludge per day per person in Guangzhou, China (Liu et al. 2017b).

In summary, this is the first China-wide survey of PBs, TCS, TCC, human metabolites and transformation products of TCC in sewage sludge. Contaminant levels detected ranged from the low ng/g to tens of μg/g and in comparison with other nations were of medium strength. Distinct differences in the distribution and concentration of contaminants were noted between sludges produced by plants processing domestic and industrial sewage. Dechlorination and transformation products of TCC were detected in China for the first time and cumulatively by far exceed concentrations of other known contaminants, such as TCS which also was assessed here. Multiple lines of evidence indicate that TCC undergoes dechlorination in the built wastewater environment, as indicated by considerable transformation product concentrations, increased ratios of levels of transformation products to parental TCC. Annual emission of 13 contaminants through sludge were estimated nationwide for the first time, indicating an inventory of about 68 t/y.

4 ASSESSMENT OF HUMAN EXPOSURE TO TRICLOCARBAN, TRICLOSAN AND FIVE PARABENS IN U.S. INDOOR DUST USING DISPERSIVE SOLID PHASE EXTRACTION FOLLOWED BY LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY

Abstract

Triclocarban, triclosan and parabens are antimicrobials widely used in consumer products. Their accumulation in indoor dust has raised concerns because of their endocrine disrupting activities and potential to promote antibiotic resistance in pathogenic bacteria. We used a modified dispersive solid phase extraction (d-SPE) followed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) to measure levels of antimicrobials in U.S. indoor dust. The method showed favorable linearity (R^2 >0.99), recovery (83–115%), and method detection limits (MDLs: 1.2–5.6 ng/g, dry weight). All analytes were found in each of the 80 dust samples collected from athletic facilities and homes, with median concentrations (ng/g) listed in decreasing order: methyl paraben (1920)>propyl paraben (965)>triclosan (390)>triclocarban (270)>ethyl paraben (195)>butyl paraben (80)>benzyl paraben (6). Triclosan levels in athletic facilities were significantly higher than those found in private homes. Estimated daily intake (EDI) of these antimicrobials (median, ng/kg-body weight/d) via dust ingestion was lowest for adults (1.9) and higher for more sensitive subpopulations, including infants (19.8), toddlers (23.6), children (11.8) and teenagers (4.6). This first application of d-SPE approach to determine antimicrobials in dust produced baseline data for triclosan and triclocarban levels in indoor dust prior to the 2017 U.S. ban of their use in antiseptic washes.

Introduction

Triclocarban (3,4,4'-trichlorocarbanilide; TCC) and triclosan (5-chloro-2-(2,4-dichlorophenoxy) phenol; TCS) are antimicrobial agents widely used in various personal care products such as liquid and bar soap, dish detergent, toothpaste, and medical disinfectants at levels of up to 2% and 0.3% (w/w), respectively (Dann and Hontela 2011, Halden and Paull 2005). They are also formulated into carpets, toys, paints and building materials (Halden et al. 2017). Parabens are a group of compounds that have been extensively used as preservatives in lotions, face wash, facial creams, food, beverages, and industrial products due to their broad spectrum of antimicrobial activity, good stability over a wide pH range, and moderate solubility (Bledzka et al. 2014). The most commonly used parabens in commercial products include methyl paraben (MePB), ethyl paraben (EtPB), propyl paraben (PrPB), butyl paraben (BuPB) and benzyl paraben (BePB). Parabens are found in more than 22,000 cosmetic products with levels up to 0.4% (by weight) for any individual paraben and 0.8% in combination. In pharmaceuticals, maximum paraben content may exceed 1% (Haman et al. 2015). Widespread use of antimicrobial chemicals has led to ubiquitous human exposure and environmental occurrence in various diverse environmental matrices, including indoor dust, wastewater influent and effluent, surface water, and sewage sludge (Hartmann et al. 2016b, Li et al. 2015, Pycke et al. 2014b), as well as in biological matrices such as breast milk, serum, urine, cord blood and amniotic fluid (Calafat et al. 2010, Philippat et al. 2013, Pycke et al. 2015, Pycke et al. 2014a, Ye et al. 2008, Ye et al. 2016).

Concerns over the potential risks of the above mentioned antimicrobial agents on human and animal health have been raised in the past decades (Halden et al. 2017, Soni et al. 2005). These compounds represent a group of emerging endocrine disruptors that cause immune dysfunction and affect human reproductive outcomes (Ahn et al. 2008, Boberg et al. 2010, Dann and Hontela

2011, Routledge et al. 1998, Smith et al. 2013). Studies have shown their toxicities to aquatic organisms, such as algae, fish and invertebrates (Brausch and Rand 2011, Chalew and Halden 2009, Madsen et al. 2001, Terasaki et al. 2015, Yamamoto et al. 2011). Potential links have been suggested between human exposure to parabens and the etiology of breast cancer (Charles and Darbre 2013, Darbre et al. 2004). There are also studies showing positive associations between the occurrence of antimicrobials and the detection frequency of antibiotic-resistance genes (Carey et al. 2016, Hartmann et al. 2016b). In September 2016, the U.S. Food and Drug Administration (FDA) issued a final rule banning 19 antimicrobial ingredients including TCS and TCC, in over-the-counter (OTC) consumer antiseptic wash products, and the rule took effect starting from September 2017 (FDA 2016). With inadequate evaluation of the impact of these emerging contaminants on ecosystems and human health, it is necessary to keep monitoring their occurrence in the environment and levels of human exposure. In addition, continuous monitoring of the occurrence of these antimicrobials in the environment will help understand the effectiveness of certain regulatory practices.

In developed countries, people spend over 90% of time in indoor environment, and the quality of the indoor environment has received increasing attention because of its implication for public health (Ma et al. 2014, Mitro et al. 2016). Indoor dust is known to be a sink for semi-volatile organic compounds (SVOC) and particle-bound organic matter and thus has frequently been used as a matrix to assess indoor contamination and human indoor exposure (Butte and Heinzow 2002, Liao et al. 2012, Liu et al. 2017a). Exposure to contaminants in dust can occur via ingestion through direct contact with indoor dust and hand-to-mouth movements, as well as indirect contact as dust deposits on food or consumer products, which are later ingested. Inhalation and dermal absorption are also possible routes of exposure to contaminants deposited in dust. Children are the

most susceptible population to contaminants in indoor dust, due to their rapidly developing organs and neurological system, greater intake of dust relative to body size and weight, and their activities on and in proximity to the floor, which leads to potential elevated contact with contaminants (Mercier et al. 2011).

So far, only a limited number of studies have reported the presence of parabens and TCS in indoor dust (Ao et al. 2017, Canosa et al. 2007a, Canosa et al. 2007b, Fan et al. 2010b, Geens et al. 2009, Rudel et al. 2003, Tran et al. 2016, Wang et al. 2012a), and only one prior study worldwide has quantified TCC in dust (Hartmann et al. 2016a). Due to its complex composition, challenges exist for sensitive and accurate measurement of trace level contaminants in dust. In these studies, sample preparation often involves extraction followed by further cleanup, such as pressurized liquid extraction (PLE) with in-cell cleanup (Canosa et al. 2007a), matrix solid phase extraction (MSPD) (Canosa et al. 2007b), pressurized hot water extraction (PHWE) (Ramírez et al. 2011), solvent extraction by mechanical shaking or sonication followed by solid phase extraction (SPE) (Geens et al. 2009, Hartmann et al. 2016a, Tran et al. 2016, Wang et al. 2012a), and accelerated solvent extraction (ASE) followed by SPE (Ao et al. 2017). Instrument analysis involves liquid chromatography-tandem mass spectrometry (LC-MS/MS) (Geens et al. 2009, Hartmann et al. 2016a, Tran et al. 2016, Wang et al. 2012a), gas chromatography-mass spectrometry (GC-MS) (Fan et al. 2010b, Rudel et al. 2003) and gas chromatography-tandem mass spectrometry (GC-MS/MS) (Ao et al. 2017, Canosa et al. 2007a, Canosa et al. 2007b). Although GC-MS or GC-MS/MS may have advantages on selectivity and sensitivity, they often require a pre-column derivatization step to make certain compounds suitable for GC analysis (Ao et al. 2017), which adds time and labor to an already cumbersome sample pretreatment.

In the past decades, modern sample preparation techniques such as QuEChERS (quick, easy, cheap, effective, rugged and safe) have been developed (Anastassiades et al. 2003) that require less organic solvent and are less time-consuming. The QuEChERS method is based on solvent extraction (normally utilizing acetonitrile) with an addition of salts to induce liquid-liquid partitioning, followed by a dispersive solid-phase extraction (d-SPE) for cleanup. The method was originally developed for extracting pesticides from fruits and vegetables, and later was modified and expanded to target a larger variety of chemicals in different matrices such as liver (Usui et al. 2013), urine and whole blood (Usui et al. 2012), sewage sludge (Peysson and Vulliet 2013), sediment (Berlioz-Barbier et al. 2014), and drinking water treatment sludge (Cerqueira et al. 2014). To the best of our knowledge, this method has not been used for the extraction of chemicals from indoor dust.

The aim of this paper was to: 1) adopt the QuEChERS method for the extraction of antimicrobial compounds from indoor dust; 2) assess the occurrence in different indoor dust environments of seven antimicrobials used widely in personal care products prior to the 2017 U.S. ban on 19 antimicrobials; and 3) establish a benchmark risk assessment for daily intake of antimicrobials from dust ingestion.

Experimental

Chemicals and Reagents

Methylparaben (MePB), triclosan (TCS) and triclocarban (TCC) were purchased from Aldrich (Sigma-Aldrich, St. Louis, MO); ethylparaben (EtPB), propylparaben (PrPB), butylparaben (BuPB), and benzylparaben (BePB) were purchased from RT Corp (Laramie, WY). Isotopically labeled standards $^{13}C_6$-MePB (99%), $^{13}C_6$-TCC (>99%) and $^{13}C_{12}$-TCS (>99%) were

obtained from Cambridge Isotope Laboratories (Andover, MA). d_5-EtPB, d_4-PrPB, and d_4-BuPB were purchased from C/D/N Isotopes (Quebec, Canada). LC-MS-grade (99%) methanol, water, and acetic acid were obtained from Fluka, and LC-MS-grade acetone was obtained from Sigma-Aldrich (St. Louis, MO, USA). Individual stock solutions of the native and isotopically-labeled compounds were prepared in methanol. The working standards were prepared by serial dilution of stock solutions with methanol prior to use. All stock solutions were stored in glass vials with polytetrafluoroethylene septa at –20 °C. Anhydrous magnesium sulfate ($MgSO_4$) was obtained from ACROS (New Jersey, USA) and anhydrous sodium acetate (NaAc) from Dionex (Sunnyvale, CA, USA). 2 mL DiSQuE QuEChERS tubes (150 mg $MgSO_4$, 50 mg PSA, and 50 mg C18) were purchased from Waters Corporation (Milford, MA, USA).

Dust Sample Collection

A total of 53 dust samples from 19 athletic facilities and 27 dust samples from 27 single family detached homes located in Oregon were collected using a vacuum apparatus fitted with Dustream collectors (Indoor biotechnologies, Charlottesville, VA). In the athletic facilities dust was collected separately from each of 3 spaces (typically a workout space, hallway, and office) until at least two collectors had been filled or no further apparent dust was available. For the homes study, dust was vacuumed for five minutes in the primary living space using a single dust collector. Samples were stored in a sterile plastic bag at -20ºC until processing. In some cases (n=10), when two samples were collected in the same house and space (a living or family room) at separate time points, average concentrations are reported. Dust was aliquoted into duplicate or triplicate (each at about 0.1 g) by mixing the collected sample and distributing the desired mass using sterile

forceps and spatulas in a sterile hood. Aliquots were then shipped on dry ice to Arizona State University and stored in -20 ˚C prior to extraction.

Sample Preparation

Approximately 0.1 g of dust was spiked with 30 µL of isotope-labeled standards (100 ng/mL of $^{13}C_6$-MePB, d_5-EtPB, d_4-PrPB, d_4-BuPB, $^{13}C_6$-TCC, and 1 µg/mL of $^{13}C_{12}$-TCS), and then 1 mL of MS-grade water was added. After vortexing for 30 s, 1.5 mL of acetonitrile with 0.1% acetic acid was added, and the slurry was vortexed again for 30 s and put into a sonication bath for 60 min. After sonication, 0.4 g of anhydrous magnesium sulfate and 0.1 g of anhydrous sodium acetate were added. The slurry was then vortexed immediately for 1 min, centrifuged at 4,000 g for 10 min. The upper organic layer was transferred into a 2-mL DiSQuE QuEChERS tube, vortexed for 1 min and centrifuged at 21,130 rpm for 10 min. Finally, the supernatant was transferred into a 4-mL amber vial and stored at -20 ˚C prior to analysis. 100 µL of the final extract was diluted with 100 µL of MS grade water for LC-MS/MS analysis.

Chemical Detection and Quantitation

A Shimadzu 2100 HPLC (Shimadzu Scientific, Kyoto, Japan) coupled with ABsciex API 4000 triple quadrupole mass spectrometer (Applied Biosystems, Framingham, MA) with electrospray ionization (ESI) was used for chemical analysis. Analytes were separated on a Waters X-Bridge C_8 column (4.6 × 150 mm, 3.5 µm particle size) preceded by an equivalent guard column using a gradient LC protocol. The injection volume was 10 µL. Methanol was used as mobile phase A and water as mobile phase B. The gradient program started at 60% A and then ramped up to 95% A over 4 min, held at 95% for 6 min, followed by dropping back to 60% A within 1 min,

and maintained at 60% A for 2 min. The ESI was operated under negative mode and the source parameters were set as follows: curtain gas = 172 kPa, gas 1 = 483 kPa, gas 2 = 345 kPa, IS = -4500 eV, source temperature = 500 °C, entrance potential (EP) = -10 eV, and collision activated dissociation (CAD) gas = 83 kPa. LC and MS/MS parameters for target analytes and labeled standards were included in Supplementary Information (SI, Table S4-1).

Quality Assurance/Control

Analytes and labeled standards were identified using their specific retention time and multiple reaction monitoring transitions as reported earlier (Chen et al. 2017, Hartmann et al. 2016a). As detection of background levels of these analytes is a known issue resulting from the ubiquity of these compounds (Pycke et al. 2015, Wang and Kannan 2016), all extractions were performed along with method blanks (*i.e.*, procedural controls), and a mixture of analytical grade acetonitrile containing 0.1% acetic acid and water (50/50, v/v) was injected once per 10 samples as a check to reveal potential carryover of target analytes from sample to sample. None of the analytes were detected in solvent blanks or method blanks.

Method detection limits (MDLs) were determined following the United States Geological Survey (USGS) and United States Environmental Protection Agency (USEPA) guidelines, and LOQ were determined according to the USEPA guideline as described previously (Halden et al. 2001).

Data Analysis

LC-MS/MS data were acquired with Analyst 1.5 software (Applied Biosystems, Foster City, CA). Concentrations of analytes in dust were obtained using the isotope-dilution method and

reported as ng/g. Concentrations were reported when the analyte peak height to background signal had a signal-to-noise ratio of >3, the sample peak areas fell within the dynamic range of the calibration, and calculated concentrations were above the MDLs.

Statistical analyses were performed using Microsoft Excel 2007 and R (version 3.2.2). Correlations among the concentration of individual parabens and the total parabens were analyzed using a non-parametric Spearman's rank correlation test, and the correlation coefficients were expressed using Spearman's *rho*.

Estimated daily intake (EDI, ng/kg) of ∑PBs (total parabens), TCS and TCC through dust ingestion were calculated using the following equation:

$$EDI_i = \frac{C_i * IR}{BW}$$

Where IR stands for the daily dust ingestion rate (g/d), C_i stands for the measured concentration of a specific analyte (ng/g) in dust, and BW is average body weight (kg). Based on the USEPA exposure factors handbook (2011) (EPA 2011), daily dust ingestion rate for infants, toddlers, children, teenagers and adults were 30, 60, 60, 60, and 30 mg, respectively, with average body weight for each group at 7.5, 12.6, 25.2, 64.2 and 80 kg, respectively.

Results and Discussions

Method Performance

In this study, the versatile QuEChERS method (Anastassiades et al. 2003) was modified and applied to the analysis of parabens, TCS and TCC in indoor dust followed by compound identification and quantification by LC-MS/MS. Compared with previous studies (SI, Table S4-2) using SPE, MSPD, PLE/ASE or PHWE (Ao et al. 2017, Canosa et al. 2007a, Canosa et al. 2007b,

Geens et al. 2009, Hartmann et al. 2016a, Ramírez et al. 2011, Tran et al. 2016, Wang et al. 2012a), which uses about 15 – 40 mL of organic solvents, this method only required 1.5 mL of acetonitrile with 0.1% acetic acid, without further drying, concentrating or solvent changing, and thus was more cost-effective, environmentally friendly and less labor intensive. The method performance parameters are present in Table 4-1. Isotope-corrected recoveries at two spiking levels varied from 83 – 115%, and precisions, expressed as relative standard deviations (RSDs) from triplicate analyses at two spiking levels, ranged from 3 – 30%. MDLs varied from 1.5 – 3.7 ng/g, which were comparable to the MDLs or LOQs reported in previous studies (0.1 to 10 ng/g).

These performance characteristics were in the range of those of other studies that reported average RSDs of < 18% (Fan et al. 2010b) and average percentage differences of < 20% (Rudel et al. 2003) using sieved dust samples analyzed in duplicate only. It is difficult to judge whether these modest differences in reproducibility observed were caused by the type of dust analyzed, the method used for extraction, or the pretreatment of samples performed. Some studies employed pre-fractionation of dust by sieving (Ramírez et al. 2011, Tran et al. 2016, Wang et al. 2012a) (SI, Table S4-2), noting that organic contaminant concentrations in indoor dust can increase with decreasing particle size (Butte and Heinzow 2002). However, at present there is no consensus or prescribed standard methodology on whether to fractionate dust prior to analysis and what particle cutoff size to use. Overall, application of d-SPE resulted in a fast, robust and efficient method for dust analysis with other performance characteristics similar to those of previously used methods.

Although previous studies have suggested that the majority of organic contaminants' concentrations in indoor dust increase with decreasing particle size (Butte and Heinzow 2002), and analytical results may vary significantly among different sieve sizes, there is no consensus on which particle size should be selected for analysis. It is unclear which particle size is representative

of the total dust sample and what relationships exist between the concentrations of selected contaminants and particle size. Therefore, selection of the dust fraction for analysis should depend on the aims of the study (characterization of the source, assessment of contamination or exposure) (Mercier et al. 2011). We chose not to sieve our dust samples because the aims of this study were to demonstrate the applicability of d-SPE approach for the determination of antimicrobials in indoor dust and to assess the occurrence of antimicrobials in U.S. indoor dust.

Table 4-1. Recovery, precision, method detection limit (MDL) and limit of quantification (LOQ) for individual analyte.

Compounds	Recovery (%)[a]		Precision (RSD, %)[a]		MDL (ng/g)	LOQ (ng/g)
	300 ng/g	600 ng/g	300 ng/g	600 ng/g		
MePB	109	105	7	4	2.3	7.9
EtPB	108	103	9	5	1.7	5.8
PrPB	101	104	16	13	1.6	5.6
BuPB	93	95	3	7	1.5	5.3
BePB	83	100	9	7	1.6	5.4
TCS	107	91	6	14	3.7	17.5
TCC	88	115	9	30	2.6	8.6

[a] Number of replicates (n = 3)

Occurrence of Parabens, TCS and TCC in Indoor Dust

A total of 80 dust samples were analyzed in this study, and 100% detection frequency was obtained for each analyte, indicating widespread occurrence of these antimicrobials in indoor environments. Concentrations of individual compounds (median, range; ng/g) are listed in decreasing order (also shown in Figure 4-1, detailed summary statistics in SI Table S4-3): MePB (1920, 50 – 26200) > PrPB (960, 70 – 11150) > TCS (390, 20 – 3270) > TCC (270, 20 – 9760) >

EtPB (195, 9 – 1060) > BuPB (80, 6 – 860) > BePB (6, 2 – 27). The sum of five parabens (∑PBs) ranged from 140 to 39090 ng/g, with a median at 3490 ng/g.

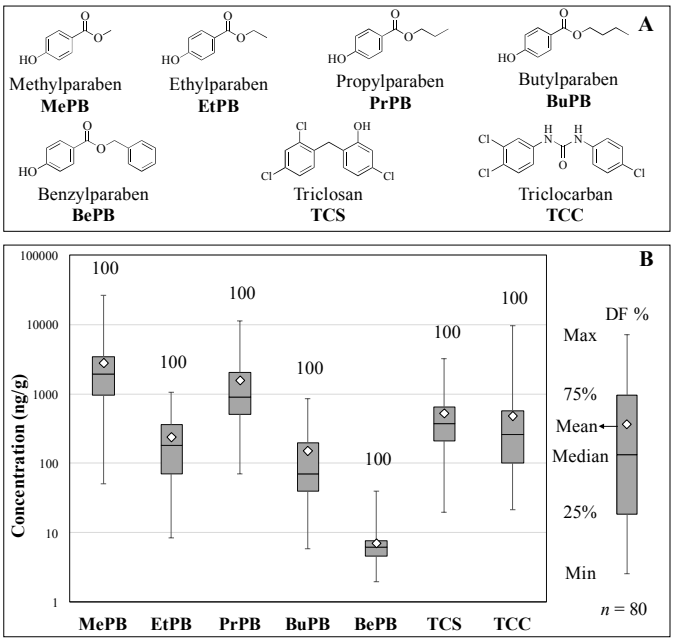

Figure 4-1. A Chemical structures, names and abbreviations of target antimicrobials; **B** Box-and-whisker plot of individual antimicrobial concentrations found in 80 indoor dust samples. DF: detection frequency.

Median paraben concentrations found in this study were similar with the levels found in South Korea, Japan, Canada and the U.S. (Table 4-2), but higher than those in Vietnam, China, Spain and Belgium. As a major source of parabens in indoor environment is from the use of cosmetics and personal care products, Wang *et al*. indicated that significantly lower levels of parabens in indoor dust from China may be related to lower per capita consumption of cosmetics and personal care products than in Japan, Korea and the U.S. (Wang et al. 2012a). Guo *et al.* had analyzed paraben concentrations in 52 personal care products (PCPs) from Tianjing, China (Guo et al. 2014), and 170 PCPs from Albany, NY, U.S. (Guo and Kannan 2013), showing that levels of parabens (SI, Table S4-4) in Chinese PCPs were similar to those from the U. S., supporting the

previous hypothesis that the differences of parabens in dust observed between China and U.S. were likely related to different usage patterns and amounts of PCPs, given similar product formulations. Indoor abundances of antimicrobial compounds may also be influenced by different rates of use of building materials that incorporate the compounds, along with construction and operation practices that would affect removal by ventilation or cleaning.

Consistent with all the other studies, MePB and PrPB were the most abundant parabens found in indoor dust, with average contributions to ∑PBs of 58% and 32%, respectively. Furthermore, individual paraben concentrations were positively correlated (Spearman's *rho* = 0.22 – 0.89, SI, Table S4-5), particularly for MePB and PrPB (Spearman's *rho* = 0.89, $p < 0.01$), indicating similar sources of parabens in the dust, which could be explained by the fact that parabens are often used in combination to improve antimicrobial activities (Guo et al. 2014). Similar positive correlations have been observed in various matrices such as urine, blood, sewage sludge, and sediment (Chen et al. 2017, Liao and Kannan 2013, Pycke et al. 2014b).

TCS levels found in this study also were similar to those reported elsewhere. Together with a previous study of only a single athletic facility in Oregon, this report constitutes the first simultaneous monitoring of TCS and TCC in indoor dust from the U.S. and it was conducted just prior to implementation of the recent Federal ban on use of 19 antimicrobials (including TCS and TCC) in antiseptic hand washes (FDA 2016).

Levels of Antimicrobials as a Function of Sampling Location

The 80 dust samples analyzed in this study originated from private homes ($n = 27$) and athletic facilities ($n = 53$). A statistical comparison (two-tailed t-test, unequal variance) of the two data sets obtained for the various analytes indicated no statistical differences ($p > 0.05$) in levels

of parabens and TCC between these two monitoring locations, but TCS levels in private homes (Mean ± SD: 311 ± 239 ng/g) were significantly lower (p <0.05) than those found in athletic facilities (684 ± 585 ng/g), which could be due to less frequent use of antiseptic wash products containing TCS in private homes than the use in athletic facilities.

Table 4-2. Comparison of median concentrations (ng/g) found in this study with the ones from other studies.

Country	Sample Size	Median concentration (ng/g) MePB	EtPB	PrPB	BuPB	BePB	TCS	Ref
Belgium	20	\	\	\	\	\	220	Geens *et al.* (2009)
	6	912[a]	276[a]	425[a]	212[a]	\	\	Ramírez *et al.* (2011)
Spain	10	455	58	415	43	\	525	Canosa *et al.* (2007a)
	10	451	135	226	106	\	880	Canosa *et al.* (2007b)
Canada	63	1080	25	463	59	<8	378	Fan *et al.* (2010)
Vietnam	41	58.2	12.7	15	14.9	0.92	\	Tran *et al.* (2016)
China	110	\	\	\	\	\	260	Ao *et al.* (2017)
	55	320	11	182	2	0.8	\	
South Korea	41	1310	46	800	40	1.85	\	Wang *et al.* (2012)
Japan	22	1470	127	228	45	\	\	
	40	760	33	706	24	0.7	\	
U.S.	118	978	<200	\	<200	\	\	Rudal *et al.* (2003)
	23	1020	60	380	60	<MDL	200	Hartmann *et al.* (2016)
	80	1920	180	965	80	6	390	This study

[a] Mean concentration; \: Analyte not included in the study.

Estimated Daily Intake from Dust Ingestion and Comparison with Other Exposure Routes

Estimated daily intake of total parabens, TCS and TCC were calculated with the same approach used in previous studies (Tran et al. 2016, Wang et al. 2012a). A summary of maximum, median and mean EDIs are listed in Table 4-3. Median EDIs (ng/kg-bw/d) varied from 1.3 (adults) to 16.6 (toddlers), 0.1-1.9, and 0.1-1.3 for total parabens, TCS and TCC, respectively. Generally, infants and toddlers had about 10-fold higher EDIs than the ones for adults.

Table 4-3. Estimated daily intake (EDI, ng/kg-bw/d) of ∑PBs, TCS and TCC via dust ingestion for different age groups.

EDI (ng/kg-bw/d)	Parabens			TCS			TCC		
	Max	Median	Mean	Max	Median	Mean	Max	Median	Mean
Infants	156.4	14.0	19.3	13.1	1.6	2.2	39.0	1.1	2.0
Toddlers	186.1	16.6	23.0	15.6	1.9	2.7	46.5	1.3	2.4
Children	93.1	8.3	11.5	7.8	0.9	1.3	23.2	0.6	1.2
Teenagers	36.5	3.3	4.5	3.1	0.4	0.5	9.1	0.3	0.5
Adults	14.7	1.3	1.8	1.2	0.1	0.2	3.7	0.1	0.2

Exposure to parabens through dust ingestion calculated in this study (median EDI, ng/kg-bw/d) were slightly higher than those reported for Koreans (1.11-5.42) and Japanese (1.18-5.38), and much higher than those for Chinese (0.2-0.98) (Wang et al. 2012a) and Vietnamese (0.11-0.53) (Tran et al. 2016). Two studies have estimated daily intake of TCS from dust ingestion for Belgians (Geens et al. 2009) and Chinese (Ao et al. 2017); EDIs for TCS were similar between this study and the Chinese study, but slightly higher than the Belgian one. No other studies to date have estimated TCC intakes from dust ingestion.

Intakes of these antimicrobials through other exposure routes have been reported earlier (Figure 4-2) (Asimakopoulos et al. 2016, Guo and Kannan 2013, Liao and Kannan 2014, Liao et

al. 2013b, Rodricks et al. 2010). Liao *et al.* have estimated mean daily dietary intakes of parabens for U.S. children and adults to be 470 and 307 ng/kg-bw/d, respectively, which were 40- and 170-times higher than the mean intakes of parabens estimated for children and adults in this study. Intakes of total parabens for children and adults from biomonitoring data were estimated to be 60300 and 53800 ng/kg-bw/d, respectively (Liao et al. 2013b). Intakes from dust ingestion calculated in this study would thus contribute <0.15% and <0.03% to total paraben exposure for children and adults, respectively, indicating dust ingestion is a minor route of paraben exposure for U.S. children and adults. While total exposure of parabens for infants and toddlers were not available, use of PCPs has been considered as the major route to parabens exposure. Median dermal intakes of six parabens for U.S. infants and toddlers from use of PCPs have been estimated to be 200 and 120 ng/kg-bw/d, (Guo and Kannan 2013), median paraben intakes from dust ingestion account for 7% and 14% of the exposure from PCPs for infants and toddlers, respectively. Thus, dust ingestion may contribute more to the intake of total parabens for infants and toddlers than it does for children and adults.

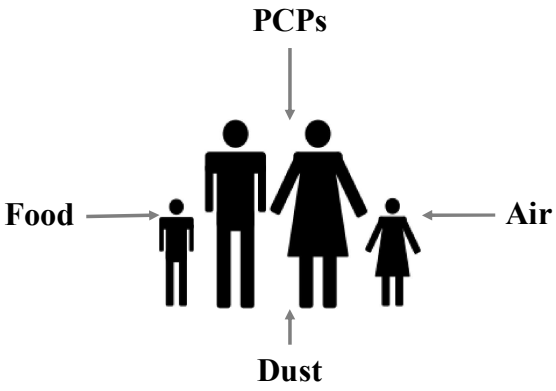

Figure 4-2. Routes of human exposure to target antimicrobials.

Rodricks *et al.* had estimated TCS daily intake based on median urinary concentrations of TCS reported in the NHANES 2003-2004 survey, to be 200 and 100 ng/kg-bw/d for adults and children, respectively (Rodricks et al. 2010), whereas median intakes of TCS through dust ingestion in this study were 0.1 and 0.9 ng/kg-bw/d for adults and children, respectively, contributing less than 0.1% towards total exposure to TCS. It is anticipated that children under 6 would use fewer products containing TCS than children and adults, therefore similar to paraben exposure, dust ingestion may contribute more to total TCS intake for infants and toddlers than for children and adults.

Migration of parabens, TCS and TCC from baby teethers collected from the U.S. market has been reported earlier (Asimakopoulos et al. 2016). Median daily intakes of six parabens were 0.59 and 0.63 ng/kg-bw/d for male and female infants, respectively, which were over 20-times lower than the intakes from dust ingestion. Median intakes for TCS and TCC from teethers were 0.004 ng/kg-bw/d, about three orders of magnitude lower than the intake from dust ingestion.

Although use of TCS and TCC in hand washes and soaps will be banned in 2017 in the U.S., they can still be used in other PCPs (e.g. toothpaste, body lotion and deodorant), building materials, household products and textiles. The system exposure doses (SED) of TCS from toothpaste, hand soap and body soap were calculated as 23.4, 6.6 and 26.8 μg/kg-bw/d (SCCP 2009) for adults, accounting for 40%, 11% and 46% of total intakes from common-use PCPs, respectively. After the ban, the use of TCS-containing toothpaste may constitute the bulk of total TCS exposure. Similarly, SEDs for dermal exposure to bar soap, liquid soap and body wash containing TCC for a 60-kg adult were 7.4, 19.5 and 5.2 μg/kg-bw/d, respectively, and total aggregate exposure was 32.1 μg/kg-bw/d (SCCP 2005). After the ban, use of other consumer products containing TCC may contribute more to total TCC intake. The data presented in this study

can serve as a baseline of pre-ban antimicrobial concentrations in U.S. dust and, as such, may help to discern the impact of the 2017 U.S. restrictions on antimicrobial use in consumer products on inhalation hazards from indoor dust in public and private spaces.

Limitations

Differences of antimicrobials levels observed among different studies could be the result of different usage patterns and volumes of consumer products containing these compounds, but it could also be influenced by different sampling methods, sieved fractions, sample preparation techniques and analytical methods. For future studies aimed at comparing antimicrobial levels in indoor dust before and after the 2017 FDA ban on antimicrobials in antiseptic washes, similar sample treatments and analytical approaches should be taken to deliver the most comparable results. For the assessment of human exposure to contaminants via dust ingestion, selection of different dust fractions may hinder a comparison of results from different studies. Current studies are limited by the lack of data on the daily exposure to different size fractions of dust, the distribution of contaminant concentrations with different particle sizes, and the bioavailability of contaminants as a function of particle size. More studies are needed to tackle these issues to better assess human exposure to organic contaminants via dust ingestion.

5 MONITORING ALCOHOL AND NICOTINE CONSUMPTION TRENDS IN THREE U.S. COMMUNITIES USING WASTEWATER-BASED EPIDEMIOLOGY

Abstract

Wastewater-based epidemiology (WBE) is an emerging public health tool for monitoring substance abuse in near real-time but applications to U.S. populations are lacking. In this longitudinal study, raw sewage was collected monthly from three U.S. cities as 24-h weekday composites and analyzed for evidence of alcohol and tobacco consumption. Over the 11-month sampling period, biomarkers of substance use were detected by liquid chromatography-tandem mass spectrometry in units of μg/L, including ethyl sulfate (1.6-25.1), nicotine (0.6-26.7), cotinine (0.2-3.8) and 3-hydroxycotinine (0.3-3.8). Average stimulant consumption rates in the three communities were calculated using detected biomarker levels in conjunction with wastewater flow rates, metabolic excretion factors, and population size data. For ages 15+ years, computed average per-capita consumption rates of alcohol (13.4 ± 5.6 L/y) and daily consumption of nicotine by smokers (14.2 ± 3.6 cigarettes/d) were in good agreement with U.S. survey data (9.0 L/y/person of alcohol; 14.2 cigarettes/d per adult smoker). The WBE approach also captured impacts of transient populations on substance consumption patterns. This first WBE study to track U.S. recreational use of stimulants longitudinally and concurrently in multiple U.S. cities highlights opportunities for collecting public health information from wastewater anonymously, economically and in near real-time.

Introduction

Community wastewater has long been known to contain indicators of public health, including toxic chemicals, pathogenic microorganisms, and drugs of abuse. The science of extracting and interpreting public health indicators in raw sewage of communities, towns and cities is still evolving, however. European nations have led the way in implementing multi-city monitoring studies to investigate substance use and abuse (Boogaerts et al. 2016, Gatidou et al. 2016, Gonzalez-Marino et al. 2016). The approach used, typically referred to as wastewater-based epidemiology (WBE) or sewage epidemiology, relies on pooled urine and stool samples from large populations, collected as raw wastewater most frequently at the intake of large, municipal wastewater treatment plants (WWTPs) serving the study population. The general approach is to measure levels of parent compounds or metabolites in sewage (wastewater influent), and to then obtain daily mass load (g/d) of the target compounds by combining concentrations with flow rate of wastewater. Daily mass loads can then be used to estimate consumption of a certain substance when considering human metabolism and excretion. Normalization of data to the population count in the sewershed examined then enables the computation of substance use or abuse per day per 1000 inhabitants (van Nuijs et al. 2011a). This approach has been applied for monitoring the use of alcohol (Andres-Costa et al. 2016, Boogaerts et al. 2016, Mastroianni et al. 2014, Rodriguez-Alvarez et al. 2014a, Ryu et al. 2016), tobacco (Castiglioni et al. 2015, Mackul'ak et al. 2015, Rodriguez-Alvarez et al. 2014b, Wang et al. 2016, Zheng et al. 2017) and a number of stimulants, pharmaceuticals and illicit drugs (Bijlsma et al. 2014, Burgard et al. 2013, Castiglioni et al. 2011, Li et al. 2014, Panawennage et al. 2011, van Nuijs et al. 2011b). Recent European studies have demonstrated that the data collected can be in good or excellent agreement with information collected by traditional, orthogonal means (Boogaerts et al. 2016, Gonzalez-Marino et al. 2016).

Studies are motivated by the potential of WBE to characterize behaviors, health status and activities in large populations quickly, objectively and cost-effectively (Castiglioni et al. 2014, Daughton 2018).

According to World Health Organization (WHO), each year, about 3.3 million people die from the harmful use of alcohol and almost 6 million people die from tobacco use (including direct use and second-hand smoking) (WHO 2014b). Alcohol and tobacco use also are known to cause social and economic issues and to adversely impact the quality of life in communities. Public health interventions aimed at reducing the prevalence of drinking and smoking have to be customized to the population targeted and require ongoing surveillance of the effectiveness of implemented interventions as a feedback tool (World Health Organization 2017). Conventional surveillance methods include conducting census, administering surveys and questionnaires, and extrapolating data from sales statistics (WHO 2014a). However, these approaches are often time-delayed, time-consuming, costly and also can yield information that is biased due to self-reporting and, in the case of alcohol and tobacco products for example, stockpiling (Mastroianni et al. 2014, Smith et al. 1990).

Ethyl sulfate (EtS), a minor phase-II metabolite of alcohol (Høiseth et al. 2008), has been measured in wastewater as a biomarker of alcohol consumption to estimate population alcohol consumption. Another alcohol metabolite, ethyl glucuronide (EtG), also has been evaluated but was found to be of lesser informational value because of its instability in sewage (Reid et al. 2011). Daily alcohol consumption per capita has been reported to vary from 6.4 to 44.3 mL/d/person in 20 European, Australian and Canadian communities, with a population-weighted average at 20 mL/d/capita. Similar levels have been reported previously in Norway (Reid et al. 2011), Belgium (Boogaerts et al. 2016), Spain (Mastroianni et al. 2014) and Greek (Gatidou et al. 2016). These

local, national and international studies have shown the ability of the WBE approach to capture spatial and temporal variations of alcohol consumption.

Two metabolites of nicotine (NIC), cotinine (COT) and 3-hydroxycotinine (3-OH-COT) (Hukkanen et al. 2005), have been measured in wastewater to estimate tobacco consumption in Italy (Castiglioni et al. 2015), Spain (Rodriguez-Alvarez et al. 2014b), and China (Wang et al. 2016), Czech Republic and Slovakia (Mackul'ak et al. 2015). Generally, results obtained using this approach matched those from prevalence data.

While most of previous WBE studies were conducted in European countries, to the best of our knowledge, no multi community studies have been performed in the United States to assess the use of alcohol and tobacco using this approach. The objectives for this study were to (i) conduct the first study using WBE to monitor alcohol and tobacco use in three distinct communities in the U. S.; (ii) track the trends of alcohol and tobacco consumption over an one year period; (iii) and then to compare the back-calculated results from WBE with national survey data from WHO (WHO 2014a), Center for Disease Control and Prevention (CDC) (Center for Disease Control and Prevention 2015), National Institute of Alcohol Abuse and Alcoholism (NIAAA) (Haughwout and Slater 2017), and Euromonitor (Euromonitor International 2014).

Experimental

Materials and Reagents

Ethyl sulfate (EtS) and sodium ethyl sulfate-d_5 (EtS-d_5) were purchased from Toronto Research Chemicals (Toronto, Canada). Nicotine (NIC), cotinine (COT), 3-hydroxycotinine (3-OH-COT) and cotinine-d_3 (COT-d_3) and acetone were purchased from Sigma-Aldrich (St. Louis, MO, USA). LC-MS (liquid chromatography-mass spectrometry) grade water and methanol were

obtained from Fisher Chemical (Houston, TX, USA), formic acid was obtained from Fluka. Stock solutions of EtS and EtS-d_5 were prepared in water, while NIC, COT, 3-OH-COT and COT-d_3 were prepared in methanol. Working solutions were prepared by serial dilution of stock solutions with the corresponding solvent. EtS and EtS-d_5 solutions were stored at 4 ˚C. NIC, COT, 3-OH-COT and COT-d_3 solutions were stored at -20 ˚C. All glassware was washed with detergent, rinsed with LC-MS grade water and heated at 550 ˚C for 4 h prior to use. Chemical structures and selected physiochemical properties of target analytes were shown in Supplementary Information, SI Table S5-1.

Sewage Samples

Time-averaged 24-h composites of raw wastewater were collected on a random weekday every month for 11 months (March 2015 to March 2016) at three WWTPs serving three communities located at different states, representing a combined population of 222,000 (see characteristics of each WWTP in SI, Table S5-2). The samples were collected using automatic samplers, placed in polyethylene bottles, and sent to Arizona State University (Tempe, AZ, U.S.) overnight on dry ice. Upon receipt, samples were stored at –80 ˚C, thawed at room temperature, and processed by extraction as described in the following.

Sample Preparation

For EtS, 5 mL of raw wastewater was centrifuged (Eppendorf Centrifuge 5810 R, Hamburg, Germany) at 4,000 *g* for 10 min, and then 500 µL of the supernatant was taken, fortified with 10 µL of 100 ppb EtS-d_5 and diluted with 490 µL of DI water to arrive at a 1 mL sample extract for analysis.

For NIC, COT and 3-OH-COT analysis, 200 mL of raw wastewater was spiked with 50 ng of COT-d_3, and then passed through an Oasis HLB cartridge (150 mg, 6 cc, Waters, Milford, MA, USA) that was previously conditioned with 5 mL of methanol and equilibrated with 5 mL of water. After loading, the cartridge was rinsed with 5 mL of water, dried under nitrogen for 10 min, and target analytes were then eluted with 4 mL of methanol/acetone (50/50, v/v) containing 0.5% formic acid. Finally, 200 µL of eluate was taken, dried under a gentle flow of nitrogen at 40˚C and reconstituted in 200 µL of methanol/water (50/50, v/v) for LC-MS/MS analysis. Each sample was prepared in duplicate.

High-performance Liquid Chromatography Tandem Mass Spectrometry **(HPLC-MS/MS)**

A Shimadzu 2100 HPLC (Shimadzu Scientific, Kyoto, Japan) coupled with ABsciex API 4000 triple quadrupole mass spectrometer (Applied Biosystems, Framingham, MA) with electrospray ionization (ESI) was used for the chemical analysis. Analytes and their corresponding isotope-labeled standards were identified using compound specific retention times and ion transitions from multiple reaction monitoring in the MS/MS (SI, Table S5-3).

For EtS, a Waters Symmetry C_{18} column (4.6 × 150 mm, 3.5 µm particle size) was used in conjunction with an equivalent guard column and a gradient LC protocol. The injection volume was 10 µL. Methanol was used as mobile phase A and water with 0.2% formic acid as mobile phase B. The flow rate was 0.4 mL/min and the gradient program started at 10% A for 1 min, ramped up to 95% A in 3 min, and held at 95% for 3 min, followed by dropping back to 10% A within 1 min, and maintained at 10% A for 3 min. The ESI was operated under negative mode and the source parameters were set as follows: curtain gas = 10 psi, gas 1 = 40 psi, gas 2 = 25 psi, IS = −3500 eV, source temperature = 500 °C, and collision induced dissociation (CID) gas = 12 psi.

For NIC, COT and 3-OH-COT, a Waters XBridge C_8 column (4.6 × 150 mm, 3.5 μm particle size) preceded by an equivalent guard column was employed in gradient LC. The injection volume was 10 μL. Methanol was used as mobile phase A and water as mobile phase B. Flow rate was 0.5 mL/min and the gradient program started at 40% A for 0.5 min, ramped up to 80% A in 1.5 min, and then slowly ramped up to 85% A in 4 min, finally reached 98% A in another 4 min, and then dropped back to 40% A within 1 min, and finally maintained at 40% A for 3 min. The ESI was operated in positive mode with source parameters set as follows: curtain gas = 10 psi, gas 1 = 80 psi, gas 2 = 80 psi, IS = 4500 eV, source temperature = 500°C, and collision induced dissociation (CID) gas = 10 psi.

Quality Assurance/Quality Control

All reported concentrations were determined based on standard curves containing between 5 and 8 data points at concentrations ranging from 0.01 to 50 ng/mL, featuring minimum coefficients of determination r^2 of ≥0.99. Average recoveries were determined based on matrix spike-recovery experiments and ranged from 73% to 109% for all the analytes. All extractions were performed along with method blanks and a pure methanol/water mixture (50/50, v/v) was injected once per 10 samples as a check for carryover of target chemicals from sample to sample. None of the analytes were found to be present in solvent blanks or method blanks, indicating no contamination during sample preparation and absence of carryover between injections.

Method detection limits (MDLs) and limits of quantification (LOQs) were determined following the U. S. Geological Survey (USGS) (Childress et al. 1999) and U. S. Environmental Protection Agency (USPEA) (EPA. 1984) guidelines. MDLs were determined as 73, 21, 2 and 6 ng/L for EtS, NIC, COT and 3-OH-COT, respectively. Every sample was prepared in duplicate,

relative percentage difference (RPD) for duplicate samples were calculated using the following equation:

$$RPD(\%) = \frac{|C_{sample} - C_{duplicate}|}{\frac{C_{sample} + C_{duplicate}}{2}} \times 100 \qquad Eq.\ 1$$

Where C_{sample} and $C_{duplicate}$ are the concentrations detected in the original sample and in its duplicate, respectively. Average RPDs for all the analytes were within 30%, indicating good repeatability of the sample preparation and analytical methods.

Back-calculations and Data Analysis

Per capita daily alcohol consumption (Q, mL/d/capita) was calculated using *Eq. 2*:

$$Q = \frac{C_{EtS} \times 10^{-6} \times F \times Cf_{EtS}}{Pop \times \rho_{EtOH}} \qquad Eq.\ 2$$

Where C_{EtS} is the concentration of EtS measured in wastewater (µg/L); F is the flow rate (L/d) on the day of sampling; *Pop* is the total population served by each WWTP (SI, Table S5-2), ρ_{EtOH} is the density of alcohol (0.789 g/mL), and Cf_{EtS} is a correction factor calculated using *Eq. 3*.

$$Cf_{EtS} = \frac{M_{EtOH}}{M_{EtS} \times \chi_{EtS}} \qquad Eq.\ 3$$

Where M_{EtOH} and M_{EtS} are the molecular weights of alcohol (EtOH) and EtS, respectively, and χ_{EtS} is the mean ratio (0.012%) of alcohol excreted as EtS which has been used in several previous studies (Boogaerts et al. 2016, Rodríguez-Álvarez et al. 2015, Ryu et al. 2016). An illustration of metabolism pathway and excretion file of alcohol to EtS was shown on Figure 5-1A.

To facilitate comparison of our results with survey reporting alcohol consumption for population over 15 years old (15+), we further calculated alcohol consumption for 15+ population (Q_{15}, mL/d/capita) using *Eq. 4*:

$$Q_{15} = \frac{C_{EtS} \times 10^{-6} \times F \times Cf_{EtS}}{Pop \times R_{15} \times \rho_{EtOH}} = \frac{Q}{R_{15}} \qquad Eq.\ 4$$

Where R_{15} is the average percentage of U.S. population over 15 years old (assumed to be 80%) (WHO 2014a).

As only weekday samples were collected in this study, and several other studies have suggested alcohol consumption on weekends were higher than those on weekdays (Boogaerts et al. 2016, Rodriguez-Alvarez et al. 2014a, Ryu et al. 2016, van Wel et al. 2016). By analyzing data from these studies (Table S5-4), a correction factor of 1.18 was used (*Eq. 5*).

$$Q_{15c} = Q_{15} \times 1.18 \qquad Eq.\ 5$$

Nicotine consumption was calculated following the approach detailed by Castiglioni *et al*. (Castiglioni et al. 2015), assuming an average of 74% (weight-basis) of nicotine was excreted as COT and 3-OH-COT, and a correction factor of 1.35 (1/0.74) was used for back-calculation. Absorbed nicotine (NIC_{abs}, mg/d/capita) was calculated using *Eq. 6*:

$$NIC_{abs} = \frac{(C_{COT} + C_{3\text{-}OH\text{-}COT}) \times 10^{-3} \times F \times Cf}{Pop} \qquad Eq.\ 6$$

Where C_{COT} and $C_{3\text{-}OH\text{-}COT}$ represent measured concentrations (μg/L) of COT and 3-OH-COT in wastewater influent and *Cf* is the correction factor (1.35).

To compare our results with data from other sources, numbers of cigarettes smoked per day by the population aged 15+ (n_{15+}) and by adult smokers (18+) were calculated using *Eq.7* and *Eq. 8*, respectively:

$$n_{15+} = \frac{NIC_{abs}}{D \times R_{15}} \qquad Eq.\ 7$$

$$n_{smoker} = \frac{NIC_{abs}}{D \times R_{18} \times \chi_{smoker}} \qquad Eq.\ 8$$

Where D is the content of NIC per cigarette smoked (1.25 mg) (Castiglioni et al. 2015), R_{18} is the percentage of U.S. population over 18 years old (77.1% in 2015 from U.S. census), and χ_{smoker} is the state-specific prevalence of current smoking among adults (18+) in 2015 obtained from State tobacco activities tracking and evaluation (STATE) system, Center of Disease Control and Prevention (CDC) (CDC 2017).

For comparison between results from this study and results from surveys, RPDs were calculated using *Eq. 1*. Statistical analyses were performed with the software R (version 3.2.2). To investigate if there were any differences in per-capita daily alcohol and nicotine consumption, or in the number of cigarettes smoked by smokers among three communities, one-way ANOVA or Kruskal-Wallis test were used, depending on whether the data were normally distributed and featured equal variances, followed by Tukey's HSD (honest significant difference) or Nemenyi test as post-hoc tests. Pearson's correlation was performed to determine if there was a correlation between alcohol and nicotine consumption at each community. The limit for significance in all cases was set at $p < 0.05$.

Results and Discussion

Occurrence of EtS, NIC, COT and 3-OH-COT in Community Wastewater

All four target analytes showed 100% detection in all samples analyzed (n = 33) over 11 months in monthly sampling campaigns of community wastewater using a 24-h composites obtained on a random workday in three U.S. communities (SI Table S5-5). Concentrations detected in units of μg/L varied by about one order of magnitude for EtS (1.6–25.1), NIC (0.6–26.7), COT (0.2–3.8), and 3-OH-COT (0.3–3.8) and were similar to those reported in prior studies from other countries worldwide (Table 5-1), where EtS, NIC, COT, and 3-OH-COT were found in the range

of <1–70.7 µg/L (Andrés-Costa et al. 2017, Boogaerts et al. 2016, Gatidou et al. 2016, Mastroianni et al. 2014, Reid et al. 2011, Rodriguez-Alvarez et al. 2014a, Ryu et al. 2016), (Mackul'ak et al. 2015, Rodriguez-Alvarez et al. 2014b, Wang et al. 2016, Zheng et al. 2017).

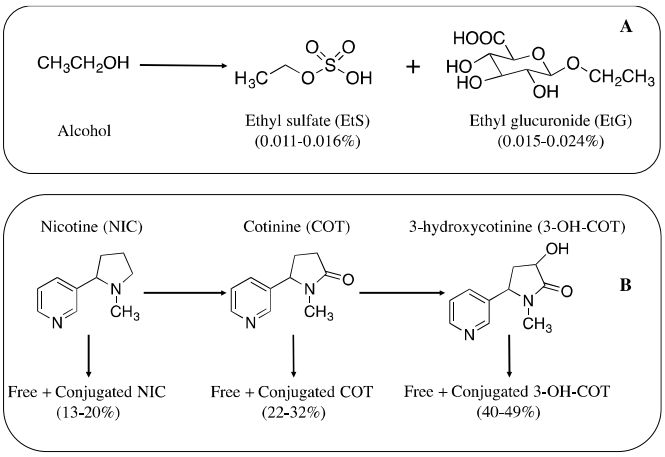

Figure 5-1. Human metabolites and excretion profiles of (A) alcohol and (B) nicotine consumption. Numbers in parentheses represent excretion rates (%) of selected metabolites normalized to alcohol and nicotine doses administered. Data source: (Hukkanen et al. 2005) and (Wurst et al. 2006).

Similar to other studies, NIC levels were generally higher than those of COT and 3-OH-COT, since only about 13-20% of absorbed nicotine is excreted as free or conjugated nicotine (Figure 5-1B), and nicotine can have other sources not leading to direct inhalation exposure, such as disposal of cigarettes and cigarette butts into wastewater (Castiglioni et al. 2015, Rodriguez-Alvarez et al. 2014b). Ideally, assuming human consumption of all nicotine detected, the resultant expected NIC/COT ratio should be around 0.6 (Zheng et al. 2017). In this work, NIC/COT ratios ranging from 0.6 to 9.2 were observed, indicating significant other sources of NIC into the WWTPs. Therefore, measured NIC levels in wastewater was not suitable to be used for back-calculating actual nicotine consumption.

Table 5-1. Comparison of alcohol and nicotine consumption data obtained in this work and in prior studies around the world using wastewater epidemiology.

	Country	WWTP (#)	City (#)	Population	Sampling period	EtS concentration (μg/L)	Daily consumption (range, mean, mL/d/capita)	Annual consumption (mean, L/y/capita)	Ref
Alcohol	Spain	1	1	1,157,000	One week	5.5-32.5	18 (total) 21 (15+)	8 (15+)	(Mastroianni et al. 2014)
	Spain	1	1	100,000	One week in Apr 2012	4-12	9.8-23.5, 16.3 (total)	5.9 (total)	(Rodriguez-Alvarez et al. 2014a)
	Spain	3	1	1,500,000	Mar 3-20th, 2016, including Fallas festivity	1.5-19.9	1.1-56.1 (15+)	\	(Andres-Costa et al. 2016)
	Belgium	8	8	1,600,000	One week sample per year during 2013-2015	1.7-32.7	5.3-33.3 (15+)	5.6 (15+)	(Boogaerts et al. 2016)
	11 countries	23	20	17,000,000	One week in Mar 2014, or Feb and Mar 2015	<1-70.7	6.4-44.3; 20.6 (total)	5.9 (total)	(Ryu et al. 2016)
	Greece	3	\	28,850	One week between Feb and Mar 2015	1.7-12.2	1.7-11.2, 5.3 (total)	1.9 (total)	(Gatidou et al. 2016)
	U.S.	3	3	222,000	A random weekday every month from Mar 2015 to Mar 2016	1.6-25.1	5.7-92.9 29.4 (total)	10.7 (total) 13.4 (15+)	This study

	Country	WWTP (#)	City (#)	Population	Sampling period	Concentration (μg/L)	Daily consumption (mg/d/capita)	No. of cigarettes by smokers per day (mean)	Ref
Nicotine	Spain	1	1	130,000	One week each year from 2012-2014	NIC: 0.7-9.4 COT 0.3-1.8 3-OH-COT: 1.0-3.3	0.9-2.3, 1.8	\	(Rodriguez-Alvarez et al. 2014b)
	Czech Republic & Slovakia	5	3	541,500	End of Jun to early Sep 2014, including several festivals	COT: 0.8-6.8	2-16	\	(Mackul'ak et al. 2015)
	China	10	8	4,000,000	Two consecutive days in Aug 2016	NIC: 2.6-37.7 COT: 1.1-2.4	2.39	16.9*	(Zheng et al. 2017)
	China	11	1	2,200,000	Two consecutive weekdays in Jun 2015	NIC: 0.7-29.2 COT: 1.0-3.6 3-OH-COT: 1.6-4.4	0.25-4.22, 1.92	14.6	(Wang et al. 2016)
	U.S.	3	3	222,000	A random weekday every month from Mar 2015 to Mar 2016	NIC: 0.6-26.7 COT: 0.2-3.8 3-OH-COT: 0.3-3.8	1.7-4.1 2.7	14.2	This study

WWTP: wastewater treatment plant; \: not available or not provided by reference; *Median; total: total population; 15+: population over 15 years old.

Consumptions of Alcohol Estimated for Entire Population

Estimated per capita weekday alcohol consumption (mean, range, unit: mL/d/capita) were as follows: Community A: 24.7,15.5–43.3, Community B: 17.9, 5.7–46.4, and Community C: 49.8, 21.5–92.9, and the population-weighted average of three communities was 29.4 mL/d/capita

(Table 5-2). Similar values have been reported from other countries (Table 5-1). For instance, an average of 18 mL of alcohol was found to be consumed per day per person in Barcelona, Spain (Mastroianni et al. 2014). In an international study investigating alcohol consumption in 20 cities from 11 countries, the average daily per capita alcohol consumption ranged from 6.4 to 44.3 mL, and the population-weighted average was 20.6 mL (Ryu et al. 2016). A Kruskal-Wallis test showed alcohol consumption varied significantly among the three communities ($p = 0.0002$), and a post-hoc test demonstrated no significant difference was found between Community A and B, but daily per capita alcohol consumption in Community C was significantly higher than in other two communities. In this study, population used for back-calculation of each community was provided by WWTPs, and not corrected for any uncertainties due to transport, events or tourist's activities. Therefore, the unusually high levels calculated for Community C may be attributable to the fact that Community C is located near a tourist destination (national park), where influx of tourists coming to the community was not available to be included in the back-calculation, leading to an overestimation of alcohol consumption for residents.

Consumptions of Nicotine Estimated for Entire Population

Estimated weekday nicotine consumption per capita (mg/d/capita) ranged from 1.7–3.6, 0.8–4.1 and 1.7–3.4, with averages of 2.7, 2.4 and 2.6 for Community A, B and C, respectively (Table 5-1). The estimated levels (mg/d/capita) for the three U.S. communities were similar to the values reported for cities in Spain (1.1–2.7) (Rodriguez-Alvarez et al. 2014b) and China (0.25–4.22) (Wang et al. 2016) (Table 5-1). A Kruskal-Wallis test showed no statistical differences of nicotine consumption among three communities ($p = 0.96$), indicating similar cigarette usage among three communities during the sampling period, which was different from the profile of

alcohol consumption. One plausible explanation is that on one hand, Community C (a tourist location) is in a state with higher excise taxes on cigarettes and lower prevalence of smoking (15%) than the states where other the two communities are at (21%) (CDC 2017), therefore, when normalized to the entire population, daily nicotine consumption rate could be lower than the ones in the other two communities. On the other hand, mass loads of nicotine could increase with incoming tourists, leading to an overestimation of per capita nicotine consumption when normalized to residents only. Thus, a combination of these two factors can potentially lead to similar nicotine consumption rates with other two communities.

Table 5-2. Average per capita weekday consumption (mean ± SD, n=11) of alcohol (mL/d/capita) and nicotine (mg/d/capita) estimated for three communities during March 2015-March 2016.

Community	Population			Per capita daily alcohol consumption (mL/d/capita)		Per capita daily nicotine consumption (mg/d/capita)		
	Total	15+	18+ smoker	Total	15+	Total	15+	18+ smoker
A	125000	100000	19950	24.7±7.8	30.9±9.8	2.7±0.6	3.4±0.7	17.2±3.7
B	44000	35200	6988	17.9±11.6	22.3±14.5	2.4±1.2	3.0±1.4	15.3±7.3
C	53000	42400	6211	49.8±23.5	62.2±19.4	2.6±0.5	3.3±0.6	22.5±3.9
Population-weighted average				29.4	36.7	2.7	3.3	17.8

Temporal Variations of Alcohol and Nicotine Consumptions

In this study, alcohol and nicotine consumptions on a typical weekday were monitored for a time period of 11 consecutive months to enable a temporal, longitudinal analysis. Figure 5-2A and Figure 5-2C showed detected concentrations of EtS and COT+3-OH-COT throughout a year, while Figure 5-2B and Figure 5-2D represented the back-calculated per capita alcohol and nicotine consumptions on sampling weekdays over a year. Since the flow of WWTPs on weekdays tended not to change much throughout a year, and the population count of each community used for back-

calculation was also constant, the profiles of concentration and calculated consumption were similar. Except that although EtS concentrations in Community C were similar to those from other two communities, the calculated alcohol consumptions were much higher. Similarly, COT+3OH-COT concentrations in Community C were generally lower than those from other two communities, but the calculated nicotine consumption levels were similar among three communities. These could be explained by the fact that Community C had a larger industrial wastewater input than the other two communities, potentially leading to dilution of analytes in influent more, and the population used for consumption calculation in Community C might be underestimated because of unaccounted tourists coming to Community C.

Since Community A and B are small communities with little tourism activities, alcohol consumption remained relatively consistent throughout the sampling period in these two communities (Figure 5-2B). Because all sampling days were weekdays, so the drinking patterns may also stay relatively constant in these two communities, and the small fluctuations could be explained by different drinking behaviors on different days. However, there was a clear trend of increasing alcohol consumption from June to September 2015 in Community C, which can partially be a result of different drinking habits for residents in Community C at different time of a year, but more likely, can be attributed to increasing tourism activities during that period. The temporal trends observed here in Community C match the fact that the national park near Community 3 has restrictions in place for use of water in the winter, and the peak season of tourism is summer. Therefore, although exact numbers of the amount of people present on each sampling day were not available, trends of alcohol consumption calculated based on the residents living in Community C partially reflect the impact of tourist influx.

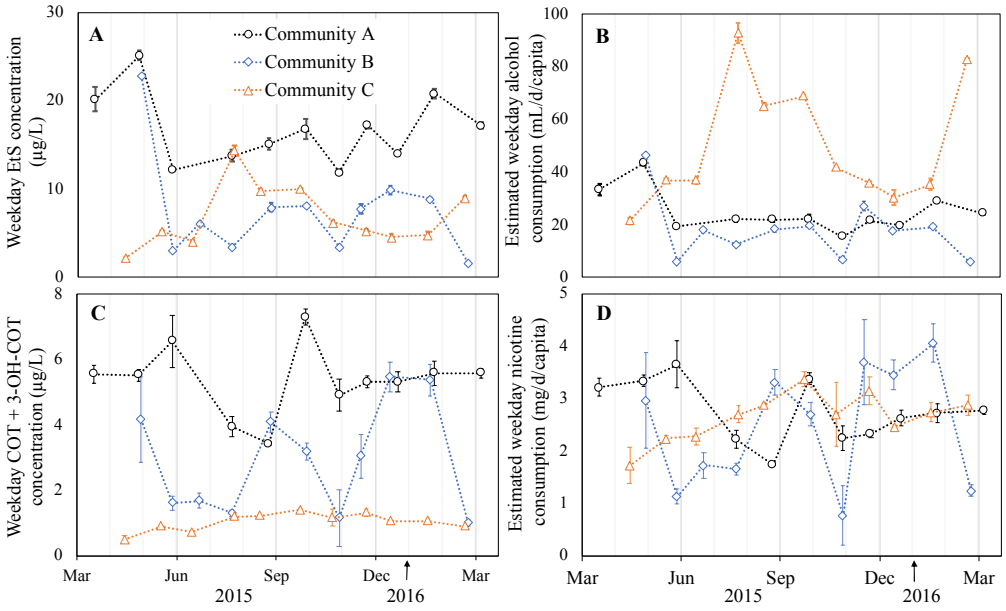

Figure 5-2. Profiles of measured concentrations of EtS (A), and COT+3-OH-COT (C) and estimated daily per capita consumption of alcohol (B) and nicotine (D) determined via sampling on a random weekday every month between March 2015 to March 2016 in three U.S. communities. Error bars indicate minimum and maximum values from duplicate analyses.

As cigarette use is on a daily basis and not clearly related to recreational activities (Rodriguez-Alvarez et al. 2014b), it was not surprising to detect less temporal variability in nicotine consumption (Figure 5-2D) when compared to that of alcohol. Per capita nicotine consumption among the three communities was relatively stable over one year. In addition, Pearson's correlation analysis was performed on daily per capita alcohol and nicotine consumption estimates from each community (SI, Figure S5-1). Weak, positive correlations were observed with Pearson's *r* values ranging from 0.42 to 0.63 and *p* values varying from 0.04 to 0.20. Positive correlations indicate common releasing sources of alcohol and nicotine into the sewage (mostly from human excretion), and the weak associations could be related to different use patterns of alcohol and nicotine.

Comparison with Survey Statistics

To compare results from this study with those from surveys, consumption of alcohol and nicotine were calculated for different groups of populations including population over 15 years old (15+), population over 18 years old (18+), and adult (18+) smokers. Assuming 80% (national average) of the population were 15 years old and older (WHO 2014a), average daily per capita (15+) alcohol consumptions for Community A, B and C were 30.9, 22.3 and 62.2 mL, respectively (Table 5-1), which were equivalent to 11.3, 8.2 and 22.7 L per year and the population-weighted average was 13.4 L. These results were compared with state-specific and national average alcohol consumption data reported by NIAAA (Haughwout and Slater 2017). As shown on Figure 5-3A, although an individual city may not be representative of the entire state or the whole country, alcohol consumptions estimated using the WBE approach matched well with the state-specific data for Community A and B, with RPDs of 28% and 1%, respectively. In addition, higher alcohol consumption was observed in Community A than that of Community B in this study, which also correlated well with state-specific data profiles, indicating the ability of WBE to observe spatial variations. As discussed earlier, the influence of tourism could have led to an overestimation of per capita alcohol consumption for Community C, therefore it was not surprising that the estimated alcohol consumption for Community C was 180% higher than state-specific survey data. Per capita alcohol consumption was reported by NIAAA to be 8.8 L in 2015 for U.S. population over 14 years old, and WHO projected the value to be 9.0 L in 2015 for U.S. population over 15 years old (WHO 2014a). The population-weighted average estimated in this study was 40% higher than the U.S. averages reported from surveys, which can be mainly attributed to the overestimated values from Community C. Weighted average of Community A and B was 10.5 L/y, which was only 16% higher than the WHO predicted value.

It should be noted that only weekday samples were taken in this study, although parts of weekend alcohol consumption could potentially be captured by Friday and Monday samples (Boogaerts et al. 2016), actual alcohol consumption was expected to be higher than the estimated values reported in this study. We looked at weekday (Monday to Friday) and weekend (Saturday and Sunday) daily alcohol consumption rates reported in other studies (SI Table S5-4) (Andres-Costa et al. 2016, Boogaerts et al. 2016, Gatidou et al. 2016, Mastroianni et al. 2014, Rodriguez-Alvarez et al. 2014a, Ryu et al. 2016), and observed 48–105% (mean: 70 ± 20%) higher alcohol consumption rates estimated for weekends compared to weekdays. Furthermore, daily consumption rates calculated including weekdays and weekends were 13–22% (mean: 18 ± 3%) higher than the ones calculated from weekday samples only. Although people in the U.S. may have different drinking habits compared to people from other countries, we used the above information to correct our estimate by a factor of 1.18, yielding an annual alcohol consumption of 13.3, 9.6 and 26.8 L for 15+ population in Community A, B and C, respectively, and a population-weighted average of 15.8 L/y. RPDs between state-specific alcohol consumption data reported by NIAAA and corrected estimates were 44%, 16% and 106%, respectively.

For smoking, an average of 3.4, 3.0 and 3.3 mg of nicotine was consumed per capita over 15 years old in Community A, B and C, respectively **(**Table 5-1). Assuming 1.25 mg of nicotine was absorbed by smoking one cigarette (Castiglioni et al. 2015), numbers of cigarettes consumed per day per capita (15+) were 2.7, 2.4 and 2.6, respectively, with a population-weighted average of 2.7, equaling 970 cigarettes a year. According to Euromonitor, an average of 1083 cigarettes were smoked per capita (15+) in U.S. in 2014 (Euromonitor International 2014). Therefore, RPD between result from this study and the one from Euromonitor was only 11%.

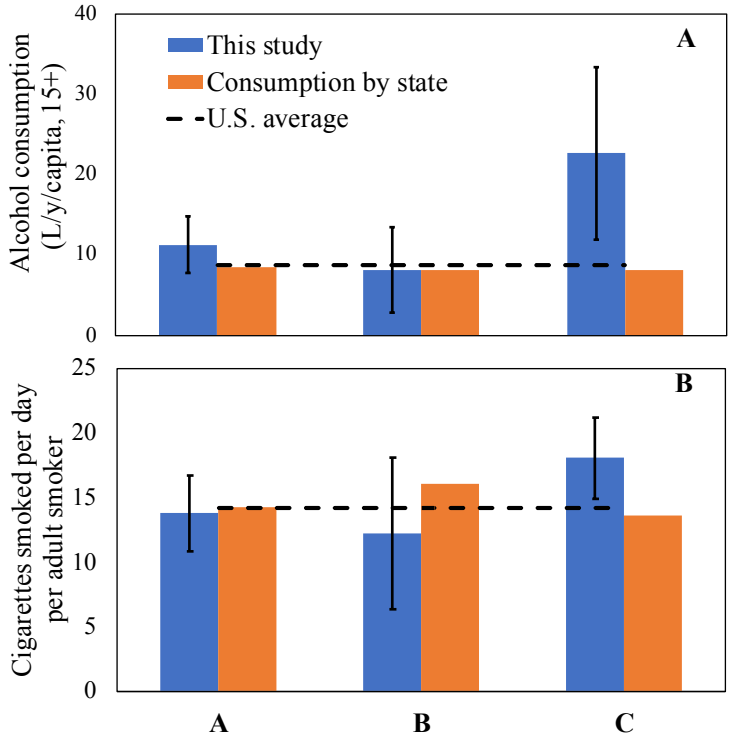

Figure 5-3. Comparison of annual average alcohol (A) and nicotine (B) consumption estimated in this study (blue column) with state-specific (orange column) and national average (black dash line) results reported from surveys. A: per capita alcohol consumption for 15+ population per year estimated in this study, compared with data reported by NIAAA; B: number of cigarettes smoked per adult smoker per day estimated in this study, compared with data from STATE-CDC. Error bars represent standard deviations calculated from 11-month data in this study.

In addition, CDC reported an average of 14.2 cigarettes were consumed per adult smoker per day in the U.S. in 2015 (Jamal 2016). In this study, the average number of cigarettes consumed by adult smokers per day were 13.8, 12.2 and 18.0 for Community A, B and C, respectively, and the population-weighted average was 14.2, which was the same as the number reported by CDC. State-specific cigarettes use data were extracted from STATE-CDC and compared against our results as shown in Figure 5-3B. Similar to alcohol consumption, the number of cigarettes used by adult smoker in Community C was higher than the ones in other two communities and the numbers

reported by survey, which could also be attributed the tourists' contribution to total nicotine mass loads, leading to an overestimation when normalized to local smokers.

Another approach for comparison is to assume every smoker consumes 14.2 cigarettes per day as reported by CDC, which equals 17.75 mg absorbed nicotine (assuming 1.25 nicotine/cigarette (Castiglioni et al. 2015)). The number of smokers in each community can be calculated by dividing the total mass loads of absorbed nicotine by 17.75 mg nicotine. Percentage of smokers in each community can then be obtained by dividing the number of smokers by the number of population over 18 years old. Using this approach, estimated prevalence of smokers in each community was 20%, 18% and 19%, respectively. Compared with state-specific cigarettes smoking prevalence data (CDC 2017), the RPDs between estimated results and the survey were 2%, 8% and 12%, respectively. Estimated percentage of smokers in Community C was 27% higher than the survey data, which was most likely due to the contribution of tourists who smoke, but can also come from the differences of smoking prevalence between state statistics and specific cities within the state.

Smoking has been suggested as a daily habit and is not clearly related to recreational activities, therefore, no apparent differences were expected between weekday and weekend consumptions (Rodriguez-Alvarez et al. 2014b), so the results may not be impacted or impacted to a less extent than that of alcohol, therefore, no correction were made for nicotine consumption.

Study Limitations

This study, similar to other prior surveys, suffered from several limitations (Castiglioni et al. 2013, Castiglioni et al. 2014, van Nuijs et al. 2011a). 1) This work did not consider potential degradation/transformation of target chemicals in the sewer line prior to arrival at the sampling

location of the WWTPs. Banks *et al.* had assessed the potential impact of sewer conditions on these biomarkers using sewer reactors, and found COT and 3-OH-COT to be relatively stable under the simulated field conditions, while in-sewer loss of EtS should be accounted for when using this metabolites as a biomarker for alcohol (Banks et al. 2017). In this study, the stability during sample storage was tested by a 12-month storage test at -20 ˚C. No significant degradation during this period was observed for any of the analytes (SI, Figure S5-2); 2) For back-calculation, the current study used single values of the metabolic correction factors (Hukkanen et al. 2005, Wurst et al. 2006) for estimating average population consumption, whereas factors including age, gender, race and health conditions could also affect the metabolisms of substances on different individuals; 3) One of the major obstacles in the field of WBE is the estimation of population size during sampling. As suggested in several other studies, tourism or certain festivals/events could lead to great variations of population on specific days. While some of the papers including this study used census data or data calculated from number of household connected to WWTPs (Rodriguez-Alvarez et al. 2014b), some studies (O'Brien et al. 2014) have used total nitrogen, total phosphorus, biochemical oxygen demand (BOD), chemical oxygen demand (COD) (van Nuijs et al. 2011b), ammonium (Been et al. 2014), and artificial sweeteners (O'Brien et al. 2014) for population calculation or normalization. The drawback of using census data is that it cannot reflect the change of population due to commute, tourism or specific events/festivals, leading to overestimation or underestimation of actual consumptions. Most of the population markers that have been used in the other studies were not specific enough that they could also be contributed by industrial discharge, agricultural input and/or animal input (such as BOD and COD), and there is no consensus on which population marker works best. Therefore, more research is needed in the future to find an ideal population biomarker that is stable, sensitive and specific enough to reflect

actual number of population in a timely manner; 4) As mentioned in the last section, this study did not collect weekend samples which could lead to an underestimation of alcohol consumption. On the other hand, the fact that only weekday samples were included in this study may have helped alleviate the uncertainties associated with population size, since the population may stay relatively consistent on weekdays throughout a year, at least for Community A and B. For Community C, although exact numbers for the population were not available for each sampling day, the alcohol consumption estimated for residents may partially reflect the intensity of tourism during different periods of the year. Future studies should consider sampling at least one consecutive week each month to get better estimates; 5) For the calculation of number of cigarettes smoked, a fixed value of 1.25 mg nicotine was assumed to be consumed by smoking one cigarette in the current study, and different values have been used by other studies such as 0.6 mg (Zheng et al. 2017) and 0.8 mg (Mackul'ak et al. 2015, Rodriguez-Alvarez et al. 2014b). This could also bring uncertainties because nicotine content varies among different cigarettes and the uptake of nicotine also varies among individuals. Furthermore, apart from conventional cigarettes, nicotine could also come from the use of nicotine patch/gum, electronic cigarettes and other tobacco products (van Wel et al. 2016), which could lead to an overestimation when calculating the number of cigarettes smoked. Additionally, with the increasing use of electronic cigarettes (McMillen et al. 2014), future studies may be needed to differentiate the use of conventional cigarettes with electronic cigarettes for more specific monitoring.

Conclusions

This study was the first in the U.S. to assess population-level alcohol and nicotine consumption using wastewater-based epidemiology, doing so for three communities for one-year period. It suggested the ability of WBE to capture spatial and temporal variations of population

consumption of alcohol and nicotine at community level. While results from WHO were based on sales statistics and interviews (WHO 2014a), NIAAA report used state sales or shipment data (Haughwout and Slater 2017), and CDC-STATE system conducted questionnaires by a random-digit dialing system to select samples of adults with household or cellular phones (CDC 2017), these approaches often take a long time to conduct, and have limitations, such as not being able to reflect actual consumption because of cross-border sales from neighboring states, time delay between sales records and actual consumption, exclusion of alcohol/nicotine contained in medications and food, unrecorded legal home production and illicit production, importation and sales (Rehm et al. 2014), and could be subject to self-reporting bias. The WBE method can provide timely, cost-effective and objective information on consumptions and exposures to chemicals. Estimated results by WBE in this study were in good agreement with statistics from other sources, demonstrating the potential of WBE to be used as a tool to provide complementary information on the use of alcohol and nicotine. Despite several limitations such as lacking a good estimate of population, not taking into account degradation or transformation of target compounds in the sewer and during sampling, only sampling weekday samples, and errors from back-calculating, when the same procedure was taken for longitudinal monitoring in small communities, this approach has the potential to provide useful information on relative trends of drinking and smoking, which could help evaluating the efficacy of certain regulations or policies.

6 CONCLUSIONS, RESEARCH GAPS AND FUTURE DIRECTIONS

The previous chapters explored several environmental monitoring strategies in assessing human exposure to chemicals that concern public health, by investigating the occurrence of selected emerging contaminants in sewage sludge and indoor dust, as well as the human metabolites of alcohol and nicotine in wastewater influent. Chapter 2 described the first comprehensive U.S. study on paraben in U.S. sewage sludges from nine states, revealing nationwide types and concentrations of parabens, temporal trends in parabens concentrations over the course of a year, and provided a first estimate of the estrogenic activity of parabens in U.S. sewage sludge relative to other estrogenic compounds. This work established a baseline of paraben levels in U.S. sewage sludge and revealed information useful for future monitoring and risk assessment. Followed by Chapter 3, the first nationwide survey on occurrence of parabens, TCS, TCC, human metabolites and transformation products of TCC in sewage sludge collected from China, demonstrating the widespread presence of these emerging contaminants in Chinese sewage sludge. It constitutes the first time the human metabolites and transformation products of TCC were measured in the Chinese built wastewater environment, and the results suggested ubiquitous TCC transformation going on in the WWTPs. Annual mass emissions of these contaminants through sludge in China was estimated to be up to 400 metric tons. In Chapter 4, an easy, quick, cost-effective and effective sample preparation followed by LC-MS/MS method was applied for the measurement of parabens, TCS and TCC in indoor dust. All analytes were found in 80 indoor dust samples collected from athletic facilities and private homes in Oregon, U.S., providing baseline levels of TCS and TCC before the U.S. FDA ban on their use in over-the-counter antiseptic washes. Daily human intake of these contaminants was estimated for different age groups, and compared with exposure doses from other routes (mainly from use of PPCPs),

suggesting exposure to these antimicrobials via dust ingestion was a minor exposure route for adults, but could be a major contributor to exposure of young children. In Chapter 5, a wastewater-based epidemiology approach was used for the first time in the U.S. for assessing alcohol and nicotine consumption in three communities from different U.S. regions, and good agreement was observed between WBE estimated results and data from other sources, suggesting the capability of WBE in providing complimentary information on alcohol and nicotine consumption to conventional surveys.

Despite some noted limitations, the findings obtained provide valuable information on chemical occurrence in different environmental compartments and implicate potential human exposure levels through different pathways, limitations do exist which need to be addressed and research gaps need to be fulfilled in future studies:

(1) Only a selected group of emerging contaminants were measured in this work, because of increasing evidences in their toxicity, endocrine disrupting activity, persistence in the environment, bioaccumulation or biomagnification in the human body or other organisms (Halden et al. 2017, Soni et al. 2005), and because of the accessibility of reference standards and targeted analysis techniques. However, as there are many more chemicals present in the built and natural environment that pose potential risks to human health, "non-targeted analysis" or "suspect screening" could help expand the field of environmental monitoring to better prioritize emerging contaminants and protect human health (Bletsou et al. 2015, Diamond et al. 2011, Singer et al. 2016). For example, non-targeted screening of chemicals using high resolution mass spectrometry (HRMS) coupled with exposure and toxicity forecast can be used to help prioritize chemicals in indoor dust (Rager et al. 2016), as well as help identify transformation products of emerging chemicals or regulated chemicals in the environment (Bletsou et al. 2015);

(2) Since environmental samples are complicated matrices, developing quick, easy, accurate, and reproducible sample preparation methods remains a challenging work in future studies. This dissertation demonstrated an example of using a QuEChERS method for the sample pretreatment of sewage sludge (Chapter 3) and indoor dust (Chapter 4), a method that can be easier, quicker and more cost-effective compared to conventional methods using liquid-liquid extraction followed by solid phase extraction (Chapter 1). Depending on the chemical and physical properties of analytes, this method can be further modified and expanded to a wider range of chemicals;

(3) In Chapter 3, nationwide survey on selected emerging contaminants were measured in sewage sludge from China, however, the sample distribution was not even across provinces. For example, 25 samples were collected from Shanghai, while in some provinces, only one or two samples were available. Better sampling strategies need to be designed for future monitoring studies. In addition, although dechlorination products of TCC were measured for the first time in Chinese wastewater system, more research is needed to characterize the dechlorination mechanisms, and the efficiencies of different wastewater and sludge treatment techniques on TCC transformation, which can potentially help future design and operation of wastewater and sludge treatment to removal certain contaminants;

(4) In Chapter 4, although daily intakes of dust-borne antimicrobials were estimated for different age groups, and compared to estimated intakes from other routes (e.g., PPCPs and teethers) to suggest the contribution of dust ingestion to total exposures, all of which were based on estimated data thus need further verification. Concurrent measurement of environmental samples and biological specimens, together with questionnaires will help better understand exposure sources/pathways. For instance, measurement of organophosphate flame retardants (PFRs) levels in indoor dust, hand wipes, and urine from residents suggested hand-to-mouth

contact or dermal absorption may be important pathways of exposure to PFRs (Hoffman et al. 2015).

(5) In Chapter 5, as discussed previously, several limitations exist and WBE is still in its early stages but remains promising for better application in the future. One of the biggest challenges is the reliable estimation of the actual population present in the monitoring network, which has been suggested to contribute up to 55% uncertainty under the best practice protocol (Castiglioni et al. 2014). More studies are needed to find human-specific markers which can reflect population fluctuation in a timely manner. Another limitation is the scarcity of pharmacokinetics studies regarding human excretion profiles of certain substance, like alcohol, only one study had reported its excretion to ethyl sulfate on six volunteers, which could lead to large variations for WBE back-calculation considering the significant differences of alcohol (Wurst et al. 2006) metabolism among individuals of different sex, race and health conditions. In addition, the biodegradation or transformation of target analyte after human excretion and prior to sampling and lab analysis need to be considered, and while most of the studies were done in the lab mimicking real-world conditions, in-sewer experiments (Plosz et al. 2013) and additional modeling studies would be highly desired to investigate in-pipe biotransformation of target analyte to advance the field of WBE (Castiglioni et al. 2014). Finally, WBE studies conducted in line with questionnaires during the same period would help offer better validation of WBE results. For example, conducting questionnaires in local community on alcohol and cigarettes consumption during the WBE sampling period would be a better practice than comparing results with national or state surveys.

In summary, this dissertation explored different environmental monitoring strategies in informing human exposure to chemicals that threat public health. Compared to biomonitoring and questionnaires, environmental monitoring can be more cost-effective and bears less ethical risks,

and can provide information to help identify human exposure pathways and inform public health regulators in the prioritizing of chemicals of concern, in drafting regulations to reduce human exposure risks and in evaluating the effectiveness of interventions.

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APPENDIX A

SUPPORTING INFORMATION

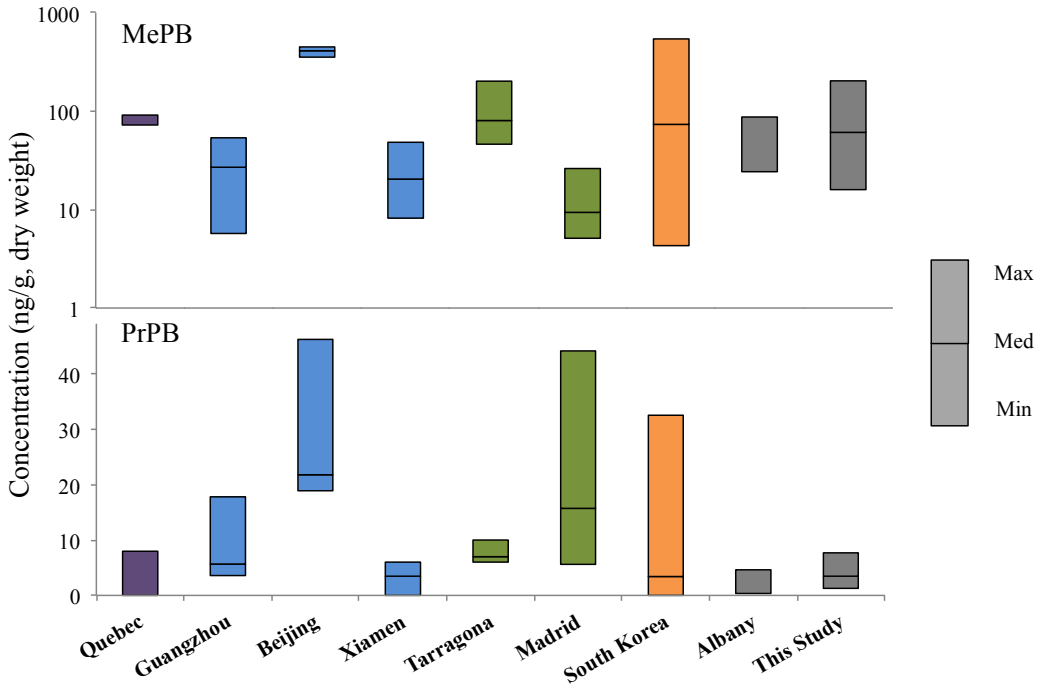


Figure S2-1. MePB and PrPB concentrations (ng/g, dw) in sewage sludge from different countries. The upper and lower edge of the box indicates maximum and minimum concentrations, respectively, and the line inside the box indicates the median concentration. The bar colors indicate different countries: Purple for Canada; Blue for China; Green for Spain, Orange for South Korea and Grey for U.S. (references for these studies are provided in Table 2-1).

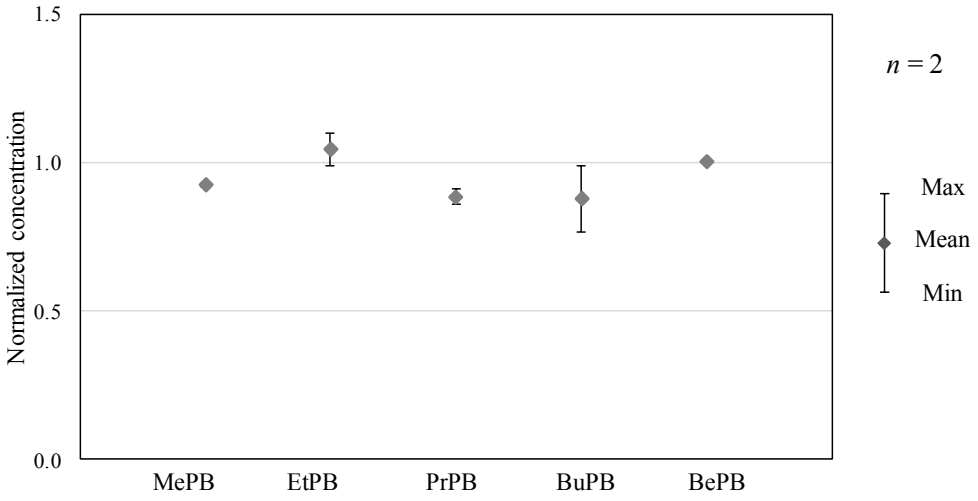


Figure S2-2. Stability test for the five parabens in two sludge samples during storage at –80^{o}C analyzed over a period of 20 months. Concentrations were normalized to the average initial concentration detected. MDL/√2 was used for non-detects (BePB). Levels of MePB, EtPB, PrPB, and BuPB did not show appreciable changes during storage. Error bar indicates minimum and maximum values, where the middle point indicates average values for the two sludge samples.

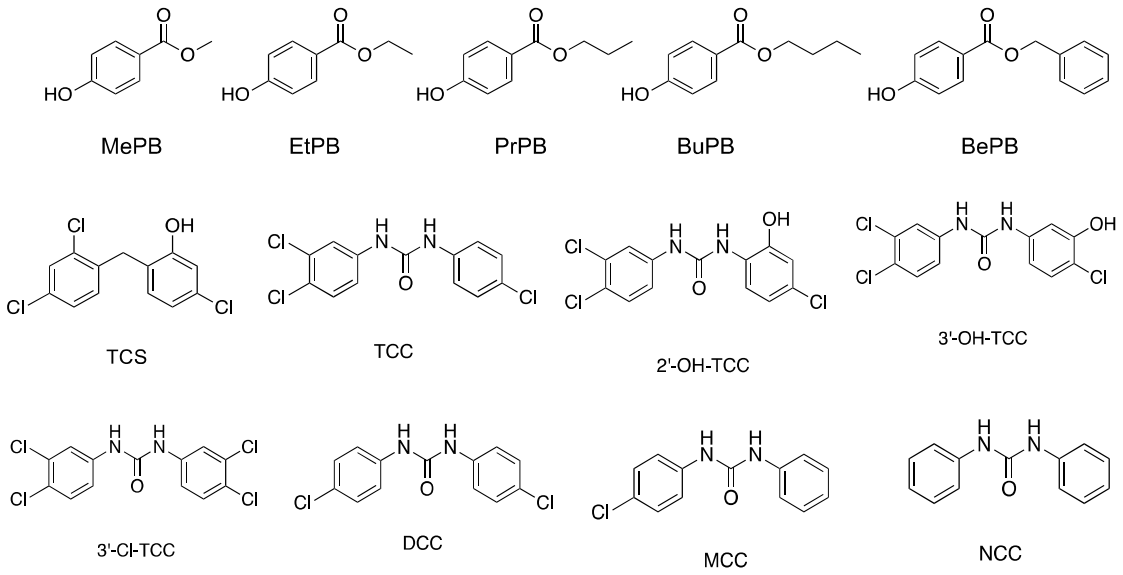


Figure S3-1. Chemical structures of target analytes

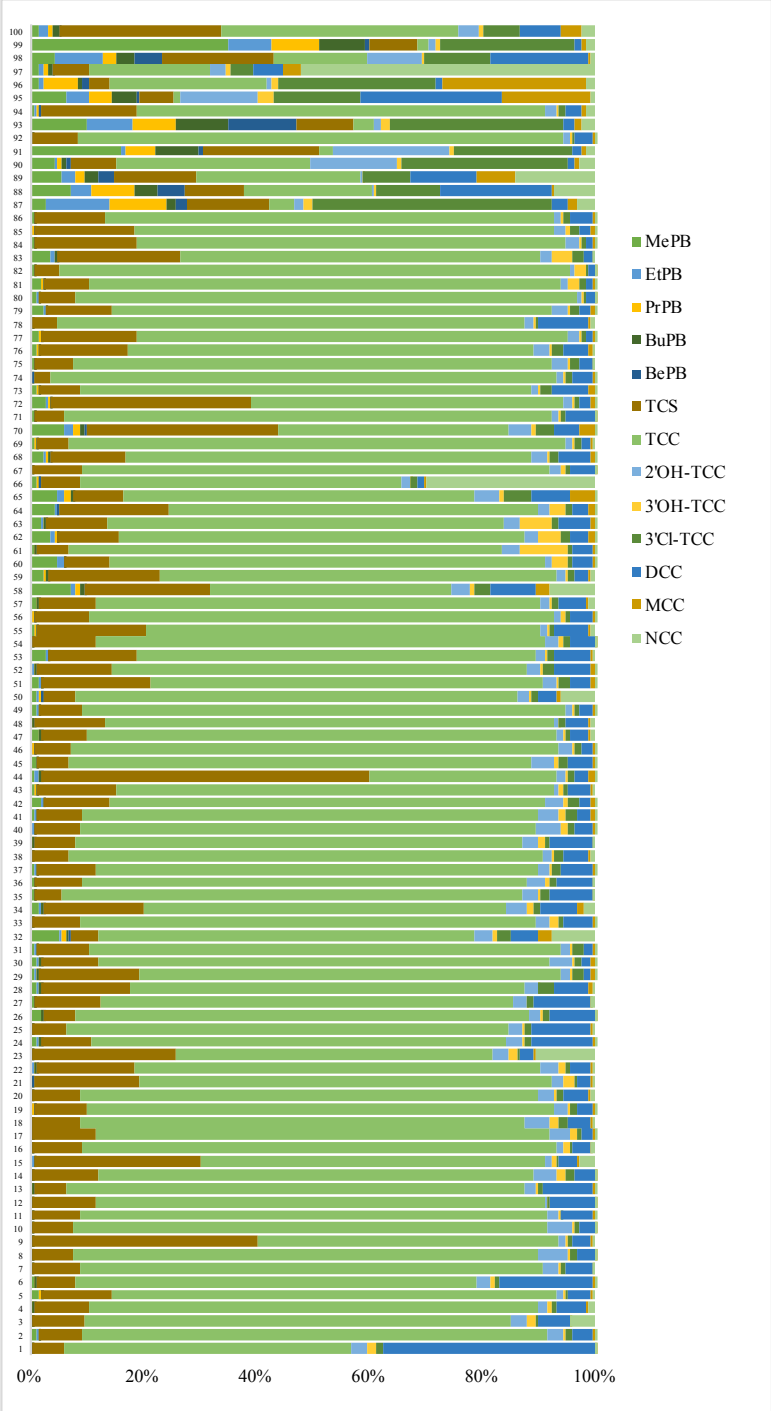


Figure S3-2. Composition distribution of individual contaminant in 100 sludge samples collected across China (Sample 1-86 represent sludge from municipal WWTPs, and 87-100 represent sludge sample from industrial WWTPs).

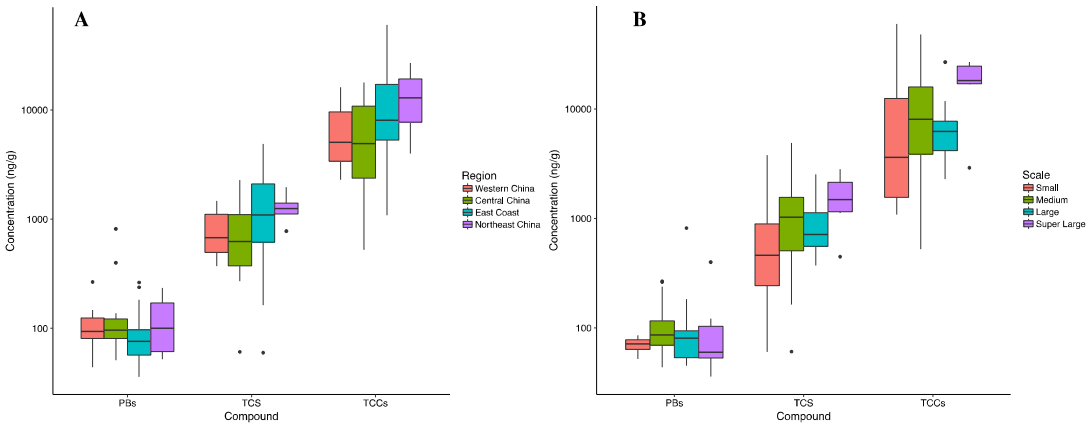


Figure S3-3. Box-and-whisker plots of ∑PBs, TCS and ∑TCCs levels with respect to A: four economic regions in China; B: different treatment capacities of WWTPs.

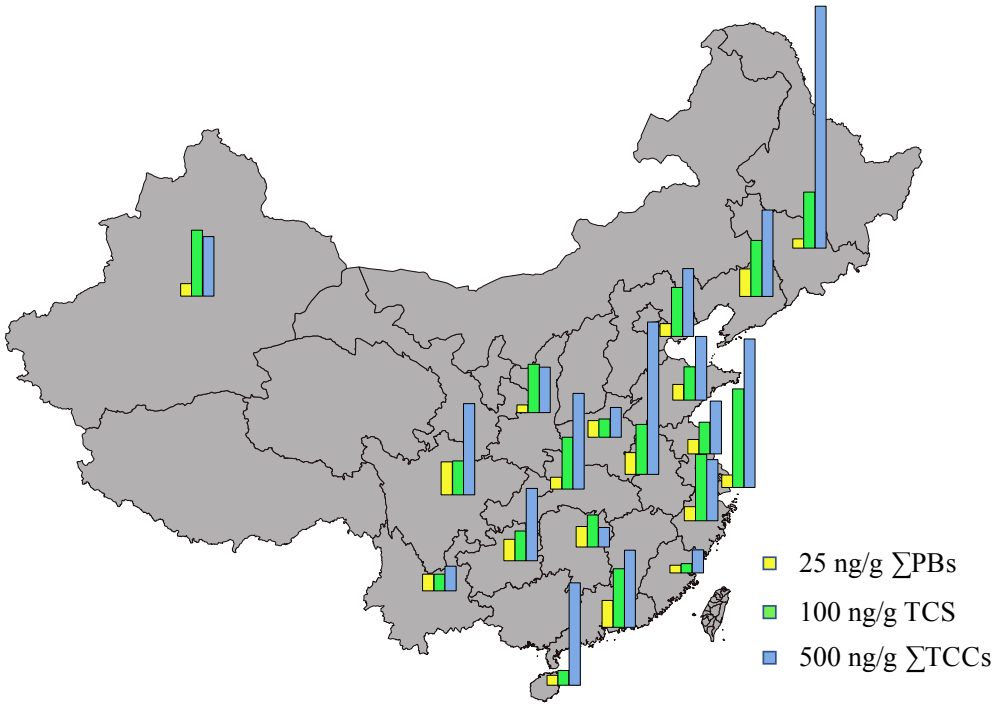


Figure S3-4 Spatial distribution of geometric mean (GM) concentrations (ng/g) of ∑PBs, TCS and ∑TCCs in sludge from different provinces.

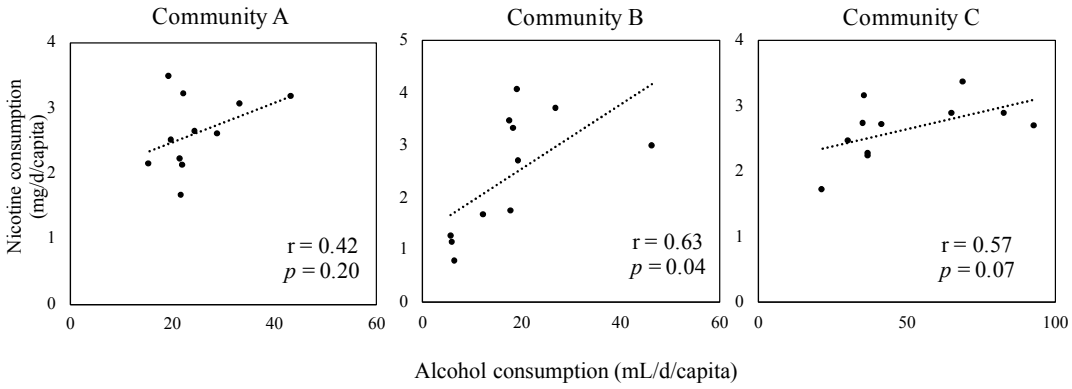


Figure S5-1. Correlations of estimated per capita alcohol and nicotine consumptions in each community (n = 11)

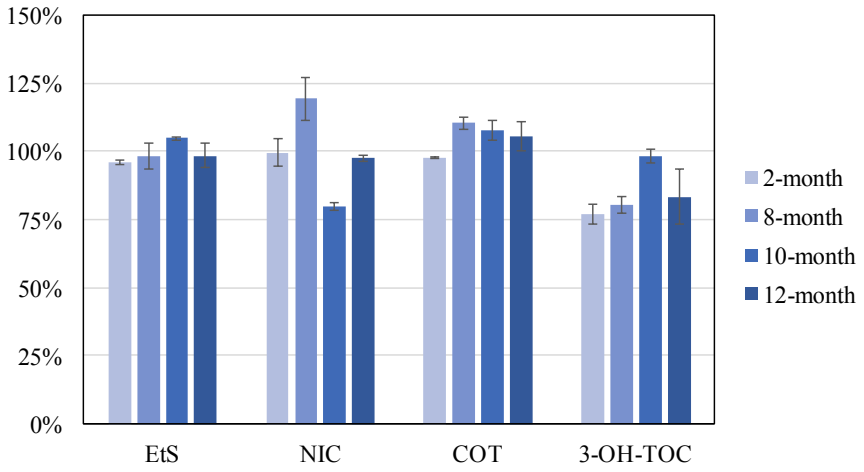


Figure S5-2. Relative abundance (%) of EtS, NIC, COT and 3-OH-TOC after 2-, 8-, 10- and 12-month storage at -20˚C compared to the initial concentration. Error bars indicate minimum and maximum values from duplicate analysis.

Storage Stability Test Procedure

2 L of wastewater influent from a local WWTP was collected in 2016 February, brought back to lab and aliquoted for storage test.

For EtS, because the concentrations of EtS were normally at μg/L, no spiking was needed. 10 aliquots of 1 mL wastewater were prepared and two of the samples were analyzed for EtS right after sample was received, and another 8 aliquots were stored at -20 ˚C freezer. After 2, 8, 10 and 12 months, two of the stored samples were taken out and analyzed for EtS.

For NIC, COT and 3-OH-COT, aliquots of 100 mL wastewater were put into 10 polyethylene bottles, and spiked with 500 ng of standards, respectively. Then two of the aliquots were extracted and analyzed for the three target analytes using the protocol described in Experimental section, another eight aliquots were stored at -20˚C for 2, 8, 10, and 12 months, respectively, and then extracted and analyzed by LC-MS/MS.

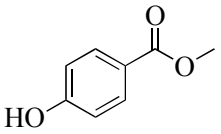
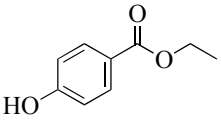
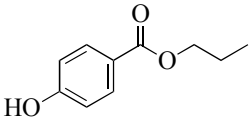
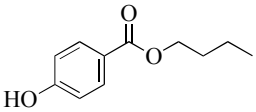
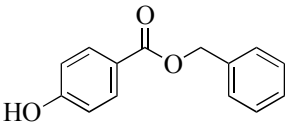
To get the relative abundance (%) of each chemical after certain storage time, peak areas of 2-, 8-, 10-, and 12-month samples of EtS, NIC, COT and 3-OH-COT were compared with the initial peak areas of each analyte, as shown in *Eq S1*.

$$Relative\ Abundance_{c,n}\ (\%) = \frac{Peak\ Area_{c,n}}{Peak\ Area_{c,0}} \times 100 \qquad Eq\ S1.$$

Where $Peak\ Area_{c,n}$ represent the peak area of individual analyte (EtS, NIC, COT, and 3-OH-COT) after storing for n (2, 8, 10 and 12) months, and $Peak\ Area_{c,0}$ was the initial peak area of each analyte.

The results of storage stability test were shown in **Figure S5-2**.

Table S2-1. Target analytes and selected physicochemical properties

Chemical name	Acronym	Chemical structure	Molecular Formula	MW	Log K_{ow} [a]	Boiling Point (˚C) [a]
Methylparaben	MePB		$C_8H_8O_3$	152.15	1.96	252
Ethylparaben	EtPB		$C_9H_{10}O_3$	166.17	2.49	269
Propylparaben	PrPB		$C_{10}H_{12}O_3$	180.08	2.98	285
Butylparaben	BuPB		$C_{11}H_{14}O_3$	194.23	3.47	300
Benzylparaben	BePB		$C_{14}H_{12}O_3$	228.24	3.70	355

[a] Predicted data cited from chemspider.com, which is generated using US Environmental Protection Agency's EPISuite

MW: molecular weight

Table S2-2. Information on wastewater treatment plants (WWTPs) sampled for the study, including flow, sewage sludge classification and treatment approaches.

Plant ID	Sample ID	WWTP Flow (MLD)	Classification	Sewage Sludge Treatment Approach
A	A1	>150	NA	\
	A2		Class B	Anaerobic digestion
	A3		Class A	Anaerobic digestion + Composting
B	B1	>150	NA	\
	B2		Class B	Anaerobic digestion
C	C	<10	NA	\
D	D	<10	Class B	Storing
E	E	>150	Class B	Anaerobic digestion
F	F	20-100	NR	Anaerobic digestion
G	G1	>150	Class B	Anaerobic digestion
	G2		Class A	Anaerobic digestion + Dewatering
H	H1	<10	NR	Anaerobic digestion
	H2		NR	Anaerobic digestion + Dewatering
I	I	<10	Class B	Anaerobic digestion
J	J	<10	NR	Anaerobic digestion + Composting
K	K	>150	Class B	Anaerobic digestion + Aging
L	L	20–100	-	Aerobic digestion + Aerobic digestion
M	M	20–100	Class A	Anaerobic digestion + Storing
N	N	>150	Class A	Anaerobic digestion + Storing + Composting

MLD: million liters per day; NA: not available; NR: not rated; \: no treatment

Table S2-3. LC and MS/MS parameters for target analytes and labeled standards

Analyte	RT (min)	MS/MS Transition [a]	DP (V)	CE (V)	CXP (V)	DW (ms)
MePB	5.30	150 > **92**, 136	-60	-30	-5	50
$^{13}C_6$-MePB	5.29	157 > **98**	-60	-30	-5	50
EtPB	5.82	165 > **92**, 136	-55	-30	-15	50
d_5-EtPB	5.78	170 > **92**	-55	-30	-15	50
PrPB	6.39	179 > **92**, 136	-55	-30	-13	50
d_4-PrPB	6.36	183 > **96**	-55	-30	-13	50
BuPB	6.88	193 > **92**, 136	-55	-38	-1	50
d_4-BuPB	6.86	197 > **96**	-55	-38	-1	50
BePB	6.73	227 > **92**, 136	-65	-36	-1	50

[a] Parent ion > **Quantification ion**, Confirmation ion

RT: Retention Time; DP: Declustering Potential; CE: Collision Energy; CXP: Collision Cell Exist Potential; DW: Dwell Time

Table S2-4. Method performance characteristics

Compound	LOD (ng/L)	MDL (ng/g, dw)	LOQ (ng/g, dw)	Relative recovery (%) [a]	Precision (%) [c]
MePB	0.5	0.97	3.2	78 ± 11	2-36
EtPB	0.2	0.59	1.9	107 ± 6	1-34
PrPB	0.4	0.28	0.9	113 ± 8	0-36
BuPB	0.4	0.36	1.1	113 ± 6	2-28
BePB	0.3	0.42	1.3	109 ± 3 [b]	3-27

LOD: limit of detection; LOQ: limit of quantification; MDL: method detection limit

[a] Matrix spike at 20 ng/g dw level for 6 replicates

[b] d_4-BuPB was used as internal standard for BePB

[c] Precision include the RPD (relative percentage difference) of concentration detected in duplicate extraction and RSD (relative standard deviation) of concentration from triplicate extraction

Table S2-5. EEF (Estradiol Equivalency Factor) values for EEQ calculation

References	EEF				
	MePB	**EtPB**	**PrPB**	**BuPB**	**BePB**
(Lange et al. 2014)	1.25×10^{-7}	6.48×10^{-7}	2.39×10^{-6}	8.09×10^{-6}	1.35×10^{-5}
(van Meeuwen et al. 2008)	5.0×10^{-7}	2.00×10^{-6}	7.94×10^{-6}	1.00×10^{-5}	2.00×10^{-5}
(Schultis and Metzger 2004)	7.20×10^{-7}	2.04×10^{-6}	0.17×10^{-6}	8.98×10^{-6}	10^{-4}
Minimum	1.25×10^{-7}	6.48×10^{-7}	0.17×10^{-6}	8.09×10^{-6}	1.35×10^{-5}
Maximum	7.20×10^{-7}	2.04×10^{-6}	7.94×10^{-6}	1.00×10^{-5}	10^{-4}
Average	4.49×10^{-7}	1.56×10^{-6}	3.50×10^{-6}	9.02×10^{-6}	4.45×10^{-5}

Table S2-6. Detection frequency, concentration and individual contribution of parabens in U.S. sludge (n = 19)

	DF (%)	Concentration (ng/g, dw)				Individual contribution (%)			
		Range	Median	Mean	SD	Range	Median	Mean	SD
MePB	100	15.9–203.0	61.0	81.4	55.0	75.2–96.0	91.9	89.4	6.1
EtPB	63	<0.6–2.6	0.9	1.1	0.7	0.3–11.6	1.2	1.8	2.5
PrPB	100	0.5–7.7	3.5	4.2	2.1	2.2–14.5	5.3	5.7	3.1
BuPB	42	<0.4–4.3	0.25	1.0	1.2	0.1–9.1	0.7	1.5	2.3
BePB	26	<0.4–3.3	0.3	0.8	1.0	0.1–9.5	0.6	1.6	2.7
∑PBs	NA	21.2–213.2	71.2	88.5	56.5		NA		

DF: detection frequency

"NA" indicates not applicable

The values were calculated based on substituting non-detects with MDL/√2

Table S2-7. Median concentration of individual and total parabens in untreated, digested and digested with additional treatment sludges

Sludge Type	Mean concentration (ng/g, dw)					
	MePB	**EtPB**	**PrPB**	**BuPB**	**BePB**	**∑PBs**
Untreated (n=3)	163.1	1.4	5.8	1.6	0.3	172.1
Digested (n=8)	86.4	1.2	4.0	0.9	0.3	92.3
Digested with additional treatment (n=8)	41.3	0.6	2.7	0.3	0.3	45.1

Table S2-8. Spearman correlations among daily average temperature (ºC), concentrations (ng/g, dw) of individual parabens and the total parabens in sewage sludge from WWTP A from 2009-2010 (*n* =18)

Spearman's *Rho*	**Temperature**	**MePB**	**EtPB**	**PrPB**	**BuPB**
MePB	-0.77***				
EtPB	-0.38	0.63**			
PrPB	-0.77***	0.78***	0.71***		
BePB	-0.55*	0.67*	0.60**	0.75***	
∑PBs	-0.78***	0.99***	0.66**	0.81***	0.70***

* Correlation is significant at the 0.05 level (2-tailed)

** Correlation is significant at the 0.01 level (2-tailed)

*** Correlation is significant at the 0.001 level (2-tailed)

Table S2-9. Daily average temperature (ºC) on sampling date (n = 18) at WWTP A between 2009-2010

Date	Temperature (ºC)*
3/25/09	17
5/7/09	28
5/20/09	22
6/3/09	27
6/17/09	32
7/9/09	33
7/21/09	33
8/5/09	33
8/19/09	32
9/2/09	29
9/17/09	24
9/30/09	26
11/4/09	18
12/2/09	8
1/6/10	7
2/3/10	9
3/3/10	11
4/7/10	20

* Data obtained from https://www.wunderground.com

Table S3-1. Sample information

Sample ID	Province	City/District	Sampling date (Month-Year)	Average Treatment Capacity (F, million tons per day)
1	Shanghai	Yangpu	Dec-09	<10
2		Qingpu	Mar-10	10-100
3		Pudong	Apr-10	10-100
4		Jiading	May-10	10-100
5		Qingpu	May-10	10-100
6			May-10	<10
7			May-10	10-100
8		Baoshan	May-10	10-100
9		Puduo	May-10	10-100
10		Changning	May-10	10-100
11		Baoshan	May-10	10-100
12		Qingpu	May-10	10-100
13			May-10	10-100
14		Xuhui	Jun-10	10-100
15		Fengxian	Jul-10	NA
16		Pudong	Jul-10	10-100
17		Minhang	Aug-10	10-100
18		Songjiang	Aug-10	100-300
19			Aug-10	10-100
20		Pudong	Sep-10	100-300
21		Fengxian	Sep-10	100-300
22			Sep-10	10-100
23		Jinshan	Sep-10	10-100
24	Anhui	Hefei	Apr-14	10-100
25			Nov-13	10-100
26			Nov-13	>300
27			Apr-14	>300
			May-14	
28			Jan-12	100-300
29	Jiangsu	Wuxi	Aug-13	100-300
30			Aug-13	10-100
31			Aug-13	100-300
32			Dec-12	<10
33			Dec-12	10-100
34			Dec-12	<10
35		Zhenjiang	Nov-13	10-100

36			May-14	10-100
37			May-14	100-300
38		Suzhou	Apr-14	10-100
39			Apr-14	10-100
40		Wuxi	Aug-13	100-300
41			Jul-13	100-300
42		NA	Oct-13	NA
43		NA	Oct-13	NA
44		Suzhou	Jul-12	10-100
45			Oct-13	10-100
46	Shangdong	Jinan	Nov-12	100-300
47		Heze	Mar-14	10-100
48		Jining	Apr-14	100-300
49		Qingdao	Mar-14	10-100
50		NA	Nov-13	NA
51		Jining	Apr-14	10-100
52	Zhejiang	Jiaxing	Dec-12	>300
53		Lishui	Nov-13	10-100
54		NA	Nov-12	NA
55		Taizhou	Mar-14	10-100
56	Hubei	Xiangyang	Dec-12	10-100
57	Hunan	Changsha	Mar-14	100-300
58		Yongzhou	Mar-14	10-100
59		Changsha	Mar-14	10-100
60	Henan	Kaifeng	Dec-12	100-300
61			Dec-12	10-100
62			Dec-12	10-100
63			Dec-12	10-100
64			Dec-12	10-100
65		Hebi	Dec-12	10-100
66			Dec-12	10-100
67		Kaifeng	Dec-12	10-100
68			Dec-12	10-100
69		Zhengzhou	Nov-12	100-300
70		Hebi	Dec-12	10-100
71	Guangdong	NA	Nov-13	NA
72		Guangzhou	Mar-14	100-300
73	Fujian	Quanzhou	Dec-12	10-100
74	Hainan	NA	Nov-12	NA
75	Liaoning	NA	Mar-14	10-100
76		Jinzhou	Apr-14	10-100
77			Mar-14	10-100

78	Jilin	Changchun	Nov-12	>300
79	Yunnan	Honghe	Apr-14	10-100
80	Sichuan	Mianyang	Mar-14	10-100
81			Oct-13	100-300
82	Guizhou	Guiyang	Mar-14	10-100
83			May-14	100-300
84	Xinjiang	Yili	Aug-12	10-100
85	Shaanxi	NA	Nov-12	NA
86	Tianjin	NA	Nov-12	NA
87*	Shanghai	NA	Nov-13	NA
88*			Mar-14	NA
89*	Fujian	NA	Aug-12	NA
90*	Guangxi	NA	May-13	NA
91*		NA	May-13	NA
92*	Helongjiang	Heilongjiang	Oct-12	NA
93*	Guangdong	NA	Nov-12	NA
94*		NA	May-13	NA
95*	Zhejiang	NA	Nov-12	NA
96*		NA	Jun-13	NA
97*	Jiangsu	NA	Feb-13	NA
98*		NA	Nov-13	NA
99*		NA	Jan-14	NA
100*		NA	Aug-12	NA

* Sample ID from 87-100 were samples from industrial WWTPs

NA: not available

Table S3-2. LC and MS/MS parameters for target analytes and labeled standards

Analyte	RT (min)	MS/MS Transition [a]	DP (V)	EP (V)	CE (V)	CXP (V)	DW (ms)
MePB	5.3	151 > **92**, 136	-60	-10	-30	-5	50
$^{13}C_6$-MePB	5.3	157 > **98**	-60	-10	-30	-5	50
EtPB	5.9	165 > **92**, 136	-55	-10	-30	-15	50
d_5-EtPB	5.8	170 > **92**	-55	-10	-30	-15	50
PrPB	6.4	179 > **92**, 136	-55	-10	-30	-13	50
d_4-PrPB	6.4	183 > **96**	-55	-10	-30	-13	50
BuPB	6.9	193 > **92**, 136	61	10	37	14	50
d_4-BuPB	6.9	197 > **96**	-55	-10	-38	-1	50
BePB	6.8	227 > **92**, 136	-65	-10	-36	-1	50
TCS	8.2	287 > **35**	-50	-10	-30	-3	50
$^{13}C_{12}$-TCS	8.2	300 > **35**	-55	-10	-28	-3	50
TCC	8.0	313 > **160**, 126	-80	-10	-18	-9	50
$^{13}C_6$-TCC	8.0	319 > **160**	-50	-10	-20	-25	50
DCC	7.5	279 > **126**	-70	-10	-20	-11	50
MCC	7.0	244 > **126**	-56	-10	-16	-4	50
NCC	6.2	211 > **126**	-60	-10	-24	-3	50
3'-Cl-TCC	8.4	347 > **160**	-80	-10	-22	-11	50
2'-OH-TCC	7.9	329 > **168**	-65	-10	-18	-9	50
3'-OH-TCC	7.2	329 > **168**	-75	-10	-16	-9	50

[a] Parent ion > **Quantification ion**, Confirmation ion. RT: Retention Time; DP: Declustering Potential; EP: Entrace Potential, CE: Collision Energy; CXP: Collision Cell Exist Potential; DW: Dwell Time

Table S3-3. Method performance parameters including MDLs, LOQs and relative recoveries

Chemical	MDL (ng/g)	LOQ (ng/g)	Recovery (%, n=3)		
			60 ng/g*	300 ng/g*	600 ng/g*
MePB	2.3	7.9	114 ± 3	109 ± 7	105 ± 4
EtPB	1.7	5.8	97 ± 3	97 ± 10	94 ± 5
PrPB	1.6	5.6	99 ± 3	104 ± 3	102 ± 7
BuPB	1.5	5.3	97 ± 1	102 ± 6	98 ± 1
BePB	1.6	5.4	90 ± 2	96 ± 7	98 ± 2
TCS	3.7	17.5	NA	111 ± 21	115 ± 35
TCC	2.6	8.6	NA	88 ± 8	101 ± 14
2'-OH-TCC	1.4	4.7	128 ± 44	105 ± 5	99 ± 2
3'-OH-TCC	1.3	4.4	97 ± 18	100 ± 6	86 ± 4
3'-Cl-TCC	1.5	5.1	100 ± 23	86 ± 4	83 ± 6
DCC	2.0	9.4	NA	90 ± 31	87 ± 17
MCC	1.5	5.1	NA	120 ± 26	108 ± 8
NCC	2.6	8.6	NA	126 ± 27	119 ± 15

*Spiking level

NA: not applicable. The spiking level was too low to generate good recoveries due to the pre-existed target analytes existed in the matrix

Table S3-4. Summary of detection frequencies and concentrations of target analytes in sewage sludge samples collected across China

Chemical	All samples (*n* = 100)				Municipal samples (*n* = 86)				Industrial samples (*n* = 14)			
	DF (%)	Concentration* (ng/g, dw)			DF (%)	Concentration* (ng/g, dw)			DF (%)	Concentration* (ng/g, dw)		
		Range	Median	Mean		Range	Median	Mean		Range	Median	Mean
MePB	98	5-630	46	67	100	12-630	54	74	86	6-40	15	17
EtPB	96	6-170	9	10	99	7-170	9	13	79	6-19	8	9
PrPB	99	5-27	9	9	100	6-27	9	9	93	6-20	6	8
BuPB	89	7-11	8	8	91	7-11	9	9	79	7-11	8	8
BePB	7	9-12	11	10	2	10-11	11	11	36	9-12	11	10
TCS	99	7-4870	780	1100	10	60-4870	970	1240	93	7-1310	28	170
TCC	95	30-43300	6130	8900	100	380-43300	6700	9630	64	30-13940	110	1860
2'-OH-TCC	94	20-2340	150	290	100	32-2340	170	307	57	20-180	25	47
3'-OH-TCC	91	6-1250	50	108	100	6-1250	50	110	21	7-74	12	31
3'-Cl-TCC	100	22-580	82	113	100	29-580	90	125	86	22-102	28	36
DCC	94	38-23890	280	760	100	43-23890	310	820	57	39-520	50	111
MCC	92	22-120	31	35	99	22-120	31	35	50	24-32	27	28
NCC	90	4-1340	33	100	97	6-1340	31	100	50	4-480	35	94
∑PBs	\	5-810	75	95	\	35-810	80	105	\	6-70	38	39
∑All	\	44-63740	8070	10850	\	680-63740	8740	12340	\	44-16210	190	1540

***** Concentration ranges, medians and means were based on detects only.

Table S3-5. Geometric mean (GM) concentrations (ng/g) of ∑PBs, TCS and ∑TCCs in sludge from different provinces, and province-specific per capita sludge production (kg/d) and per capita GDP (thousand dollars)

Province	**NO. of samples**	**GM concentration (ng/g)**			**Per capita sludge production (kg/d)** (Yang et al. 2015)	**Per capita GDP (thousand dollars)** (Yang et al. 2015)
		∑PBs	TCS	∑TCCs		
Guizhou	2	121	661	8051	2.36	3.7
Xinjiang	1	70	1468	6614	2.78	3.7
Yunnan	1	93	378	2771	2.4	4
Anhui	5	121	1116	16952	3.58	5.1
Sichuan	2	186	754	10137	2.82	5.3
Henan	11	94	407	3304	4.14	5.4
Shaanxi	1	44	1082	5041	2.82	5.6
Hainan	1	57	336	11398	4.28	5.6
Hunan	3	116	713	3295	3.4	5.9
Hubei	1	66	1153	10647	4.54	6.9
Jilin	1	52	1251	26893	3.98	7.5
Shandong	6	87	742	7076	5.34	8.9
Fujian	1	44	204	2588	4.96	9.3
Guangdong	2	151	1312	8612	7.62	9.3
Liaoning	3	154	1251	9624	5.84	9.9
Zhejiang	4	80	1477	6793	8.32	11
Jiangsu	17	80	703	5848	7.32	11.8
Shanghai	23	72	2201	16511	12.6	14.5
Tianjin	1	71	1091	7552	7.36	16.3

Table S4-1. LC and MS/MS parameters for target analytes and labeled standards

Analyte	RT (min)	MS/MS Transition [a]	DP (V)	EP (V)	CE (V)	CXP (V)	DW (ms)
MePB	5.3	151 > **92**, 136	-60	-10	-30	-5	50
$^{13}C_6$-MePB	5.3	157 > **98**	-60	-10	-30	-5	50
EtPB	5.9	165 > **92**, 136	-55	-10	-30	-15	50
d_5-EtPB	5.8	170 > **92**	-55	-10	-30	-15	50
PrPB	6.4	179 > **92**, 136	-55	-10	-30	-13	50
d_4-PrPB	6.4	183 > **96**	-55	-10	-30	-13	50
BuPB	6.9	193 > **92**, 136	61	10	37	14	50
d_4-BuPB	6.9	197 > **96**	-55	-10	-38	-1	50
BePB	6.8	227 > **92**, 136	-65	-10	-36	-1	50
TCS	8.2	287 > **35**	-50	-10	-30	-3	50
$^{13}C_{12}$-TCS	8.2	300 > **35**	-55	-10	-28	-3	50
TCC	8.0	313 > **160**, 126	-80	-10	-18	-9	50
$^{13}C_6$-TCC	8.0	319 > **160**	-50	-10	-20	-25	50

[a] Parent ion > **Quantification ion**, Confirmation ion

RT: Retention Time; DP: Declustering Potential; EP: Entrace Potential, CE: Collision Energy; CXP: Collision Cell Exist Potential; DW: Dwell Time

Table S4-2. Summary of sieving sizes, sampling locations, sample preparations, analytical methods, and method performance parameters for targeted antimicrobials in indoor dust

Ref	Sieving size	Sampling location	Extraction method	Further treatment	Analytical instrument	Isotope dilution (Y/N)	Recovery	Repeatability /Reproducibility
Geens (2009)	<500 µm	Houses Offices	Liquid-solid extraction, sonication and vortex	SPE (Florisil)[a]	LC-MS/MS	Y	NA	Repeatability: RSD=2% for TCS
Ramírez (2011)	<100 µm	Private homes	Pressurized hot water extraction and stir bar sorption extraction	Derivatization	GC-MS	N	40-80%	Repeatability 2-8% Reproducibility 2-10 %
Canosa (2007a)	<60 µm	Private homes	Matrix solid-phase dispersion	Filtration Derivatization	GC-MS/MS	N[b]	80-114%	Repeatability 3-6% Reproducibility 3-13 %
Canosa (2007b)	<75 µm	Private homes Office buildings	Pressurized liquid extraction	Derivatization	GC-MS/MS	N[b]	76-98%	Repeatability 5-8% Reproducibility 6-11%
Fan (2010)	<80 µm	Private homes	Liquid-solid extraction, sonication and vortex	SPE (HLB) Derivatization	GC-MS	Y	76-92%	Repeatability 4-15% Reproducibility 11-16% Average RSD from samples<18%
Tran (2016)	<150 µm	Homes Supermarkets Electronic stores Pharmacies Labs Offices	Liquid-solid extraction, mechanical shaking	SPE (MCX)	LC-MS/MS	Y	69-97%	NA
Ao (2017)	<75 µm	Residences Dorms Offices	Accelerated solvent extraction	SPE (HLB)	GC-MS/MS	Y	71-95%[c]	RSDs <6%[c]
Wang (2012)	<2 mm	Houses Apartments Offices Labs	Liquid-solid extraction, mechanical shaking	SPE (MCX)	LC-MS/MS	Y	97-115%	Selected duplicate CV<15%
Rudal (2003)	<150 µm	Homes	Liquid-solid extraction, sonication	Derivatization	GC-MS	Y	NA[d]	Average percent difference<20%
Hartmann (2016)	No sieving	Complex building	Liquid-solid extraction, sonication	SPE (HLB)	LC-MS/MS	Y	79-97%	NA
This study	No sieving	Athletic facilities Private homes	Liquid-liquid extraction, sonication and vortex	d-SPE	LC-MS/MS	Y	83-115%	Repeatability 3-30% Average RSDs from all the samples<38%

NA: not available; SPE: solid-phase extraction; d-SPE: dispersive solid-phase extraction

[a] Cartridges used for SPE were included in parentheses;

[b] Quantified using external calibration;

[c] Determined by spiking in blank samples;

[d] Recoveries reported from 40% to 220% for all analytes, but recoveries for parabens were not available;

Ref (Ao et al. 2017, Canosa et al. 2007a, Canosa et al. 2007b, Fan et al. 2010b, Geens et al. 2009, Hartmann et al. 2016a, Ramírez et al. 2011, Rudel et al. 2003, Tran et al. 2016, Wang et al. 2012a)

Table S4-3. Summary statistics of individual paraben, total parabens, triclosan and triclocarban concentrations in 80 indoor dust

Concentration (ng/g)	MePB	EtPB	PrPB	BuPB	BePB	∑PBs	TCS	TCC
Min	50	9	70	6	2	140	20	20
Q1	983	83	485	50	5	1750	263	110
Median	1920	195	965	80	6	3490	390	270
Q3	3413	368	2095	210	8	6278	768	648
Max	26200	1060	11150	860	27	39090	3270	9760
Mean	2816	253	1594	162	7	4832	558	497
SD	3635	222	1825	186	4	5604	525	1106
GM	1848	161	1011	91	6	3240	387	247

SD: standard deviation

GM: geometric mean

Table S4-5. Weighted average concentrations of parabens found in personal care products (PCPs) from the United States and China, respectively.

Weighted-average concentration (μg/g)	MePB	EtPB	PrPB	BuPB	BePB	Ref
New York State, U.S. (*n*=170)	490	78	191	52	0.13	(Guo and Kannan 2013)
Tianjing, China (*n*=52)	666	20	376	10	\	(Guo et al. 2014)

Table S4-66. Spearman correlations among concentrations of individual antimicrobials in indoor dust (n = 80)

Spearman's *Rho*	MePB	EtPB	PrPB	BuPB	BePB	TCS
EtPB	0.70**					
PrPB	0.89**	0.71**				
BuPB	0.68**	0.70**	0.76**			
BePB	0.22	0.22	0.32**	0.22		
TCS	0.44**	0.47**	0.48**	0.42**	0.34**	
TCC	0.35**	0.42**	0.38**	0.45**	0.43**	0.57**

* $p<0.05$; ** $p<0.01$

Table S5-1. Target analytes and selected physicochemical properties

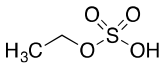
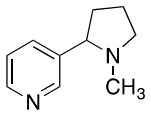
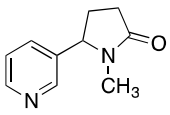
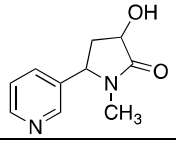
Chemical	Chemical Structure	pKa	Molecular weight	Log K_{ow}
Ethyl sulfate (EtS)		-2.1	126.13	-2.49
Nicotine (NIC)		8.0	162.23	1.17
Cotinine (COT)		4.7	176.22	0.07
3-hydroxycotinine (3-OH-COT)		13.0	192.21	-1.48

Table S5-2. Population of each community and selected characteristics of WWTPs sampled in this study

Community ID	Population	Average daily flow (MGD*)	Wastewater Source		
			Domestic	Industrial	Other
A	125000	12.6	72	28	0
B	44000	7.7	54	28	18
C	53000	27.4	55	45	0

*MGD: million gallons per day

Table S5-3. LC and MS/MS parameters for target analytes and labeled standards

Analyte	RT (min)	MS/MS Transition [a]	DP (V)	EP (V)	CE (V)	CXP (V)	DW (ms)
EtS	4.6	125 > **97**, 80	-45	-10	-22	-1	200
EtS-d_5	4.6	130 > **98**, 80	-45	-10	-26	-7	200
NIC	3.7	163 > **84**, 80	46	10	29	4	50
COT	5.2	177 > **80**, 98	61	10	37	14	50
COT-d_3	5.2	180 > **80**, 101	66	10	37	14	50
3-OH-COT	4.5	193 > **80**, 134	76	10	47	16	50

[a] Parent ion > **Quantification ion**, Confirmation ion

RT: Retention Time; DP: Declustering Potential; EP: Entrace Potential, CE: Collision Energy; CXP: Collision Cell Exist Potential; DW: Dwell Time

Table S5-4. Difference in daily alcohol consumption rate between weekend vs weekday and between entire week vs weekday from other studies.

Ref	Difference between daily weekend alcohol consumption rate vs daily weekday alcohol consumption rate[a]	Difference between overall daily alcohol consumption rate and weekday daily alcohol consumption rate[b]
(Mastroianni et al. 2014)	73%	21%
(Rodriguez-Alvarez et al. 2014a)	58%	17%
(Andres-Costa et al. 2016)	105%	19%
(Boogaerts et al. 2016)	48%	13%
(Ryu et al. 2016)	63%	18%
(Gatidou et al. 2016)	77%	22%

[a] $(\text{Consumption}_{\text{weekend}} - \text{Consumption}_{\text{weekday}}) \times 100\% / \text{Consumption}_{\text{weekday}}$

[b] $(\text{Consumption}_{\text{overall}} - \text{Consumption}_{\text{weekday}}) \times 100\% / \text{Consumption}_{\text{weekday}}$

Table S5-5. Measured concentrations (µg/L) and summary statistics of EtS, NIC, COT and 3-OH-COT.

Community A					Community B					Community C				
	Concentration (µg/L)					Concentration (µg/L)					Concentration (µg/L)			
Sampling date	EtS	NIC	COT	3-OH-COT	Sampling date	EtS	NIC	COT	3-OH-COT	Sampling date	EtS	NIC	COT	3-OH-COT
3/17/15	20.2	9.3	2.5	3.1	4/29/15	22.8	1.3	2.3	1.8	4/14/15	2.2	1.9	0.2	0.3
4/27/15	25.1	8.6	2.4	3.1	5/28/15	3.0	0.6	0.8	0.8	5/18/15	5.1	2.6	0.4	0.5
5/28/15	12.2	11.0	2.8	3.8	6/22/15	6.1	1.4	0.9	0.8	6/15/15	4.0	1.4	0.4	0.3
7/22/15	13.7	26.7	2.9	1.1	7/22/15	3.4	0.7	0.8	0.5	7/24/15	14.4	2.9	0.6	0.6
8/25/15	15.1	23.5	2.7	0.7	8/27/15	7.9	2.0	2.8	1.3	8/17/15	9.7	2.3	0.7	0.6
9/28/15	16.8	11.9	3.8	3.5	9/29/15	8.1	2.8	2.4	0.8	9/23/15	10.0	2.1	0.7	0.7
10/29/15	11.9	7.9	2.6	2.3	10/29/15	3.4	1.8	0.8	0.4	10/23/15	6.2	1.4	0.6	0.5
11/24/15	17.2	9.9	2.9	2.4	11/18/15	7.8	5.4	2.0	1.1	11/23/15	5.3	3.1	0.7	0.6
12/22/15	14.0	9.9	2.9	2.4	12/15/15	9.9	13.1	3.0	2.5	12/16/15	4.5	2.9	0.6	0.4
1/25/16	20.8	13.9	2.8	2.8	1/21/16	8.8	12.9	2.9	2.4	1/19/16	4.8	2.9	0.6	0.5
3/8/16	17.2	18.6	2.7	2.9	2/25/16	1.6	1.4	0.4	0.6	2/22/16	9.0	2.3	0.4	0.5
Min	11.9	7.9	2.4	0.7		1.6	0.6	0.4	0.4		2.2	1.4	0.2	0.3
Max	25.1	26.7	3.8	3.8		22.8	13.1	3.0	2.5		14.4	3.1	0.7	0.7
Average	16.8	13.7	2.8	2.5		7.5	3.9	1.7	1.2		6.8	2.5	0.6	0.5
SD	4.0	6.4	0.4	0.9		5.8	4.7	1.0	0.8		3.5	1.0	0.2	0.1