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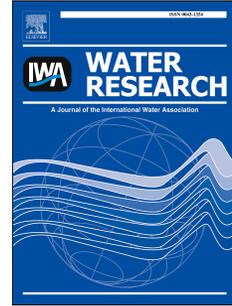
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Stability of alcohol and tobacco consumption biomarkers in a real rising main sewer

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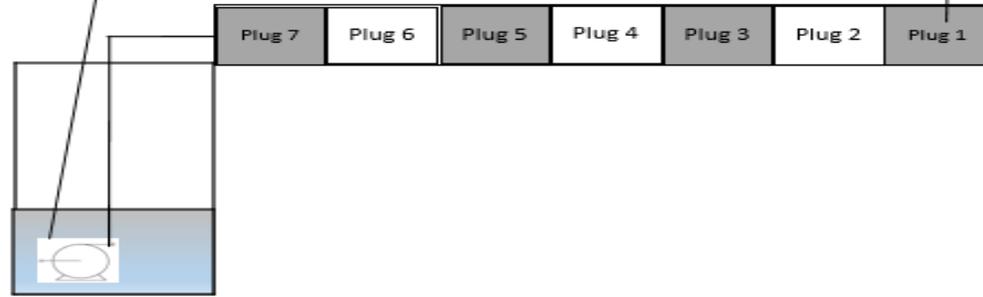
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Upstream samples

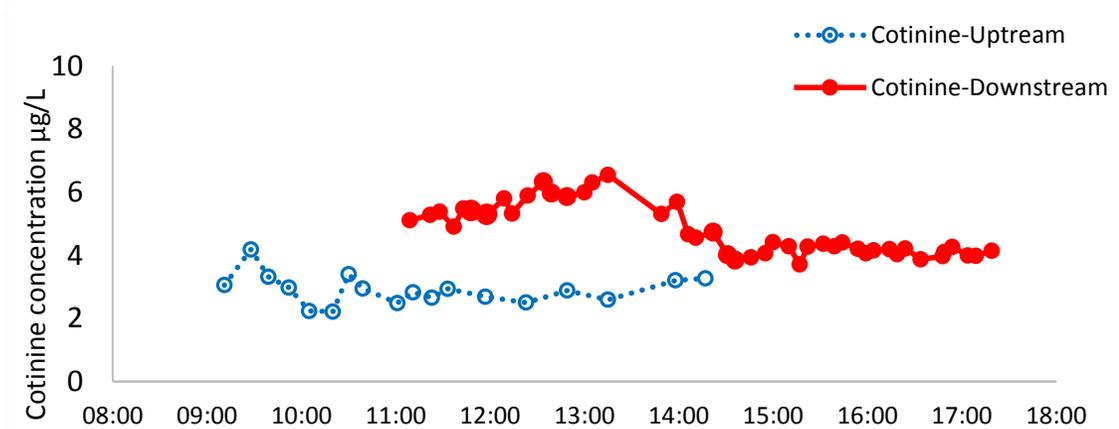
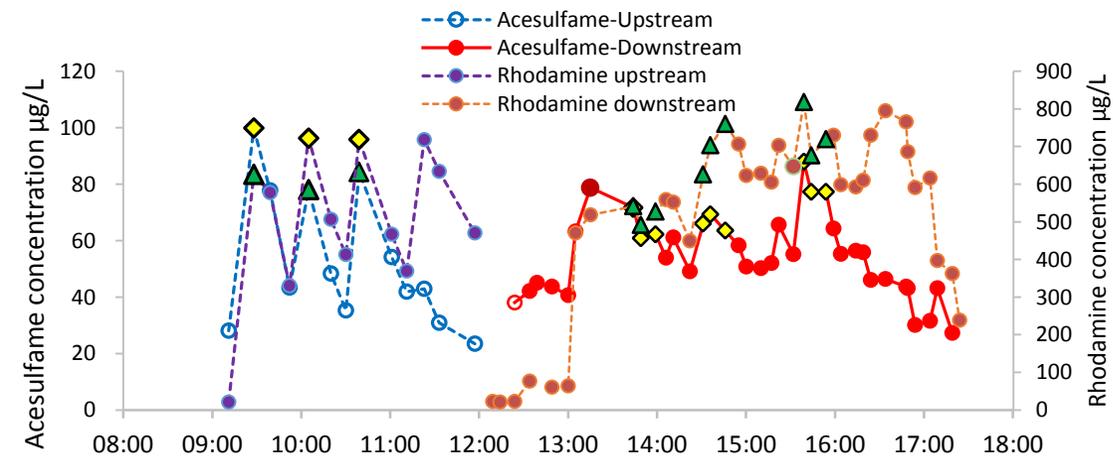


Downstream samples



Benchmarker and flow tracer

Biomarker in upstream and downstream



18 **Highlights**

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

ACCEPTED MANUSCRIPT

23 **ABSTRACT**

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

41

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

44 1. Introduction

45 Alcohol and tobacco are the most popular legal stimulants in the world (WHO 2015, 2017).
46 Consumption of alcohol and tobacco can cause considerable health problems in the population; for
47 example cardiovascular diseases and various types of cancers (Castaldelli-Maia et al. 2016, Jemal
48 et al. 2011). To develop integrated strategies to reduce the social and health burdens associated with
49 alcohol and tobacco consumption, it is very important to understand the consumption behaviour of
50 these substances in as much detail as possible. Traditional methods of consumption estimation
51 involve sales statistics (Black et al. 2011) and population surveys (Bush et al. 1998, WHO 2000),
52 which are subject to sampling limitations and usually are time-consuming and require monetary
53 resources. Wastewater-based epidemiology (WBE) is an alternative approach to monitor
54 consumption of substances in the population, including illicit drugs and psychoactive substances.
55 WBE is based on the analysis of trace level of substance residues in influent wastewater including
56 the parent drug and/or human metabolites. Using refined correction factors for human excretion
57 and stability, together with daily flow and catchment population, substance consumption in the
58 catchment population can be back-calculated. It has the advantage of cost-effectiveness and high
59 resolution sampling compared with conventional epidemiology, as the influent wastewater can be
60 treated as diluted human excretion. (EMCDDA, 2016b; Lai et al., 2016; Li et al., 2014; Thai et al.,
61 2016). Recently, alcohol and tobacco consumption in different settings has been estimated by WBE
62 to provide valuable information for temporal and geographical consumption behaviour (Andres-
63 Costa et al. 2016, Castiglioni et al. 2015, Lai et al. 2017, Mastroianni et al. 2014, Tschärke et al.
64 2016, van Wel et al. 2016).

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

70 without the presence of sewer biofilm (McCall; et al. 2015, Rodríguez-Álvarez et al. 2014,
71 Rodríguez-Alvarez et al. 2014, Tschärke et al. 2016). Only one study has investigated the stability
72 of alcohol and tobacco biomarkers in simulated sewer conditions using laboratory sewer reactors
73 (Banks et al. 2017). Since the dynamics of wastewater and activity of biofilms in actual sewers can
74 affect the degradation of chemicals in a different way than the simulated conditions (Huisman
75 2001, McCall et al. 2016, Zwiener and Frimmel 2003), the findings of the above-mentioned
76 laboratory studies need to be validated against data from real sewers. Jelic et al. (2015) and McCall
77 et al. (2017) have investigated the fate of pharmaceuticals and illicit drugs in real sewers, and
78 found degradation and formation of different chemicals in the sewer. However, a lesson to be
79 drawn from those studies is that for investigation of biomarker stability in real sewers, more
80 accurate and sufficiently long hydraulic retention time of the wastewater samples should be
81 employed, such as the recently reported study (Li et al. 2018).

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

96 approach is difficult.

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

103

104 **2. Materials and methods**

105 **2.1 Chemicals and Reagents**

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

113

114 **2.2 The UC9 sewer**

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

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

122 The rising main pipe transports domestic sewage with an average dry weather flow of
123 approximately 126 m³/day, servicing about 550 people living in the catchment. The hydraulic
124 retention time (HRT) of wastewater varies from 1.5 to 6.0 hours, depending on time of the day
125 (Guisasola et al. 2008, Mohanakrishnan et al. 2009) (as shown in **Figure S2**). Previous monitoring
126 showed that UC9 had strong anaerobic bioactivities, with sulfide and methane in the downstream
127 sampling point being in the range of 8-12 mg S/L and 20-120 mg COD/L respectively.

128

129 **2.3 Flow tracer and benchmarker spiking and sampling of wastewater**

130 Rhodamine, a pink-coloured flow tracer, was used to understand the movement of the wastewater
131 plugs in the rising main sewer. Acesulfame, an artificial sweetener that is stable under the simulated
132 sewer conditions (O'Brien et al. 2017), was used as a benchmarker. The benchmarking method is
133 similar to that used previously in the evaluation of PPCPs stability in lakes (McLachlan et al. 2017,
134 Zou et al. 2015). Mixtures of acesulfame and rhodamine were spiked to the upstream well
135 (pumping station) every two pumping events for three or four times a day. In each spiking, 100 mg
136 of acesulfame and 1100 mg of rhodamine dissolved in 300mL MilliQ water was poured into the
137 wet well after the pump stopped. The wastewater in one pumping event, having a volume of 1.8
138 m³, was treated as a “wastewater plug” (as shown in **Figure S3**). The biomarkers of alcohol and
139 tobacco consumption were not spiked, since preliminary testing showed that they are present in the
140 wastewater at quantifiable levels.

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

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

153

154 **2.4 Instrumental analysis**

155 Consumption biomarkers for alcohol, i.e. ethanol metabolites of ethyl-sulphate (EtS) and ethyl
156 glucuronide (EtG), were determined using direct injection by LC-MS/MS using a Shimadzu
157 Nexera HPLC system (Shimadzu Corp., Kyoto, Japan) coupled to a Sciex API 5500Q mass
158 spectrometer (Sciex, Concord, Ontario, Canada) equipped with an electrospray (TurboV) interface
159 (Reid et al. 2011). Ten μL of 1 mg/L labelled analogues of the analytes was added to each 1 mL
160 filtered and acidified wastewater sample, and 10 μL was injected into the column. Separation was
161 achieved using a Phenomenex EVO C18 column (50 x 2.0 mm, 1.7 μm , Phenomenex, Torrance,
162 CA) kept at 45°C and a flow rate of 0.27 mL min⁻¹. The linear gradient starts at 0% B ramped to
163 100% B in 3.0 minutes, then held at 100% for 2.0 minutes, followed by equilibration at 0% B for
164 4.0 minutes. (A = 5 mM dihexyl ammonium acetate in HPLC grade water, B = 5 mM dihexyl
165 ammonium acetate in methanol). A Gemini NX C18 column (50 x 2 mm, 3 μm , Phenomenex,
166 Torrance, CA) was used to trap mobile phase contaminants. The mass spectrometer was operated in
167 the negative ion multiple reaction-monitoring mode using nitrogen as the collision gas. Mass
168 spectrometer parameters are shown in **Table S2**.

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

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

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

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

189

190 **2.5 Data processing and statistical analysis**

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

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

$$198 \quad P_{\text{abs}} (\%) = \frac{C_{i,\text{downstream}}}{C_{i,\text{upstream}}} * 100 \quad \text{Equation 1}$$

$$199 \quad P_{\text{benmk}} (\%) = \frac{\frac{C_{i,\text{downstream}}}{C_{\text{ace},\text{downstream}}}}{\frac{C_{i,\text{upstream}}}{C_{\text{ace},\text{upstream}}}} * 100 \quad \text{Equation 2}$$

200 where

201 - P_{abs} is the percentage of biomarkers concentration in the downstream sample compared with the
202 upstream sample in the same wastewater plug;

203 - P_{benmk} is the percentage of biomarkers concentration normalized by benchmarker concentration in
204 the same plugs from downstream and upstream;

205 - $C_{i,\text{upstream}}$ is the concentration of biomarker i in the upstream sample;

206 - $C_{i,\text{downstream}}$ is the average concentration of biomarker i in the 3 samples collected in the same plug
207 downstream;

208 - $C_{\text{ace},\text{upstream}}$ is the concentration of acesulfame in the upstream sample;

209 - $C_{\text{ace},\text{downstream}}$ is the average concentration of acesulfame in the 3 samples collected in the same
210 plug downstream.

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

214 Biomarker transformation kinetics were evaluated using linear regression and first order kinetics.

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

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

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

223

224 **3. Results and discussion**

225 **3.1 Wastewater composition and bioactivity in the sewer**

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

236

237 **3.2 Profile of the flow tracer and benchmarker in the sewer**

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

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

249

250 **3.3 Stability of alcohol and tobacco biomarkers in the rising main sewer**

251 **3.3.1 EtS and EtG**

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

263 EtG degraded more rapidly than EtS in the same wastewater plugs investigated (**Figure 2**). Within
264 2.7-5.0 hours HRT, only $16\pm 11\%$ EtG remains in the downstream plugs (**Table 1**). EtG had a half-
265 life of 1.89 hours in the real rising main sewer, while in the rising main sewer reactor, the half-life
266 was 0.36 hour (Banks et al., 2017). The relatively slower degradation in the real sewer could
267 attribute to the fact that the real sewer has a lower A/V ratio (26.7 m^{-1} in UC9 compare with 72.5
268 m^{-1} in the sewer reactor) and relatively poorer mixing conditions.

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

275

276 3.3.2 Nic, Cot and OH-Cot

277 In this study, it is likely that more Nic, Cot and OH-Cot was generated in the wastewater plugs
278 during transportation in the real sewer (**Figure 3**). Within 2.7-5.0 hours HRT, Nic, Cot and OH-Cot
279 in the downstream plugs increased to $114\pm 17\%$, 170 ± 38 and $132\pm 27\%$ of their corresponding
280 upstream plugs ($n=21$), respectively (**Table 1**). The formation of Nic, Cot and OH-Cot was likely
281 attributed to the de-conjugation of the glucuronide-compounds as demonstrated by (Rodriguez-
282 Alvarez et al. 2014). After tobacco smoking, it is estimated that 3-5% of Nic intake will be
283 excreted as Nic-glucuronide (8-10% as free Nic), 12-17% will be excreted as Cot-glucuronide (10-
284 15% as free Cot) and 7-9% will be excreted as OH-Cot-glucuronide (33-40% as free OH-Cot)
285 (Benowitz et al. 2009) (see also **Figure S4**). It was assumed that both free form and conjugated
286 forms of Nic, Cot and OH-Cot in the urine of smokers in the catchment reached the pumping
287 station. Subsequently, the in-sewer de-conjugation process would increase the concentration of free
288 form chemicals in the downstream plugs. Unlike the sewer reactor, the transformation of Cot and
289 OH-Cot cannot be fitted with either linear regression or first-order kinetics, possibly due to the
290 dynamic release of free form glucuronides in the real sewer. Quick in-sewer de-glucuronidation
291 was also observed with morphine-glucuronide and codeine-glucuronide, with >95% decrease of
292 these two conjugates within 2 hours in the sewer reactor leading to significant release of free form
293 morphine and codeine (Gao et al. 2017). The observations in these studies suggest that the de-
294 glucuronidation could be relatively fast with the presence of biofilm in the sewer. In comparison,

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

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

307

308 **3.4 Performance of the benchmarking method**

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

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

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

322

323 **3.5 Biomarker stability impacts on the back-calculation of alcohol and tobacco consumption**

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

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

339

340 **3.6 Limitations and future work**

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

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

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

361

362 **4. Conclusions**

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

370

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380

381 **References:**

- 382 Andres-Costa, M.J., Escriva, U., Andreu, V. and Pico, Y. (2016) Estimation of alcohol consumption
383 during "Fallas" festivity in the wastewater of Valencia city (Spain) using ethyl sulfate as a
384 biomarker. *Science of the Total Environment* 541, 616-622.
- 385 Banks, A.P., Lai, F.Y., Mueller, J.F., Jiang, G., Carter, S. and Thai, P.K. (2017) Potential impact of
386 the sewer system on the applicability of alcohol and tobacco biomarkers in wastewater-based
387 epidemiology. *Drug Testing and Analysis*.
- 388 Benowitz, N., Hukkanen, J. and Jacob, P. (2009) *Handbook of Experimental Pharmacology*.
- 389 Black, H., Gill, J. and Chick, J. (2011) The price of a drink: levels of consumption and price paid
390 per unit of alcohol by Edinburgh's ill drinkers with a comparison to wider alcohol sales in Scotland.
391 *Addiction* 106(4), 729-736.
- 392 Bush, K., Kivlahan, D.R., McDonell, M.B., Fihn, S.D., Bradley, K.A. and for the Ambulatory Care
393 Quality Improvement, P. (1998) The audit alcohol consumption questions (audit-c): An effective
394 brief screening test for problem drinking. *Archives of Internal Medicine* 158(16), 1789-1795.

- 395 Castaldelli-Maia, J.M., Ventriglio, A. and Bhugra, D. (2016) Tobacco smoking: From ‘glamour’ to
396 ‘stigma’. *A comprehensive review. Psychiatry and clinical neurosciences* 70(1), 24-33.
- 397 Castiglioni, S., Bijlsma, L., Covaci, A., Emke, E., Hernández, F.I., Reid, M., Ort, C. and Kevin V.
398 Thomas, A.L.N.V.N., Pim de Voogt, Ettore Zuccato (2013) Evaluation of uncertainties associated
399 with the determination of community drug use through the measurement of sewage drug
400 biomarkers. *Environmental Science & Technology* 47(3), 1452-1469.
- 401 Castiglioni, S., Senta, I., Borsotti, A., Davoli, E. and Zuccato, E. (2015) A novel approach for
402 monitoring tobacco use in local communities by wastewater analysis. *Tobacco Control* 24(1), 38-
403 42.
- 404 Foley, J., Yuan, Z.G. and Lant, P. (2009) Dissolved methane in rising main sewer systems: field
405 measurements and simple model development for estimating greenhouse gas emissions. *Water
406 Science and Technology* 60(11), 2963-2971.
- 407 Gao, J., Banks, A., Li, J., Jiang, G., Lai, F.Y., Mueller, J.F. and Thai, P.K. (2017) Evaluation of in-
408 sewer transformation of selected illicit drugs and pharmaceutical biomarkers. *Science of the Total
409 Environment* 609, 1172-1181.
- 410 Guisasola, A., de Haas, D., Keller, J. and Yuan, Z. (2008) Methane formation in sewer systems.
411 *Water Research* 42(6-7), 1421-1430.
- 412 Guisasola, A., Sharma, K.R., Keller, J. and Yuan, Z.Q. (2009) Development of a model for
413 assessing methane formation in rising main sewers. *Water Research* 43(11), 2874-2884.
- 414 Gutierrez, O., Jiang, G., Sharma, K. and Yuan, Z. (2016) Biofilm development in sewer networks.
415 *Aquatic Biofilms*, 145.
- 416 Huisman, J.L. (2001) Transport and transformation processes in combined sewers.
- 417 Jemal, A., Bray, F., Center, M.M., Ferlay, J., Ward, E. and Forman, D. (2011) Global cancer
418 statistics. *CA: A Cancer Journal for Clinicians* 61(2), 69-90.
- 419 Jiang, G., Sun, J., Sharma, K.R. and Yuan, Z. (2015) Corrosion and odor management in sewer
420 systems. *Current opinion in biotechnology* 33, 192-197.

- 421 Kapo, K.E., Paschka, M., Vamshi, R., Sebasky, M. and McDonough, K. (2017) Estimation of U.S.
422 sewer residence time distributions for national-scale risk assessment of down-the-drain chemicals.
423 *Science of the Total Environment* 603-604, 445-452.
- 424 Lai, F.Y., Been, F., Covaci, A. and van Nuijs, A.L.N. (2017) Novel Wastewater-Based
425 Epidemiology Approach Based on Liquid Chromatography–Tandem Mass Spectrometry for
426 Assessing Population Exposure to Tobacco-Specific Toxicants and Carcinogens. *Analytical*
427 *Chemistry* 89(17), 9268-9278.
- 428 Li, J., Gao, J., Thai, P.K., Sun, X., Mueller, J.F., Yuan, Z. and Jiang, G. (2018) Stability of Illicit
429 Drugs as Biomarkers in Sewers: From Lab to Reality. *Environmental Science & Technology* 52(3),
430 1561-1570.
- 431 Mastroianni, N., Lopez de Alda, M. and Barcelo, D. (2014) Analysis of ethyl sulfate in raw
432 wastewater for estimation of alcohol consumption and its correlation with drugs of abuse in the city
433 of Barcelona. *Journal of Chromatography A* 1360, 93-99.
- 434 McCall, A.-K., Palmitessa, R., Blumensaat, F., Morgenroth, E. and Ort, C. (2017) Modeling in-
435 sewer transformations at catchment scale–Implications on drug consumption estimates in
436 wastewater-based epidemiology. *Water Research*.
- 437 McCall, A.K., Scheidegger, A., Madry, M.M., Steuer, A.E., Weissbrodt, D.G., Vanrolleghem, P.A.,
438 Kraemer, T., Morgenroth, E. and Ort, C. (2016) Influence of Different Sewer Biofilms on
439 Transformation Rates of Drugs. *Environmental Science & Technology* 50(24), 13351-13360.
- 440 McCall, A.-K., Bade, R., Kinyua, J., Lai, F.Y., Thai, P.K., Covaci, A., Bijlsma, L., Nuijs,
441 A.L.N.v. and Ort, C. (2015) Critical Review on the stability of illicit drugs in sewers and
442 wastewater samples. *Water Research* In press.
- 443 McLachlan, M.S., Zou, H. and Gouin, T. (2017) Using Benchmarking To Strengthen the
444 Assessment of Persistence. *Environmental Science & Technology* 51(1), 4-11.
- 445 Mohanakrishnan, J., Gutierrez, O., Sharma, K.R., Guisasola, A., Werner, U., Meyer, R.L., Keller, J.
446 and Yuan, Z. (2009) Impact of nitrate addition on biofilm properties and activities in rising main

- 447 sewers. *Water Research* 43(17), 4225-4237.
- 448 O'Brien, J.W., Banks, A.P., Novic, A.J., Mueller, J.F., Jiang, G., Ort, C., Eaglesham, G., Yuan, Z.
449 and Thai, P.K. (2017) Impact of in-Sewer Degradation of Pharmaceutical and Personal Care
450 Products (PPCPs) Population Markers on a Population Model. *Environmental Science &*
451 *Technology* 51(7), 3816-3823.
- 452 Reid, M.J., Langford, K.H., Morland, J. and Thomas, K.V. (2011) Analysis and interpretation of
453 specific ethanol metabolites, ethyl sulfate, and ethyl glucuronide in sewage effluent for the
454 quantitative measurement of regional alcohol consumption. *Alcohol Clinical and Experimental*
455 *Research* 35(9), 1593-1599.
- 456 Rieckermann, J., Bares, V., Kracht, O., Braun, D. and Gujer, W. (2007) Estimating sewer leakage
457 from continuous tracer experiments. *Water Research* 41(9), 1960-1972.
- 458 Rodríguez-Álvarez, T., Rodil, R., Cela, R. and Quintana, J.B. (2014) Ion-pair reversed-phase liquid
459 chromatography–quadrupole-time-of-flight and triple-quadrupole–mass spectrometry
460 determination of ethyl sulfate in wastewater for alcohol consumption tracing. *Journal of*
461 *Chromatography A* 1328, 35-42.
- 462 Rodríguez-Alvarez, T., Rodil, R., Rico, M., Cela, R. and Quintana, J.B. (2014) Assessment of local
463 tobacco consumption by liquid chromatography-tandem mass spectrometry sewage analysis of
464 nicotine and its metabolites, cotinine and trans-3'-hydroxycotinine, after enzymatic deconjugation.
465 *Analytical Chemistry* 86(20), 10274-10281.
- 466 Senta, I., Krizman, I., Ahel, M. and Terzic, S. (2014) Assessment of stability of drug biomarkers in
467 municipal wastewater as a factor influencing the estimation of drug consumption using sewage
468 epidemiology. *Science of the Total Environment* 487, 659-665.
- 469 Sharma, K., Ganigue, R. and Yuan, Z.G. (2013) pH dynamics in sewers and its modeling. *Water*
470 *Research* 47(16), 6086-6096.
- 471 Thai, P.K., Jiang, G., Gernjak, W., Yuan, Z., Lai, F.Y. and Mueller, J.F. (2014) Effects of sewer
472 conditions on the degradation of selected illicit drug residues in wastewater. *Water Research* 48,

- 473 538-547.
- 474 Tscharke, B.J., White, J.M. and Gerber, J.P. (2016) Estimates of tobacco use by wastewater analysis
475 of anabasine and anatabine. *Drug Testing and Analysis* 8(7), 702-707.
- 476 van Nuijs, A.L., Abdellati, K., Bervoets, L., Blust, R., Jorens, P.G., Neels, H. and Covaci, A. (2012)
477 The stability of illicit drugs and metabolites in wastewater, an important issue for sewage
478 epidemiology? *Journal of Hazardous Materials* 239-240, 19-23.
- 479 van Wel, J.H.P., Gracia-Lor, E., van Nuijs, A.L.N., Kinyua, J., Salvatore, S., Castiglioni, S.,
480 Bramness, J.G., Covaci, A. and Van Hal, G. (2016) Investigation of agreement between
481 wastewater-based epidemiology and survey data on alcohol and nicotine use in a community. *Drug*
482 *and Alcohol Dependence* 162, 170-175.
- 483 WHO (2000) International guide for monitoring alcohol consumption and related harm.
- 484 WHO (2015) WHO global report on trends in prevalence of tobacco smoking 2015, World Health
485 Organization.
- 486 WHO (2017) World Health Statistics 2017: Monitoring Health for the SDGs Sustainable
487 Development Goals, World Health Organization.
- 488 Wu, S., Zhang, L. and Chen, J. (2012) Paracetamol in the environment and its degradation by
489 microorganisms. *Applied microbiology and biotechnology* 96(4), 875-884.
- 490 Wurst, F.M., Kempter, C., Seidl, S. and Alt, A. (1999) Ethyl glucuronide--a marker of alcohol
491 consumption and a relapse marker with clinical and forensic implications. *Alcohol and alcoholism*
492 (Oxford, Oxfordshire) 34(1), 71-77.
- 493 Zou, H., MacLeod, M. and McLachlan, M.S. (2014) Evaluation of the potential of benchmarking to
494 facilitate the measurement of chemical persistence in lakes. *Chemosphere* 95, 301-309.
- 495 Zou, H., Radke, M., Kierkegaard, A., MacLeod, M. and McLachlan, M.S. (2015) Using chemical
496 benchmarking to determine the persistence of chemicals in a Swedish lake. *Environmental Science*
497 *& Technology* 49(3), 1646-1653.
- 498 Zwiener, C. and Frimmel, F. (2003) Short-term tests with a pilot sewage plant and biofilm reactors

499 for the biological degradation of the pharmaceutical compounds clofibrac acid, ibuprofen, and
500 diclofenac. The Science of The Total Environment 309(1-3), 201-211.

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

Biomarker	Stability _{conc} (%)	Stability _{benmk} (%)	<i>p</i> 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;

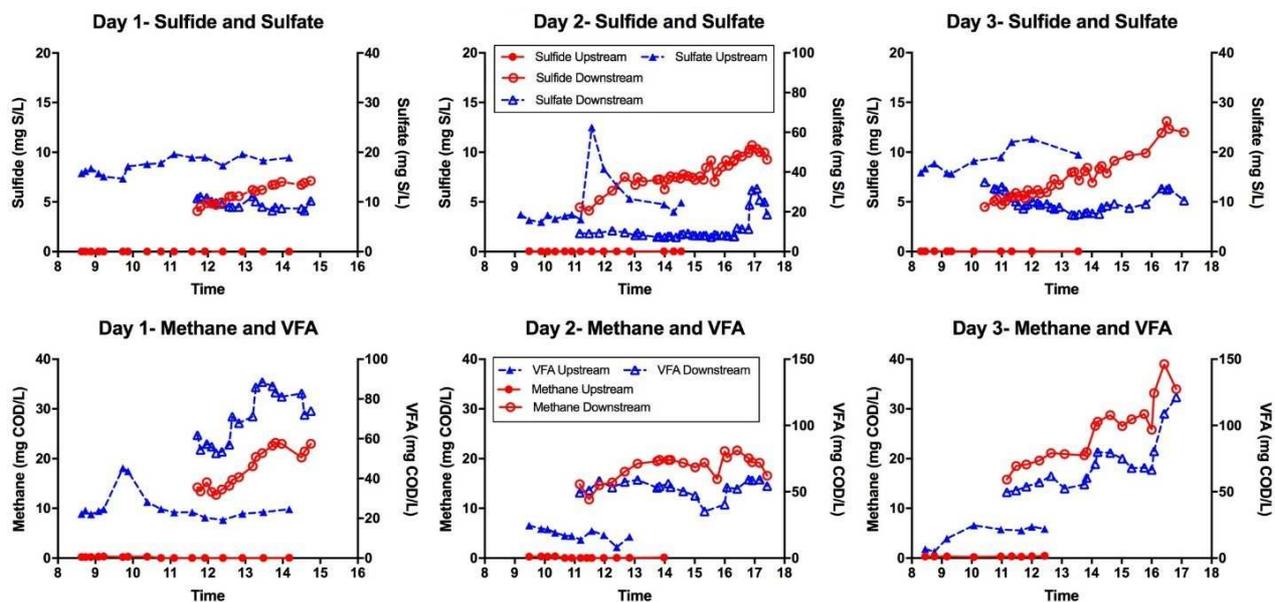


Figure 1. The wastewater parameters in upstream and downstream of UC9. The sampling was designed such that the measured plugs at the pumping station wet well were also measured at the downstream sampling point.

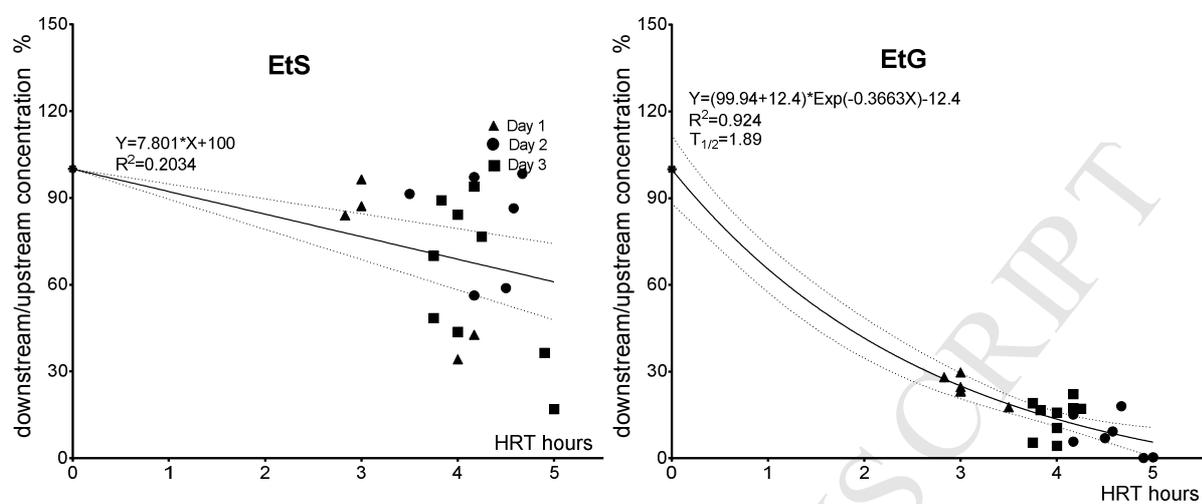


Figure 2. Transformation of alcohol biomarker, EtS & EtG, in UC9. The dash line shows the 95% confidence bands of best-fit line.

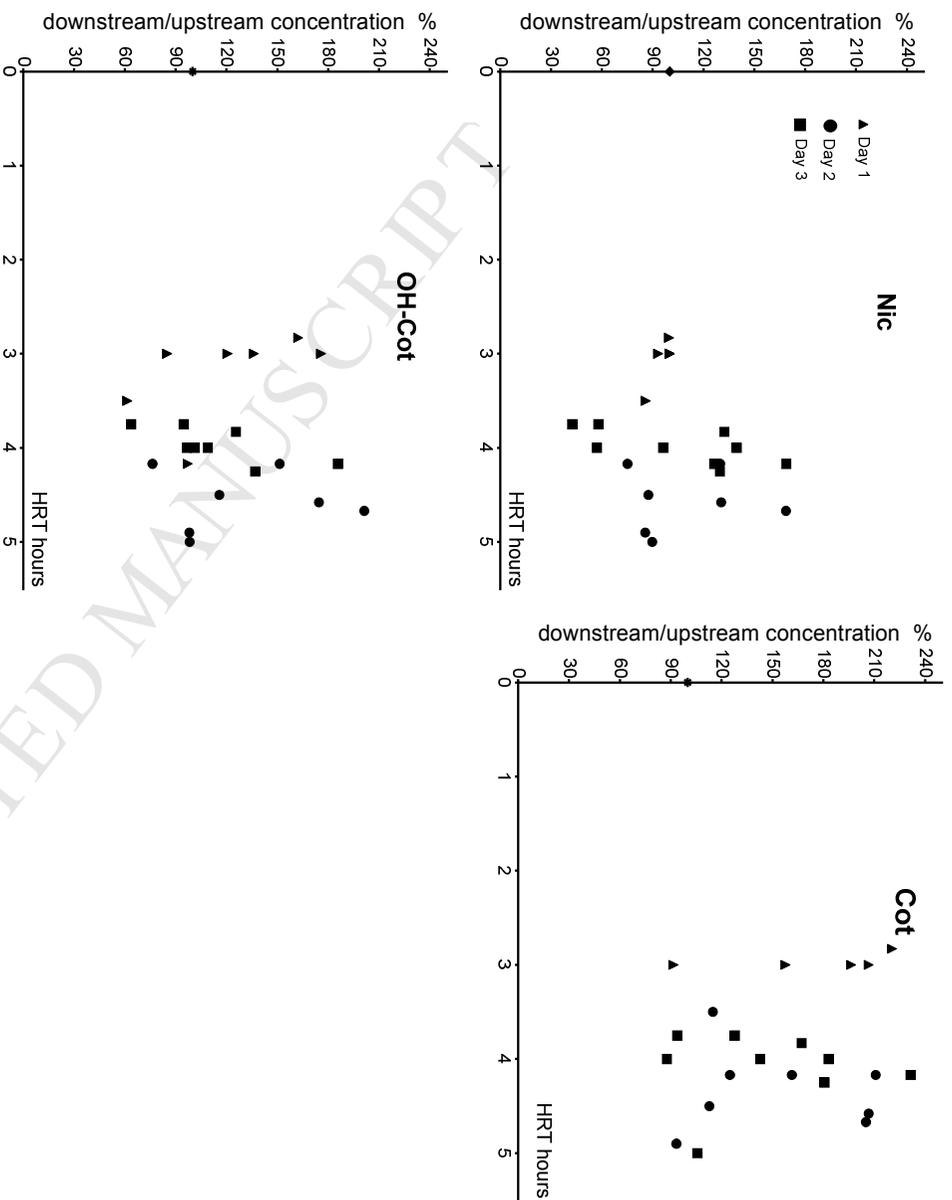


Figure 3. Transformation of tobacco biomarkers, Nic, Cot, OH-Cot, in the real sewer.

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