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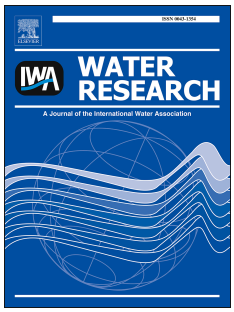

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PII: S0043-1354(18)30232-X

DOI: 10.1016/j.watres.2018.03.036

Reference: WR 13655

To appear in: *Water Research*

Received Date: 20 December 2017

Revised Date: 2 March 2018

Accepted Date: 13 March 2018

Please cite this article as: Gao, J., Li, J., Jiang, G., Yuan, Z., Eaglesham, G., Covaci, A., Mueller, J.F., Thai, P.K., Stability of alcohol and tobacco consumption biomarkers in a real rising main sewer, *Water Research* (2018), doi: 10.1016/j.watres.2018.03.036.

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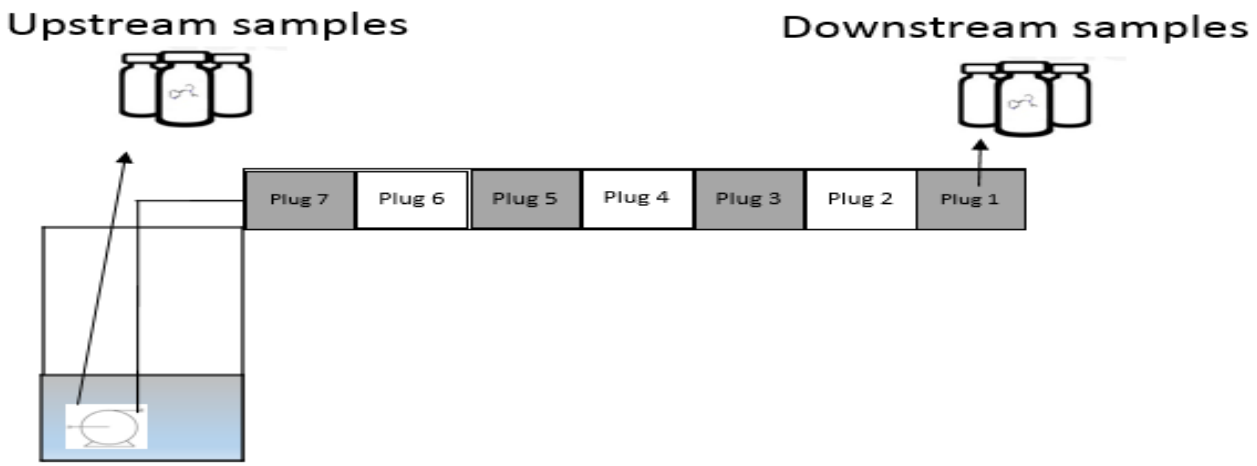
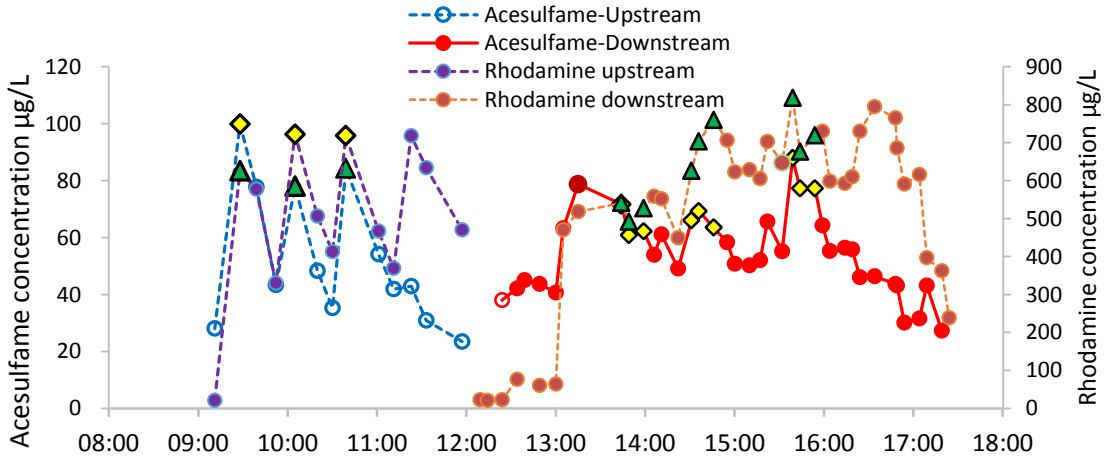
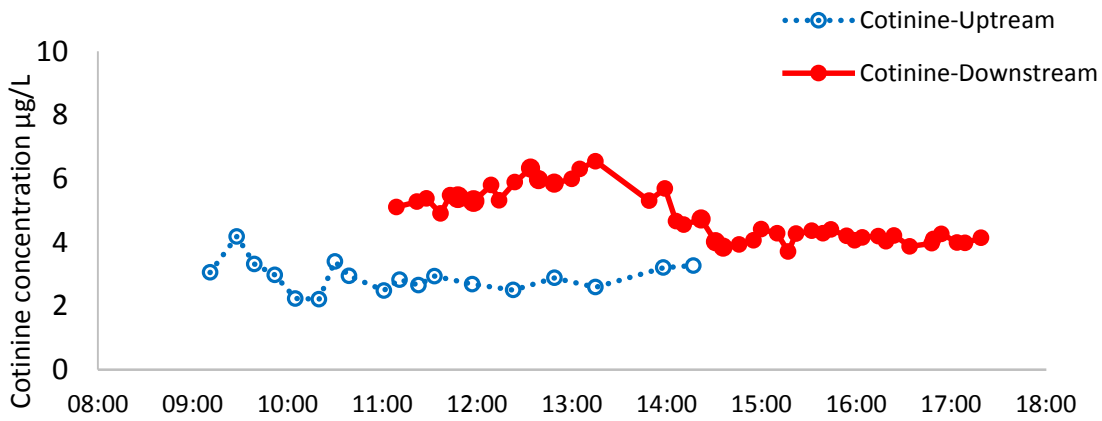


Benchmarker and flow tracer

Biomarker in upstream and downstream





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Stability of alcohol and tobacco consumption biomarkers in a real rising main sewer

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Highlights

- First study to test the stability of alcohol and tobacco biomarkers in a real sewer
- Ethyl sulfate is much more stable than ethyl glucuronide in the rising main sewer
- Strong de-conjugation in the sewer can interfere with the stability assessment
- Results from benchmarking method and absolute concentration were comparable

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ABSTRACT

Since alcohol and tobacco consumption are among the leading causes of population health harm, it is very important to understand the consumption behaviour to develop effective harm reduction strategies. Wastewater-based epidemiology (WBE) is a potential tool for estimating their consumption, but there are several uncertainties that need to be determined, including the stability of biomarkers in the sewer. Utilizing a real rising main sewer, this study investigated the stability of alcohol and tobacco consumption biomarkers. Rhodamine and acesulfame were used as flow tracer and benchmarker to understand the transportation of wastewater in the sewer with a hydraulic retention time between 2.7 and 5.0 h. Ethyl sulphate (EtS) and ethyl glucuronide (EtG), two biomarkers of alcohol consumption, were found to have different in-sewer stability, with EtS much more stable than EtG. The degradation rate of EtS is approximately 8% per hour, while EtG has a half-life of 1.9 h. Formation of nicotine, cotinine and trans-3'-hydroxycotinine, three biomarkers for tobacco consumption, was observed during the experiment, probably due to deconjugation of their glucuronide chemicals. The deconjugation process has prevented the determination of actual stability of the three chemicals. However, it is suggested that cotinine is relatively stable, while nicotine and trans-3'-hydroxycotinine degrade to a certain degree in the sewer system. According to our findings, the in-sewer degradation is more important during the interpretation of alcohol consumption estimation than for tobacco consumption estimation.

Keywords: Alcohol and tobacco; Benchmarking; Biomarker stability; LC-MS/MS; Wastewater-based epidemiology

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1. Introduction

Alcohol and tobacco are the most popular legal stimulants in the world (WHO 2015, 2017). Consumption of alcohol and tobacco can cause considerable health problems in the population; for example cardiovascular diseases and various types of cancers (Castaldelli-Maia et al. 2016, Jemal et al. 2011). To develop integrated strategies to reduce the social and health burdens associated with alcohol and tobacco consumption, it is very important to understand the consumption behaviour of these substances in as much detail as possible. Traditional methods of consumption estimation involve sales statistics (Black et al. 2011) and population surveys (Bush et al. 1998, WHO 2000), which are subject to sampling limitations and usually are time-consuming and require monetary resources. Wastewater-based epidemiology (WBE) is an alternative approach to monitor consumption of substances in the population, including illicit drugs and psychoactive substances. WBE is based on the analysis of trace level of substance residues in influent wastewater including the parent drug and/or human metabolites. Using refined correction factors for human excretion and stability, together with daily flow and catchment population, substance consumption in the catchment population can be back-calculated. It has the advantage of cost-effectiveness and high resolution sampling compared with conventional epidemiology, as the influent wastewater can be treated as diluted human excretion. (EMCDDA, 2016b; Lai et al., 2016; Li et al., 2014; Thai et al., 2016). Recently, alcohol and tobacco consumption in different settings has been estimated by WBE to provide valuable information for temporal and geographical consumption behaviour (Andres-Costa et al. 2016, Castiglioni et al. 2015, Lai et al. 2017, Mastroianni et al. 2014, Tscharke et al. 2016, van Wel et al. 2016).

The term biomarker in WBE refers to the parent drug or human metabolites of substances that can be quantitated in wastewater. Biomarker stability is recognised as an important factor contributing to the overall uncertainties of estimating consumption of substances within a catchment in WBE (Castiglioni et al. 2013, Senta et al. 2014, van Nuijs et al. 2012). For biomarkers of alcohol and tobacco, most of the stability studies so far were carried out in the laboratory with bulk wastewater

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without the presence of sewer biofilm (McCall, et al. 2015, Rodríguez-Álvarez et al. 2014, Rodriguez-Alvarez et al. 2014, Tscharke et al. 2016). Only one study has investigated the stability of alcohol and tobacco biomarkers in simulated sewer conditions using laboratory sewer reactors (Banks et al. 2017). Since the dynamics of wastewater and activity of biofilms in actual sewers can affect the degradation of chemicals in a different way than the simulated conditions (Huisman 2001, McCall et al. 2016, Zwiener and Frimmel 2003), the findings of the above-mentioned laboratory studies need to be validated against data from real sewers. Jelic et al. (2015) and McCall et al. (2017) have investigated the fate of pharmaceuticals and illicit drugs in real sewers, and found degradation and formation of different chemicals in the sewer. However, a lesson to be drawn from those studies is that for investigation of biomarker stability in real sewers, more accurate and sufficiently long hydraulic retention time of the wastewater samples should be employed, such as the recently reported study (Li et al. 2018).

The real sewer is a dynamic system regarding the wastewater flow, which leads to turbulent mixing and variable sewer HRT (hydraulic retention time), an important factor influencing the transformation of chemicals in the sewer (Kapo et al. 2017). Biofilms developed in the inner sewer surface are another important component in the organic matter transformation due to their strong bioactivity (Gutierrez et al. 2016, Jiang et al. 2015). The presence of gravity and rising main biofilms was observed to be able to enhance the degradation of biomarkers in laboratory-scale sewer reactors (Gao et al. 2017, Thai et al. 2014). Benchmarking is a method to assess chemical stability in the environment that can compensate for the dynamic flow in the sewer by using the concentration ratio of the chemical of interest against a stable chemical (McLachlan et al. 2017). Furthermore, benchmarking allows for ready comparison and ranking of the persistence of different chemicals (Zou et al., 2015). The technique was used to study the stability of PPCPs in lakes (McLachlan et al. 2017, Zou et al. 2014, Zou et al. 2015), as well as to evaluate leaks in sewers (Rieckermann et al. 2007). It is thus important to evaluate whether the benchmarking technique could be used to conduct stability tests in the real sewer when the application of mass balance

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approach is difficult.

In this study, we aimed to evaluate the stability of alcohol and tobacco consumption biomarkers, ethyl sulphate (EtS), ethyl glucuronide (EtG), nicotine (Nic), cotinine (Cot) and trans-3'-hydroxycotinine (OH-Cot) in a real rising main sewer. We also aimed to evaluate whether there are any advantages in using the benchmarking approach for stability assessment. The insights gained from this study about biomarker transportation and transformation in the sewer can improve the estimation of alcohol and tobacco consumption in WBE studies.

2. Materials and methods

2.1 Chemicals and Reagents

Acesulfame-K, ethyl-sulphate, ethyl-glucuronide, nicotine, cotinine, trans-3'-hydroxycotinine, acesulfame-d4, ethyl-sulfate-d5, ethyl-glucuronide-d5, cotinine-d3, were purchased from Sigma Aldrich (Castle Hill, Australia). The properties of the biomarkers are presented in **Table S1**. Rhodamine was purchased from Kingscote Chemicals. Dihexyl-ammonium-acetate was purchased from Sigma Aldrich (Japan). Analytical grade hydrochloric acid (32%) was purchased from Univar (Ingleburn, Australia). LCMS grade methanol was purchased from Merck (Germany). Deionized water was produced by a MilliQ system (Millipore, 0.22 μm filter, 18.2 $m\Omega \cdot cm^{-1}$).

2.2 The UC9 sewer

The experiment was carried out in a rising main sewer, named UC9 sewer, located in Gold Coast, Queensland, Australia. The UC9 sewer is 1080 m long and 150 mm in diameter, resulting in an area/volume ratio (A/V) ratio of 26.7 m^{-1}. The pump in the pumping station was operated in an ON/OFF manner. When the water level reached 19.5% of the total wet well volume, the pump was ON and when the water level dropped to 8.5% of the total wet well volume, the pump was OFF. Each pumping event typically lasted for 1-3 minutes. The map of UC9 sewer with locations of the

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upstream and downstream sampling points is provided in **Figure S1**.

The rising main pipe transports domestic sewage with an average dry weather flow of approximately 126 m^3/day, servicing about 550 people living in the catchment. The hydraulic retention time (HRT) of wastewater varies from 1.5 to 6.0 hours, depending on time of the day (Guisasola et al. 2008, Mohanakrishnan et al. 2009) (as shown in **Figure S2**). Previous monitoring showed that UC9 had strong anaerobic bioactivities, with sulfide and methane in the downstream sampling point being in the range of 8-12 mg S/L and 20-120 mg COD/L respectively.

2.3 Flow tracer and benchmarker spiking and sampling of wastewater

Rhodamine, a pink-coloured flow tracer, was used to understand the movement of the wastewater plugs in the rising main sewer. Acesulfame, an artificial sweetener that is stable under the simulated sewer conditions (O'Brien et al. 2017), was used as a benchmarker. The benchmarking method is similar to that used previously in the evaluation of PPCPs stability in lakes (McLachlan et al. 2017, Zou et al. 2015). Mixtures of acesulfame and rhodamine were spiked to the upstream well (pumping station) every two pumping events for three or four times a day. In each spiking, 100 mg of acesulfame and 1100 mg of rhodamine dissolved in 300mL MilliQ water was poured into the wet well after the pump stopped. The wastewater in one pumping event, having a volume of 1.8 m^3, was treated as a "wastewater plug" (as shown in **Figure S3**). The biomarkers of alcohol and tobacco consumption were not spiked, since preliminary testing showed that they are present in the wastewater at quantifiable levels.

Samples of wastewater were taken from the pump station wet well (upstream) and the downstream sampling point (828 m from the upstream sampling point). During pump-off period, three samples were taken upstream at water levels of 9%, 14.5% and 18% respectively using a grab sampler. Downstream samples were taken 1 min after the pump-on (during the pump event), 5 min and 15 min after the pump-off at the downstream sampling point using a peristaltic pump. Samples for

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biomarker analysis were acidified with 2 M HCl on site and transported to the lab on ice. Samples for analysis of inorganic sulfur species (sulfate, sulfide, sulfite and thiosulfate) and dissolved methane were also treated on site according to the methods described in Guisasola et al. (2008), which were subsequently measured within 24 hours. Samples for other wastewater parameters, i.e. volatile fatty acids (VFA), ammonia, total and volatile suspended solids (TSS and VSS), total and volatile chemical oxygen demand (TCOD and VCOD), were prepared in the lab within 24 hours. The experiment was conducted in triplicate (i.e. in three days).

2.4 Instrumental analysis

Consumption biomarkers for alcohol, i.e. ethanol metabolites of ethyl-sulphate (EtS) and ethyl glucuronide (EtG), were determined using direct injection by LC-MS/MS using a Shimadzu Nexera HPLC system (Shimadzu Corp., Kyoto, Japan) coupled to a Sciex API 5500Q mass spectrometer (Sciex, Concord, Ontario, Canada) equipped with an electrospray (TurboV) interface (Reid et al. 2011). Ten μL of 1 mg/L labelled analogues of the analytes was added to each 1 mL filtered and acidified wastewater sample, and 10 μL was injected into the column. Separation was achieved using a Phenomenex EVO C18 column (50 x 2.0 mm, 1.7 μm, Phenomenex, Torrance, CA) kept at 45℃ and a flow rate of 0.27 mL min^{-1}. The linear gradient starts at 0% B ramped to 100% B in 3.0 minutes, then held at 100% for 2.0 minutes, followed by equilibration at 0% B for 4.0 minutes. (A = 5 mM dihexyl ammonium acetate in HPLC grade water, B = 5 mM dihexyl ammonium acetate in methanol). A Gemini NX C18 column (50 x 2 mm, 3 μm, Phenomenex, Torrance, CA) was used to trap mobile phase contaminants. The mass spectrometer was operated in the negative ion multiple reaction-monitoring mode using nitrogen as the collision gas. Mass spectrometer parameters are shown in **Table S2**.

Tobacco consumption biomarkers, Nic, Cot and OH-Cot, and the benchmarker acesulfame were determined by the same LC-MS/MS system in direct injection mode (Banks et al. 2017). Separation was achieved using a Phenomenex Kinetex Biphenyl column (50 x 2 mm, 2.6 μm

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Phenomenex, Torrance, CA) kept at 45°C. The flow rate is 0.3 mL/min with a linear gradient starting at 5% B ramped to 100% B in 10.0 minutes then held at 100% for 4.5 minutes followed by equilibration at 5% B for 4.0 minutes. (A = 0.1% formic acid in HPLC grade water, B = 0.1% formic acid in methanol). The mass spectrometer was operated in the positive/negative ion switching, scheduled multiple reaction-monitoring mode, using nitrogen as the collision gas. Mass spectrometer parameters are shown in **Table S2**.

Positive samples were confirmed by retention time and by comparing transition intensity ratios between the sample and an appropriate concentration standard from the same run. Samples were reported as positive if the two transitions were present, retention time was within 0.15 minute of the standard and the relative intensity of the confirmation transition was within 20% of the expected value. The value reported was that for the quantitation transition. The method performance data including LOD, method accuracy, and precision are shown in **Table S3**.

Rhodamine concentration in wastewater was measured by a rhodamine monitoring system, which comprises a portable Cyclops®-7 Submersible Rhodamine Sensor coupled with a Cyclops® Explorer. The temperature of wastewater samples was measured on site using a portable meter with temperature probe (TPS Aqua-pH pH/Temp meter). Analytical methods for wastewater biological parameters are provided in the SI.

2.5 Data processing and statistical analysis

The concentration of wastewater samples taken at a water level of 18% (maximum level that triggers the pumping event) in the pumping station wet well was used to represent the upstream concentration of biomarkers and flow tracers. The average concentration of the three samples collected in the downstream sampling point was used to represent the downstream concentration of each wastewater plug. The stability was evaluated by comparing concentrations of biomarkers in upstream and downstream samples from the same wastewater plug using the absolute concentration

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and the normalised concentration biomarkers using acesulfame as shown in **Equations 1 & 2**.

$$P_{abs}\ (\%) = \frac{Ci,downsteam}{Ci,upstream} * 100 \qquad \textbf{Equation 1}$$

$$P_{benmk}\ (\%) = \frac{\frac{Ci,downstream}{Cace,downstream}}{\frac{Ci,upstream}{Cace,upstream'}} * 100 \qquad \textbf{Equation 2}$$

where

- P_{abs} is the percentage of biomarkers concentration in the downstream sample compared with the upstream sample in the same wastewater plug;
- P_{benmk} is the percentage of biomarkers concentration normalized by benchmarker concentration in the same plugs from downstream and upstream;
- $C_{i,upstream}$ is the concentration of biomarker *i* in the upstream sample;
- $C_{i,downstream}$ is the average concentration of biomarker *i* in the 3 samples collected in the same plug downstream;
- $C_{ace,upstream}$ is the concentration of acesulfame in the upstream sample;
- $C_{ace,downstream}$ is the average concentration of acesulfame in the 3 samples collected in the same plug downstream.

The HRT was calculated according to the pump operational data recorded by the online supervisory control and data acquisition (SCADA); the flow tracer concentration in upstream and downstream; and the total volume of the pipe and the volume of wastewater pumped in each pump event.

Biomarker transformation kinetics were evaluated using linear regression and first order kinetics. We assume there was no transformation with HRT 0 hour (e.g. the bulk wastewater plug right before the pump-on). The transformation in all the plugs investigated was evaluated in the two models, and the model with higher R^2 value was selected. If the R^2 value is less than 0.8, we think neither model can describe the observed transformation.

A paired nonparametric test (Wilcoxon matched-pairs signed rank test) was used to examine

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whether normalization to acesulfame concentration (benchmarking method) makes significant difference to the level of transformation. Correlation of biomarker transformation to HRT was investigated by plotting the HRT of all 21 plugs with their corresponding transformation levels.

3. Results and discussion

3.1 Wastewater composition and bioactivity in the sewer

Diurnal variations of wastewater compositions were observed due to the dynamic release of chemicals in the catchment. Sulfide and methane showed continuous generation, indicating strong anaerobic biological activity (**Figure 1**). Sulfide production rate was 1.63 ± 0.12 g S m^{-2} d^{-1} and the activity of methanogenic archaea was 4.50 ± 0.81 g COD m^{-2} d^{-1}, being comparable to the rising main sewer reactor used in our previous studies (Gao et al. 2017, Thai et al. 2014). Wastewater pH dropped by approximately 0.5 units due to the generation of acidic chemicals in sewer processes, such as the formation of VFAs through fermentation (**Figure 1**). The variations in wastewater compositions and bioactivity in this study were comparable with the previous lab-scale and full-scale monitoring in rising main sewer systems (Foley et al. 2009, Guisasola et al. 2009, Sharma et al. 2013).

3.2 Profile of the flow tracer and benchmarker in the sewer

The concentration profiles of rhodamine and acesulfame in the upstream and downstream match well. The ratio of downstream/upstream concentration of rhodamine and acesulfame is 1.10±0.47 and 1.03±0.32 (n=21) indicating good mass balance of the flow tracer and benchmarker in the sewer (**Figure S5**). The profile of rhodamine and acesulfame reflected the transportation of wastewater plugs in the UC9 sewer. The spiking of acesulfame and rhodamine to the pump station wet well increased the upstream concentration significantly. Certain dispersion and mixing was

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observed between plugs due to the high concentration difference (as shown in **Figure S5**) and the turbulence created by the pumping events. Overall, the use of rhodamine has facilitated the monitoring of sewage flow through the sampling event and hence accurate wastewater HRT for individual plugs could be calculated. The concentration profile of investigated biomarkers in upstream and downstream of UC9 sewer is also discussed in the **SI**.

3.3 Stability of alcohol and tobacco biomarkers in the rising main sewer

3.3.1 EtS and EtG

There was 77±13% EtS in the downstream plugs compared with the same plugs in the upstream wet well as calculated using the absolute concentration. Using the acesulfame benchmarking method, the degradation was slightly higher with 72±25% EtS/Ace in the downstream (**Table 1**). Unlike the results from the rising main sewer reactor (Banks et al. 2017), the transformation of EtS in this study cannot fit well with either linear regression (zero-order) or first-order kinetics as shown in **Table 2**. In the rising main sewer reactor, the degradation of EtS can be described with first-order kinetics (R^2=0.904) with a half-life of 1.27 hours (Banks et al., 2017). The discrepancy could attribute to the limited range of HRT and the more complex and dynamic conditions in the real sewer that can affect the degradation of EtS. According to the results observed in the real sewer, EtS can still be used as the alcohol consumption biomarker but in-sewer stability need to be considered in catchment with high A/V and long HRT.

EtG degraded more rapidly than EtS in the same wastewater plugs investigated (**Figure 2**). Within 2.7-5.0 hours HRT, only 16±11% EtG remains in the downstream plugs (**Table 1**). EtG had a half-life of 1.89 hours in the real rising main sewer, while in the rising main sewer reactor, the half-life was 0.36 hour (Banks et al., 2017). The relatively slower degradation in the real sewer could attribute to the fact that the real sewer has a lower A/V ratio (26.7 m^{-1} in UC9 compare with 72.5 m^{-1} in the sewer reactor) and relatively poorer mixing conditions.

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The fast degradation of EtG in the sewer made it unsuitable as the alcohol consumption biomarker in WBE. However, it could still be used as a biomarker for urine analysis in forensic applications, because in contrast with the fast degradation in the sewer, EtG was stable in urine samples stored at room temperature up to 140 hours (Wurst et al. 1999). The degradation of EtG in the control sewer reactor without biofilm also had much slower degradation than the rising main reactor, suggesting that the sewer biofilm is likely the major contributor to the in-sewer degradation of EtG.

3.3.2 Nic, Cot and OH-Cot

In this study, it is likely that more Nic, Cot and OH-Cot was generated in the wastewater plugs during transportation in the real sewer (**Figure 3**). Within 2.7-5.0 hours HRT, Nic, Cot and OH-Cot in the downstream plugs increased to 114±17%, 170±38 and 132±27% of their corresponding upstream plugs (n=21), respectively (**Table 1**). The formation of Nic, Cot and OH-Cot was likely attributed to the de-conjugation of the glucuronide-compounds as demonstrated by (Rodriguez-Alvarez et al. 2014). After tobacco smoking, it is estimated that 3-5% of Nic intake will be excreted as Nic-glucuronide (8-10% as free Nic), 12-17% will be excreted as Cot-glucuronide (10-15% as free Cot) and 7-9% will be excreted as OH-Cot-glucuronide (33-40% as free OH-Cot) (Benowitz et al. 2009) (see also **Figure S4**). It was assumed that both free form and conjugated forms of Nic, Cot and OH-Cot in the urine of smokers in the catchment reached the pumping station. Subsequently, the in-sewer de-conjugation process would increase the concentration of free form chemicals in the downstream plugs. Unlike the sewer reactor, the transformation of Cot and OH-Cot cannot be fitted with either linear regression or first-order kinetics, possibly due to the dynamic release of free form glucuronides in the real sewer. Quick in-sewer de-glucuronidation was also observed with morphine-glucuronide and codeine-glucuronide, with >95% decrease of these two conjugates within 2 hours in the sewer reactor leading to significant release of free form morphine and codeine (Gao et al. 2017). The observations in these studies suggest that the de-glucuronidation could be relatively fast with the presence of biofilm in the sewer. In comparison,

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de-conjugation of Cot-glucuronide in urine sample at 25℃ in 26 days led to only 50% increase of free Cot (Hagan et al. 1997). The slower de-glucuronidation in the urine samples compared with wastewater in the sewer indicated that the abundant microorganisms would accelerate the transformation (Wu et al. 2012).

Tscharke et al. (2016) reported that Cot and Nic were stable in wastewater only under temperatures of -20℃, 4℃ and 25℃ without addition of preservatives. With the presence of sewer biofilm, Cot and OH-Cot had approximately 10% and 40% degradation within 12 hours in the rising main sewer reactor with A/V ratio of 72.5 m^{-1} (Banks et al. 2017). The in-sewer formation of Cot and OH-Cot suggests that for the back-calculation of tobacco consumption, the excretion factors should reflect the combination of both free form and glucuronides (e.g. 30% for Cot and 44% for OH-Cot) (Castiglioni et al. 2015). Overall, Cot is a better biomarker than OH-Cot for tobacco consumption estimation in light of their in-sewer stability.

3.4 Performance of the benchmarking method

The benchmarking method using acesulfame normalization did not make any significant difference to the stability of biomarkers investigated (*p* value shown in **Table 1**). Additionally, the benchmarking method may have increased the uncertainty, i.e. the relative standard deviation of the transformation increased from 13% to 25%, 17% to 36%, 38% to 51%, 27% to 37% for EtS, Nic, Cot and OH-Cot, respectively.

On one hand, the benchmarking method can compensate for some physical dissipation in the transformation calculation; on the other hand, however, it could also introduce more uncertainty with the chemical analysis for acesulfame and the possible different behaviour of native biomarker and spiked benchmarker in the wastewater. In open systems such as river and lake with intensive mixing and high flow uncertainty, benchmarking method is a powerful tool to evaluate chemical stability. However, in the case of our study where infiltration and exfiltration of wastewater in the

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sewer is not an issue, the benchmarking method is equally or less advantageous than the calculation method using the absolute concentrations.

3.5 Biomarker stability impacts on the back-calculation of alcohol and tobacco consumption

The stability of biomarkers is important for accurate back-calculation of substance consumption in the population (Castiglioni et al., 2013). This study revealed that EtS could have some degradation in the real rising main sewer with average A/V and HRT. Hence, its stability should be considered for the back-calculation of the alcohol consumption, especially when evaluating geographical variation because the in-sewer loss could vary from catchment to catchment. The presence of glucuronide compounds has probably prevented us from determining the actual stability of tobacco biomarkers in the real sewer. Overall, there was no decrease in the concentration of Cot and OH-Cot between upstream and downstream sampling points.

For an accurate estimation of alcohol and tobacco consumption with WBE, a good understanding of the sewer catchment in regard to the distribution of the flow, A/V ratio and the HRT is essential as these factors influence the transportation and transformation of biomarkers before they are sampled in the influent of wastewater treatment plant. The geographical comparison of alcohol and tobacco consumption should also consider the catchment characters, as different infrastructure and wastewater HRT distribution would result in different levels of in-sewer transformation of biomarker.

3.6 Limitations and future work

The experiment was carried out in the upstream of a sewer catchment, where there could be considerable amounts of biomarker conjugates in the wastewater due to flushes of fresh urine. The de-conjugation led to the formation of Nic, Cot and OH-Cot, that can complicate the evaluation of stability. We could not test the downstream sewer in the catchment, where the conjugates are likely

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depleted, and the stability of biomarkers could be evaluated with the minimum interference from de-conjugation. The wastewater composition in sewers changes diurnally due to the living habits of residents in the catchment. In addition, the composition and properties of wastewater would change due to the biochemical processes in the sewer during the transportation. There could be potential impacts of wastewater composition and properties on the biomarker transformation in sewers. This was not considered in the present study but needs further research, which can be carried out using lab-scale sewer reactors under well-controlled conditions.

Due to practical reasons, because we only evaluated the biomarker transformation in 2.7-5.0 hours HRT, we cannot accurately predict the behaviour of biomarkers outside this HRT range. Further evaluation of the impacts of A/V, HRT and wastewater composition on the transformation of biomarkers is necessary. A modelling approach that can extrapolate the research observations to predict biomarker stability in a catchment with different diameter sewers would be favourable (McCall et al. 2017). As biofilms are likely the dominant power driving the degradation of chemicals, better understanding of the microbe composition in the biofilms and the variability of microorganism composition within and between catchments would provide more insights to the understanding of biomarker stability and sewer characteristics.

4. Conclusions

Our study found that EtS can degrade approximately 8% per hour in a real rising main sewer. Therefore, degradation should be considered when EtS is used to estimate consumption of alcohol by WBE. EtG is unstable in the sewer, and hence not a suitable biomarker for WBE. Rapid de-conjugation of glucuronide Nic, Cot and OH-Cot interfered with the stability assessment for those chemicals. Further study may be required to assess the stability of those chemicals in the real sewer. A good understanding of the sewer catchment would improve the interpretation of WBE results.

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Acknowledgements

The Queensland Alliance for Environmental Health Sciences, The University of Queensland gratefully acknowledges the financial support of the Queensland Department of Health. Special thanks to support from Gold Coast Water for access to the site to conduct the study. Jianfa Gao receives an ARC scholarship (DP150100645). Jiaying Li is funded by China Scholarship Council. Phong K. Thai is funded by the QUT VC Fellowship. Dr Guangming Jiang is the recipient of a Queensland State Government's Early Career Accelerate Fellowship and an Australian Research Council DECRA Fellowship (DE170100694). Thanks Dr Xiaoyan Sun and Miss Lisa Bethke for sampling assistance.

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Table 1. Stability of alcohol and tobacco biomarkers in rising main sewer

Biomarker	$Stability_{conc}$ (%)	$Stability_{benmk}$ (%)	*p* value
EtS	77±13	72±25	0.8408
EtG	16±11	14±9	0.3377
Nic	114±17	107±36	0.0696
Cot	170±38	160±51	0.4245
OH-Cot	132±27	120±37	0.5028

Note: The transformation is present as average ± standard deviation of data calculated with 21 plugs in 3 days test. 100% stability indicate absolute stable of biomarker, <100% stability indicate degradation while >100% indicate formation.

Table 2. Transformation kinetics of alcohol and tobacco biomarkers

Biomarker	Linear Regression		First-order kinetics		Kinetic model selected
	Slope (%/h)	R^2	Half-life (h)	R^2	
EtS	-7.801±1.267	0.203	~1301	0.203	NA
EtG	-17.44±1.661	0.840	1.89	0.924	First-order
Nic	4.977±6.807	0.025	NC	NC	NA
Cot	-12.16±16.46	0.027	0.0692	0.103	NA
OH-Cot	1.85±13.85	0.001	0.0833	0.067	NA

NC: not converged, NA: not applicable;

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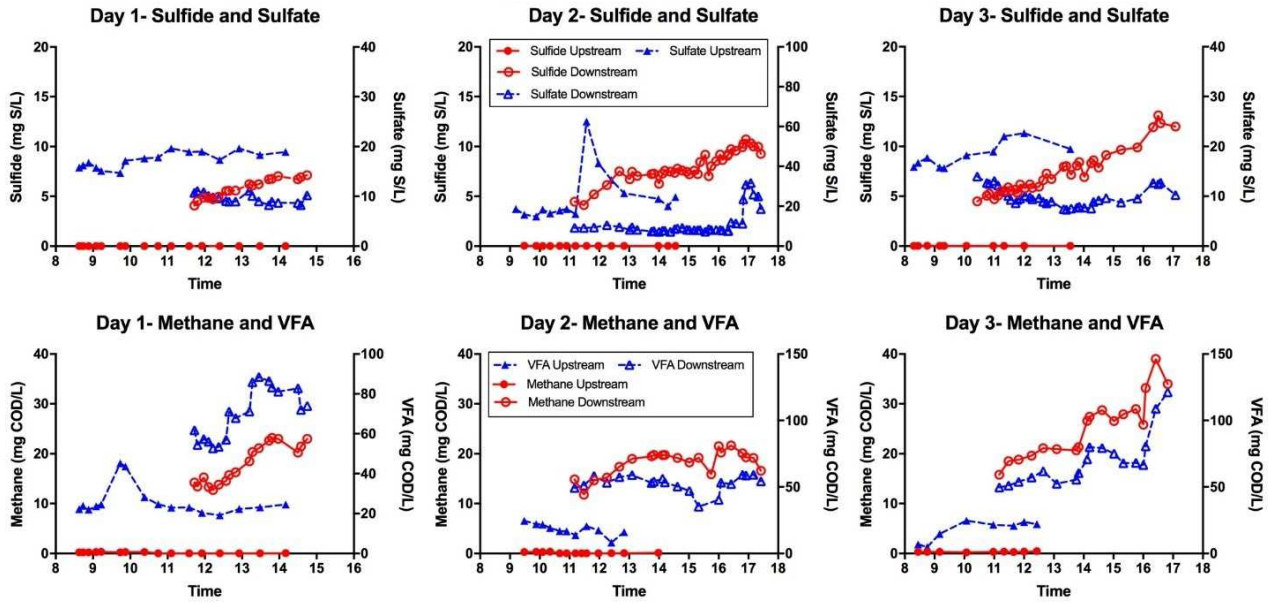

Figure 1. The wastewater parameters in upstream and downstream of UC9. The sampling was designed such that the measured plugs at the pumping station wet well were also measured at the downstream sampling point.

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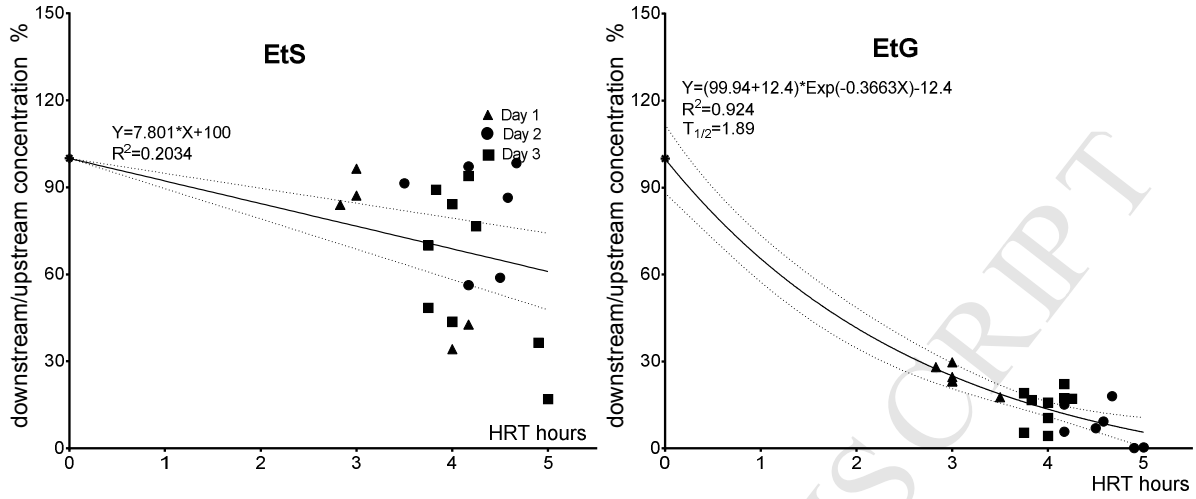

Figure 2. Transformation of alcohol biomarker, EtS & EtG, in UC9. The dash line shows the 95% confidence bands of best-fit line.

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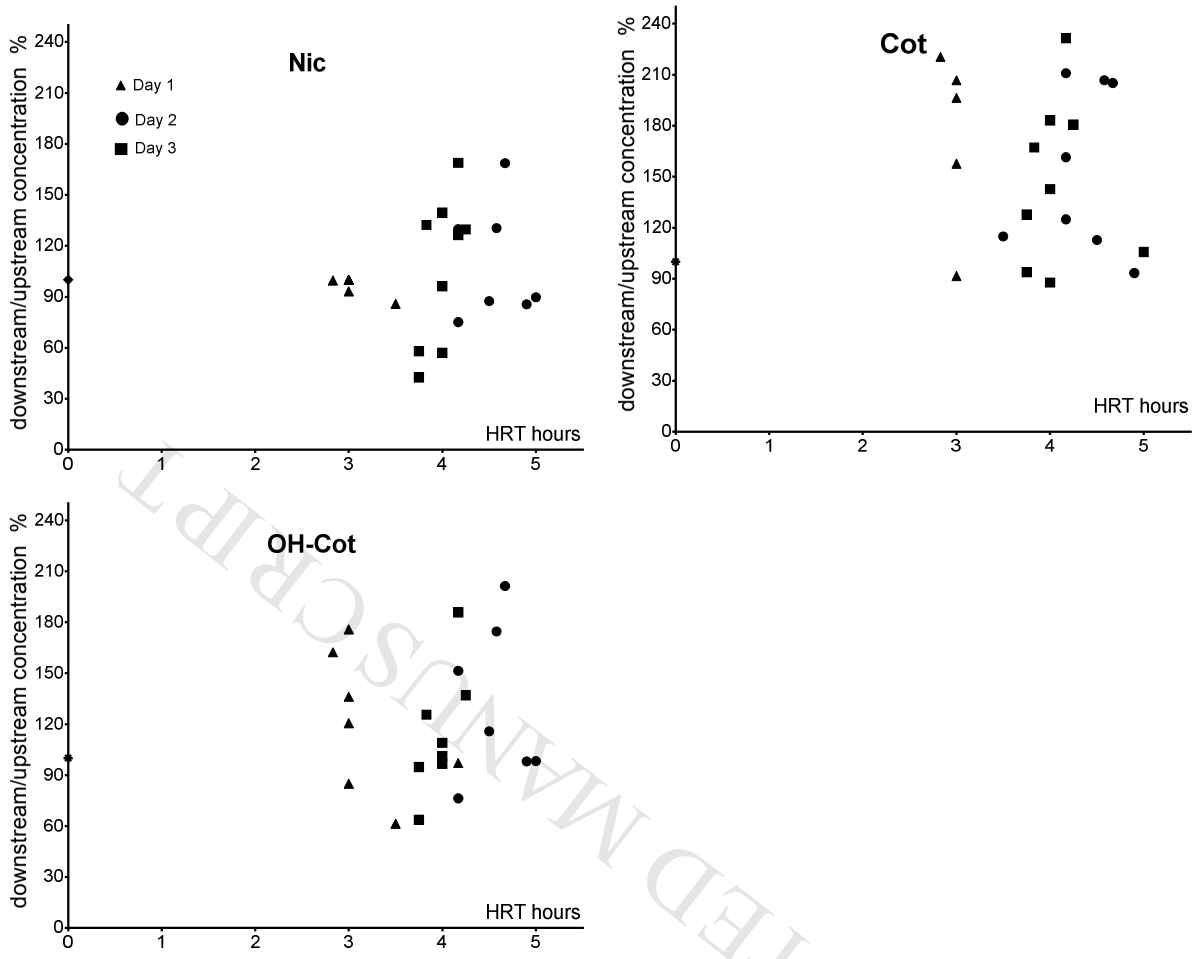

Figure 3. Transformation of tobacco biomarkers, Nic, Cot, OH-Cot, in the real sewer.

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Highlights

- First study to test the stability of alcohol and tobacco biomarkers in a real sewer
- Ethyl sulfate is much more stable than ethyl glucuronide in the rising main sewer
- Strong de-conjugation in the sewer can interfere with the stability assessment
- Results from benchmarking method and absolute concentration were comparable

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