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Spatial and temporal trends in alcohol consumption in Belgian cities : a wastewater-based approach

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21 **Abstract**

22 Background

23 In recent years, scientific evidence has emerged that wastewater-based epidemiology can deliver  
24 complementary information concerning the use of different substances of abuse. In this study, the  
25 potential of wastewater-based epidemiology in monitoring spatial and temporal trends in alcohol  
26 consumption in different populations in Belgium has been examined.

27 Methods

28 Concentrations of ethyl sulphate, a minor phase II metabolite of ethanol, in 163 influent wastewater  
29 samples from eight wastewater treatment plants in Belgium in the period 2013-2015 were measured  
30 with liquid chromatography coupled to tandem mass spectrometry and used to estimate alcohol  
31 consumption.

32 Results

33 The highest levels of alcohol consumption were detected in the metropolises Antwerp and Brussels  
34 compared to smaller villages. Annual variations were detected, with a higher alcohol consumption  
35 measured in 2013 compared with 2014. The weekly pattern showed a clear week and weekend  
36 difference in alcohol use, with intermediate levels on Monday and Friday. The results were  
37 extrapolated and a volume of 5.6 L pure alcohol per year per inhabitant aged 15+ has been estimated  
38 in Belgium. The comparison with available information on drinking habits of the Belgian population  
39 further demonstrated the usefulness of the wastewater-based epidemiology approach.

40 Conclusions

41 This largest wastewater-based epidemiology study to monitor alcohol consumption up to now  
42 demonstrates that objective and quick information on spatio-temporal trends in alcohol  
43 consumption on a local and (inter)national scale can be obtained.

44

45 **Keywords**

46 Ethyl sulphate, influent wastewater, sewage-based epidemiology, alcohol consumption, spatio-

47 temporal trends

48

49 **1.0 Introduction**

50 Alcohol (ethanol,  $\text{CH}_3\text{CH}_2\text{OH}$ ) abuse is the most common form of substance abuse and has a high  
51 impact on societies with a higher incidence of traffic accidents and criminality, economic damage and  
52 health treatment costs (WHO, 2014). Actually, abuse of alcoholic beverages and in particular binge  
53 drinking occurs so often that it has become a sociological phenomenon all over the world (Courtney  
54 & Polich, 2009). Therefore, a concern about concomitant health problems is manifesting in different  
55 countries which is demonstrated by the fact that four percent of all deaths on a global scale are  
56 caused by the (ab)use of alcoholic beverages (WHO, 2014). Because of this serious impact on human  
57 health in general, it is important to gather information on the average alcohol use in a population  
58 which can assist authorities to take measures to diminish the morbidity and mortality due to alcohol  
59 usage. The findings of the World Health Organization (WHO) suggest a worldwide annual intake of  
60 6.2 L pure ethanol per capita aged 15+ in 2010, which implies the consumption of a daily dose of 1.4  
61 standard consumptions per capita aged 15+ (NIAAA, 2010; WHO, 2014). For Belgium, the WHO  
62 suggests an annual consumption of 11 L pure ethanol per capita aged 15+ in 2010, which  
63 corresponds to 2.4 standard consumptions per day (WHO, 2014). This value is considerably higher  
64 than the finding of 1.5 standard consumption per day per capita aged 15+, reported by the Belgian  
65 Scientific Institute of Public Health in 2013 (WIV-ISP, 2013).

66 In order to better evaluate these discrepancies and to have up-to-date and timely numbers on  
67 alcohol consumption, there is a need for quick, objective and complementary approaches to  
68 estimate the amount of consumed alcohol within communities. Currently, alcohol consumption is  
69 measured mostly based on individual surveys or sales statistics, but these approaches are not always  
70 reliable (NIAAA, 2003). Sales numbers are often inaccurate because they are only an indirect  
71 measure of alcohol consumption and they do not take international purchases and wastage or stock-  
72 piling into account. Interview and questionnaire-based information on a large population scale suffer

73 from bias since these methods rely on the self-report of the users themselves (NIAAA, 2003; Smith et  
74 al., 1990).

75 Recently, scientific evidence has emerged that the analysis of wastewater for the presence of urinary  
76 biomarkers of different substances can deliver objective and complementary information on the use  
77 of these compounds within communities (Daughton, 2001). The approach, called wastewater-based  
78 epidemiology (WBE), has already proven its applicability in evaluating spatial and temporal trends in  
79 illicit drug consumption on a regional, national and international scale (Castiglioni et al., 2014; Ort &  
80 van Nuijs et al., 2014). The proven potential of the WBE approach opens possibilities for applying it to  
81 monitor the use of other substances such as alcohol (Thomas & Reid, 2011).

82 Alcohol is mainly metabolized through oxidation which involves alcohol dehydrogenase and aldehyde  
83 dehydrogenase to form acetaldehyde and acetic acid. However, a small fraction of alcohol undergoes  
84 Phase-II metabolism to form ethyl sulphate (EtS) and ethyl glucuronide (EtG) in which  
85 sulphotransferase and UDP-glucuronosyltransferase enzymes play a key role (Kurogi et al., 2012;  
86 Schwab & Skopp, 2014; Walsham & Sherwood, 2014). EtS and EtG are commonly used in clinical and  
87 forensic toxicology to monitor individual alcohol consumption via the analysis of hair, urine and  
88 blood (Crunelle et al., 2014; Walsham & Sherwood, 2012; Walsham & Sherwood, 2014). Based on  
89 this scientific evidence, both EtS and EtG form potential biomarkers that could be targeted in influent  
90 wastewater samples to determine the amount of alcohol used by a certain population. Previous  
91 studies have already proven the stability of EtS in this matrix and used it successfully on samples that  
92 originated from Italian, Spanish and Norwegian populations (Mastroianni et al., 2014; Reid et al.,  
93 2011; Rodriguez-Alvarez et al., 2014; Rodriguez-Alvarez et al., 2015). Unlike EtS, EtG was not suitable  
94 as an alcohol biomarker in wastewater due to its marked instability in wastewater (Reid et al., 2011).

95 The present study reports on the application of WBE to evaluate spatial and temporal trends in  
96 alcohol consumption in eight different communities in Belgium through the analysis of EtS in

97 wastewater samples and to compare our findings with other figures obtained through the classical  
98 approaches. The rationale of the WBE approach can be found in Figure 1.

99

## 100 **2.0 Materials and methods**

### 101 *2.1. Reagents and materials*

102 LC-grade methanol (MeOH), acetonitrile (ACN), and acetic acid (CH<sub>3</sub>COOH) were purchased from  
103 Merck (Darmstadt, Germany). Ultrapure water was obtained by purifying demineralized water in an  
104 Elga LabWater Purelab Flex system (Veolia Water Solutions & Technologies Belgium, Tienen,  
105 Belgium). Analytical standards of EtS and EtS-D<sub>5</sub> were acquired from Athena Enzyme Systems  
106 (Baltimore MD, USA) in neat powder. Dilutions of the reference standards were prepared in MeOH.  
107 Safe-Lock tubes (1.5 mL and 2 mL) were obtained from Eppendorf (Rotselaar, Belgium) and  
108 centrifugal filters with a nylon membrane with pore size 0.2 µm were purchased from VWR (Leuven,  
109 Belgium).

### 110 *2.2. Sampling*

111 Influent wastewater samples were collected from eight Belgian wastewater treatment plants  
112 (WWTPs) covering approximately 1.6 million inhabitants: Antwerpen-Zuid, Deurne, Ninove,  
113 Geraardsbergen, Lier, Oostende, Brussel-Noord and Wulpen. To obtain samples that were  
114 representative for an entire day, 24-h composite samples were collected using time- or volume-  
115 proportional techniques (Ort et al., 2010). For each WWTP, at least seven consecutive daily samples  
116 were collected over a period of three years (2013-2015); in total 163 samples were collected (see  
117 Table 1). Samples were immediately frozen after collection and stored at -20 °C until analysis.

### 118 *2.3. Analysis*

#### 119 *2.3.1. Sample preparation*

120 Prior to the analysis, samples were thawed at room temperature and afterwards vigorously shaken.  
121 One mL of influent wastewater was transferred to a 2 mL Eppendorf tube which was centrifuged at  
122 8000 rpm for 5 min. Thereafter, 190  $\mu\text{L}$  supernatant was transferred to a 1.5 mL Eppendorf tube and  
123 10  $\mu\text{L}$  of the internal standard EtS-D<sub>5</sub> at a concentration of 1 ng/ $\mu\text{L}$  was added. The sample was  
124 vortexed for 1 min, subsequently brought to a centrifugal filter (0.2  $\mu\text{m}$ ) and centrifuged for 5 min at  
125 8000 rpm. The resulting filtrate was transferred to a vial for determination with liquid  
126 chromatography coupled to tandem mass spectrometry (LC-MS/MS).

### 127 2.3.2. Liquid chromatography-tandem mass spectrometry

128 The LC system consisted of an Agilent 1290 series binary pump and an autosampler module.  
129 Separation was achieved with an Atlantis T3 column (150 mm x 2.1 mm, 3  $\mu\text{m}$ ) maintained at 30 °C  
130 and a mobile phase composed of (A) Ultrapure water with 0.1% CH<sub>3</sub>COOH and (B) ACN using a  
131 gradient as follows: 0-10 min: 2%-15% B; 10-11 min: 15%-95% B; 11-12 min: 95% B; 12-13 min: 95%-  
132 2% B; 13-20 min: 2% B for column equilibration. The flow rate was 0.18 mL/min and the injection  
133 volume was set at 4  $\mu\text{L}$ . EtS and EtS-D<sub>5</sub> eluted at 4.80 and 4.75 min, respectively.

134 The MS system was an Agilent 6460 triple quadrupole mass spectrometer equipped with an  
135 electrospray interface operating in negative ionisation mode and the following settings: gas  
136 temperature 250 °C, gas flow 12 L/min, nebulizer pressure 35 psi, sheath gas heater 325 °C, sheath  
137 gas flow 11 L/min, capillary voltage 2750 V, and nozzle voltage 750 V. Quantitative analyses was  
138 performed in multiple reaction monitoring (MRM) mode and the two most abundant fragmentation  
139 products (selected as quantifier and qualifier) were recorded. Optimized MS parameters for both  
140 analyte and internal standard are shown in Table 2. The LC flow was diverted to the waste in the first  
141 two minutes after injection and from 10 to 20 min, to avoid excessive contamination of the mass  
142 spectrometer.

143 A curve containing seven calibration points was constructed with increasing concentrations of EtS  
144 (range: 1.5 to 100  $\mu\text{g/L}$ ) and a fixed amount of EtS-D<sub>5</sub> (50  $\mu\text{g/L}$ ). The calibration was considered as

145 satisfactory when a coefficient of determination ( $R^2$ )  $>0.99$  was obtained and when the back-  
146 calculated concentrations of the calibrators had an accuracy within 85-115% (EMA, 2011). The  
147 precision of the method could be determined based on these calibrators and was  $<10\%$  relative  
148 standard deviation. The limit of quantification of the method was  $1.5 \mu\text{g/L}$  and was determined as  
149 the lowest point of the calibration curve with an accuracy of the back-calculated concentration  
150 between 80% and 120% and a precision  $<20\%$  relative standard deviation (EMA, 2011).

#### 151 *2.4. Calculations and statistical analysis*

152 Firstly, the measured concentrations (in  $\mu\text{g/L}$ ) were multiplied with a factor of 1.05 to correct for the  
153 dilution occurring during sample preparation. Corrected concentrations were then multiplied with  
154 the wastewater flow rate (L/day) recorded at the WWTPs to obtain mass loads of EtS (in g/day). To  
155 compare EtS loads for cities and villages of different population size, mass loads were divided by the  
156 number of inhabitants served by each WWTP. In order to obtain realistic figures and to compare  
157 results with existing data, only inhabitants aged 15+ were taken into account (FOD Economy, 2015).  
158 This calculation resulted in the daily per capita load of EtS, expressed in g/day/1000 inhabitants aged  
159 15+.

160 Secondly, back-calculations were made to transform to obtained population-normalized mass loads  
161 of EtS into a consumed amount of alcohol (Figure 1, see Table S1 for the all raw data). Therefore, the  
162 excretion rate (0.012%) and the molar mass ratio of EtS and alcohol (0.369) were considered,  
163 resulting in a correction factor of 3047 (Hoiseith et al., 2008; Rodriguez-Alvarez et al., 2015). Note  
164 that when the volume of pure alcohol is desired, the density ( $\rho=789 \text{ g/L}$ ) needs to be included. In  
165 order to report an amount of consumed alcohol doses per day per capita aged 15+, the value for a  
166 standard dose of alcohol needs to be known (9.86 g pure alcohol) (NIAAA, 2010).

167 Statistical analysis was performed with IBM SPSS Statistics 22 software (IBM, Armonk, USA). A One-  
168 Sample Kolmogorov-Smirnov normality test was applied to determine if parametric or non-  
169 parametric tests should be used. To investigate if there were any daily differences in alcohol

170 consumption, paired samples T-test or Wilcoxon signed pair rank test were used depending on the  
171 normal distribution. Variations between years and locations were evaluated by using the parametric  
172 One-Way Anova test, followed by a Bonferroni post-hoc test after validating the normality of these  
173 data. The limit for significance in all cases was set at  $p < 0.05$ .

174

## 175 **3.0 Results and discussion**

### 176 *3.1. Spatial analysis*

177 To evaluate spatial variations in alcohol consumption, the calculated use (in standard consumptions  
178 per day per capita 15+) in each of the seven locations that were targeted in 2015 was averaged and  
179 compared (Figure 2).

180 Two WWTPs that receive wastewater from the city of Antwerp were included in the present study:  
181 WWTP Antwerpen-Zuid covers the centre of Antwerp, whereas WWTP Deurne covers the eastern  
182 districts of this city. This is reflected in the statistically significant differences observed in the  
183 measured alcohol consumption between the two WWTPs. The alcohol consumption in the area of  
184 WWTP Deurne was considerably lower than the use in the area covered by WWTP Antwerpen-Zuid  
185 ( $p = 0.002$ , One-Way Anova). This observation can be related to the higher density of recreational  
186 accommodations (discotheques, pubs, etc.) in the centre of Antwerp compared to the suburbs of the  
187 city. Consequently, this can be linked with higher alcohol consumption in the centre of Antwerp.

188 Statistical analysis revealed that alcohol consumption in the regions covered by WWTP Antwerpen-  
189 Zuid and WWTP Brussel-Noord is different from its use in the other regions (Figure 2). These results  
190 suggest that the use of alcohol in more urbanised cities, such as Brussels and Antwerp is higher than  
191 those from smaller villages and cities, such as Ninove, Geraardsbergen, Oostende and Wulpen. The  
192 alcohol consumption measured in the neighbouring villages of Ninove and Geraardsbergen was very  
193 similar, which can be linked with the matching socio-demographic character of both villages. WWTPs

194 Oostende and Wulpen both receive wastewater from several seaside villages. Although the  
195 difference between Oostende en Wulpen was not statistically significant, it seems that the alcohol  
196 use in the catchment area of WWTP Oostende is somewhat higher than in WWTP Wulpen. Probably  
197 this can be related to the different tourist influxes and activities between these two recreational  
198 locations. This study is the first to report on the applicability of WBE to detect spatial differences in  
199 alcohol consumption by comparing results of seven cities with different demographics within one  
200 country.

201

## 202 *3.2. Temporal analysis*

### 203 3.2.1. Daily variations

204 When evaluating daily variations in alcohol consumption based on wastewater analysis, one needs to  
205 consider two important issues: 1) 24 h-composite sampling is applied to obtain representative  
206 samples and 2) EtS is excreted from the body via urine with peak concentrations after 2-4 h and with  
207 more than 90% of the total EtS excreted within 12 h after alcohol consumption (Helander et al.,  
208 2008; Wurst et al., 2011).

209 The 24-h samples are pooled samples consisting of discrete subsamples collected after a fixed  
210 interval (time or volume) (Ort et al., 2010). Consequently, in addition to the abovementioned  
211 information regarding the excretion pattern of EtS, a 24-h composite sample from e.g. Sunday  
212 contains EtS that is mostly originating from alcohol consumption on Saturday (evening) and Sunday.

213 Clear variations in alcohol consumption were observed during the week with a recurrent trend. The  
214 consumption patterns typically describe the highest use over the weekend period. Previous studies  
215 based on wastewater analysis have already shown a significantly increased consumption of alcohol  
216 during the weekend in Italy, Norway and Spain (Mastroianni et al., 2014; Reid et al., 2011; Rodriguez-  
217 Alvarez et al., 2014; Rodriguez-Alvarez et al., 2015). However this study included multiple locations

218 within Belgium and these were monitored over multiple years. In this way, a more profound and  
219 reliable statistical evaluation of the daily variation in alcohol consumption could be performed  
220 (Figure 3). Consumption from Tuesday to Thursday was lower than the consumption on Saturday and  
221 Sunday ( $p < 0.001$ , Paired Samples T-test) which can be explained by more recreational use of alcohol  
222 during the weekend. Interestingly, we observed that the alcohol consumption calculated for Friday  
223 and Monday was higher than the consumption on Tuesday to Thursday ( $p < 0.001$ , Paired Samples T-  
224 test) was lower than the consumption on Saturday and Sunday ( $p < 0.001$ , Paired Samples T-test). This  
225 probably can be linked with the fact that the samples from Friday and Monday capture parts of the  
226 typical weekend alcohol consumption.

227

### 228 3.2.2. Annual variations

229 Another interesting feature of the WBE approach is the possibility to compare alcohol consumption  
230 over the years. To this end, the results of the three WWTPs (Antwerpen-Zuid, Deurne and Ninove)  
231 sampled in all three years (2013-2015) were used for further statistical analysis (Figure 4). The  
232 alcohol consumption in 2013 was significantly higher than in 2014, however there was no significant  
233 difference between 2013 and 2015 or between 2014 and 2015.

234 A similar pattern in alcohol use was observed in WWTP Antwerpen-Zuid and Deurne between the  
235 three years with a clearly higher alcohol consumption in 2013 compared with 2014 and 2015. Alcohol  
236 consumption in 2014 was lower than in 2015 for both two locations. In the catchment area in WWTP  
237 Ninove, however, a different pattern was observed. The highest alcohol use was found in 2013,  
238 which is similar to the findings from the two WWTP areas in Antwerp, but the alcohol consumption in  
239 2014 in WWTP Ninove was higher than in 2015. These results highlight the usefulness of the WBE  
240 approach: temporal variations on a local scale can be monitored on a short-term and long-term  
241 resolution.

242

243 *3.3. Extrapolation to a national level and comparison with other studies*

244 The full data set included 163 days of sampling spread over three years and covered approximately  
245 1.6 million inhabitants from large cities and smaller villages, which corresponds to 12% of the Belgian  
246 population. These features made appropriate an extrapolation of the results to an annual amount of  
247 consumed ethanol for the whole of Belgium (11.2 million inhabitants) for the period 2013-2015. The  
248 extrapolation resulted in a use of  $1.2 \pm 0.5$  standard consumptions per day per capita aged 15+,  
249 corresponding to a yearly consumption of  $5.6 \pm 2.0$  L pure ethanol per capita aged 15+ in Belgium.  
250 This value is lower than the values reported by the WHO (2010) and the Belgian Scientific Institute  
251 for Public Health (2013), with respectively 2.4 and 1.4 standard consumptions per day per inhabitant  
252 (15+ population) (WHO, 2014; WIV-ISP, 2013). However, considering the uncertainties related to all  
253 three estimations (see 3.4.), the estimation of alcohol consumption based on the WBE approach  
254 results in a logical and realistic figure.

255 A similar WBE approach was applied by Reid et al. (2011) on the population of Oslo, Norway, by  
256 Rodríguez-Álvarez et al. (2014) on the population of a city in Galicia, Spain (exact location is unknown  
257 due to reasons of confidentiality), and by Mastroianni et al. (2014) on the population of Barcelona,  
258 Spain. These values yielded respectively 6.8, 5.9 and 8.0 L/year/inhabitant. The values found in these  
259 studies ranged within a similar magnitude as the values found in our study. It is important to point  
260 out that these previous studies only included a single urbanised location in their analysis, unlike the  
261 present study which also took into account smaller villages, such as Geraardsbergen and Ninove,  
262 which reflects better the general population within a country. Because various locations were  
263 integrated in this research, the tested population was also larger than the populations examined in  
264 the above-mentioned studies. The presented results are originating from the largest sampling  
265 campaign up to now in the frame of WBE to detect alcohol consumption, which increases the  
266 statistical validity of the observed results. .

267

268 *3.4. Uncertainties of the methods*

269 The WBE approach to assess alcohol consumption in populations has evidently some uncertainties  
270 (Castiglioni et al., 2013). A first factor is the applied sampling frequency and sampling mode. Since  
271 pulses or losses of compounds of interest may exist within this method of wastewater sampling, it  
272 must be performed with a high frequency to take these changes into consideration (Ort et al., 2010).  
273 The sampling mode for the locations included in this study was already evaluated in the frame of a  
274 Europe-wide WBE study to monitor illicit drug consumption and it was concluded that the applied  
275 discrete levels of the sampling mode were considered to be small enough to minimize errors related  
276 to sampling (Castiglioni et al., 2013). Considering that the prevalence of alcohol consumption is much  
277 higher than the illicit drug use prevalence, it is valid to assume that the applied sampling schemes are  
278 appropriate to apply WBE to evaluate alcohol consumption (Castiglioni et al., 2013; Ort et al., 2010).  
279 Another uncertainty arising in the WBE approach is the possibility of (bio)transformation of the  
280 compounds during the in-sewer transport from the place of excretion to the WWTP. However, in the  
281 case of EtS analysis, this is minimalised since Reid et al. (2011) have already proven the stability of  
282 EtS in wastewater and its exclusive formation through metabolism. The estimation of the use of  
283 alcohol from measured concentrations of EtS and its pharmacokinetic information (absorption,  
284 distribution, metabolism and excretion) creates additional uncertainties to the approach. The small  
285 amounts (0.012%) of EtS formed from alcohol result in the necessity of a large correction factor with  
286 a relatively high uncertainty to be used in the back-calculations. In this light, it is important to  
287 mention that only one study has been published which reports on the excretion percentage of EtS  
288 after alcohol intake (Hoiseith et al., 2008). This highlights the absolute need for future  
289 pharmacokinetic studies on the excretion of alcohol as EtS to confirm or update the value of 0.012%.  
290 Another critical factor is the normalization of the data for the population number that is contributing  
291 to a wastewater sample. At the moment, a fixed number of people are used while some variations in

292 population amounts could occur due to commuting, tourist activities,... Finally, the use of equipment,  
293 like LC-MS/MS or flow rate measurement systems are linked with a certain degree of uncertainty. For  
294 all these reasons, further investigations are needed to validate this approach. Nevertheless, WBE  
295 offers a quick and objective methodology to monitor spatio-temporal trends in alcohol consumption  
296 and could deliver information that is complementary to the classical methods used by the WHO and  
297 the Belgian Scientific Institute for Public Health.

298

#### 299 **4.0 Conclusions**

300 This study is the largest study to date to demonstrate the applicability of WBE in monitoring spatio-  
301 temporal trends in alcohol consumption on a local and national scale, by measuring an alcohol  
302 metabolite (EtS) in 163 daily influent wastewater samples from eight locations in Belgium spread  
303 over three years. Higher alcohol consumption was evidenced in the metropolises Antwerp and  
304 Brussels, compared to smaller cities such as Ninove, Geraardsbergen, Oostende and Wulpen. Clear  
305 daily variations in alcohol consumption could be observed with lower alcohol use from Tuesday to  
306 Thursday compared to Saturday-Sunday. Interestingly, use on Monday and Friday was higher than  
307 the use on Tuesday-Thursday but lower than the use on Saturday-Sunday. On an annual level, 2013  
308 has been shown different from 2014, but no differences were found between 2013 and 2015 or  
309 between 2014 and 2015. Finally, alcohol consumption was extrapolated to a national level which  
310 resulted in a daily use of 1.2 standard consumptions per capita aged 15+. This value is in agreement  
311 with findings on alcohol use in Belgium by the Belgian Scientific Institute of Public Health, but is  
312 lower than the value found by the WHO. Future monitoring campaigns based on WBE could provide  
313 timely, valuable and reliable information on the amount of alcohol used by the general population  
314 simultaneously on a spatial and temporal resolution.

315

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317 Nothing declared

318 **Conflict of Interest**

319 No conflict declared

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326 **Supplementary material**

327 Supplementary material for this work is available.

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408

409 Table 1. Summary of the sampling locations and periods. WWTP = wastewater treatment plant

WWTP	Main area served by WWTP	Population served (census data)	Sampling mode	Sampling dates
Antwerpen-Zuid	Antwerp (centre)	130,218	Time-proportional	06/03/2013-12/03/2013 09/04/2014-15/04/2014 18/03/2015-26/03/2015
Brussel-Noord	Brussels	953,987	Volume-proportional	18/03/2015-25/03/2015
Deurne	Antwerp (suburbs)	213,876	Time-proportional	06/03/2013-12/03/2013 09/04/2014-15/04/2014 18/03/2015-25/03/2015
Geraardsbergen	Geraardsbergen	29,047	Time-proportional	19/03/2014-25/03/2014 11/03/2015-18/03/2015
Lier	Lier	31,539	Time-proportional	1/09/2014-14/09/2014 29/09/2014-13/10/2014 28/10/2014-10/11/2014 25/11/2014-11/12/2014
Ninove	Ninove	36,179	Time-proportional	06/03/2013-12/03/2013 19/03/2014-25/03/2014 11/03/2015-17/03/2015
Oostende	Oostende	160,000	Time-proportional	23/04/2015-30/04/2015
Wulpen	Koksijde	78,441	Time-proportional	11/03/2015-18/03/2015*

410 \* Sampling on 12/03/2015 encountered technical problems and sample was thus not available

411

412 Table 2. MS/MS acquisition parameters. EtS = ethyl sulphate ; Q = quantifier ; q = qualifier

	Precursor ion	Product ion	Dwell time (msec)	Fragmentor voltage (V)	Collision Energy (eV)	Cell Accelerator voltage (V)
<b>EtS-D<sub>5</sub></b>	130.0	97.9 (Q)	50	90	14	4
<b>EtS-D<sub>5</sub></b>	130.0	80.0 (q)	50	90	38	4
<b>EtS</b>	125.0	96.9 (Q)	50	84	14	4
<b>EtS</b>	125.0	80.0 (q)	50	84	38	4

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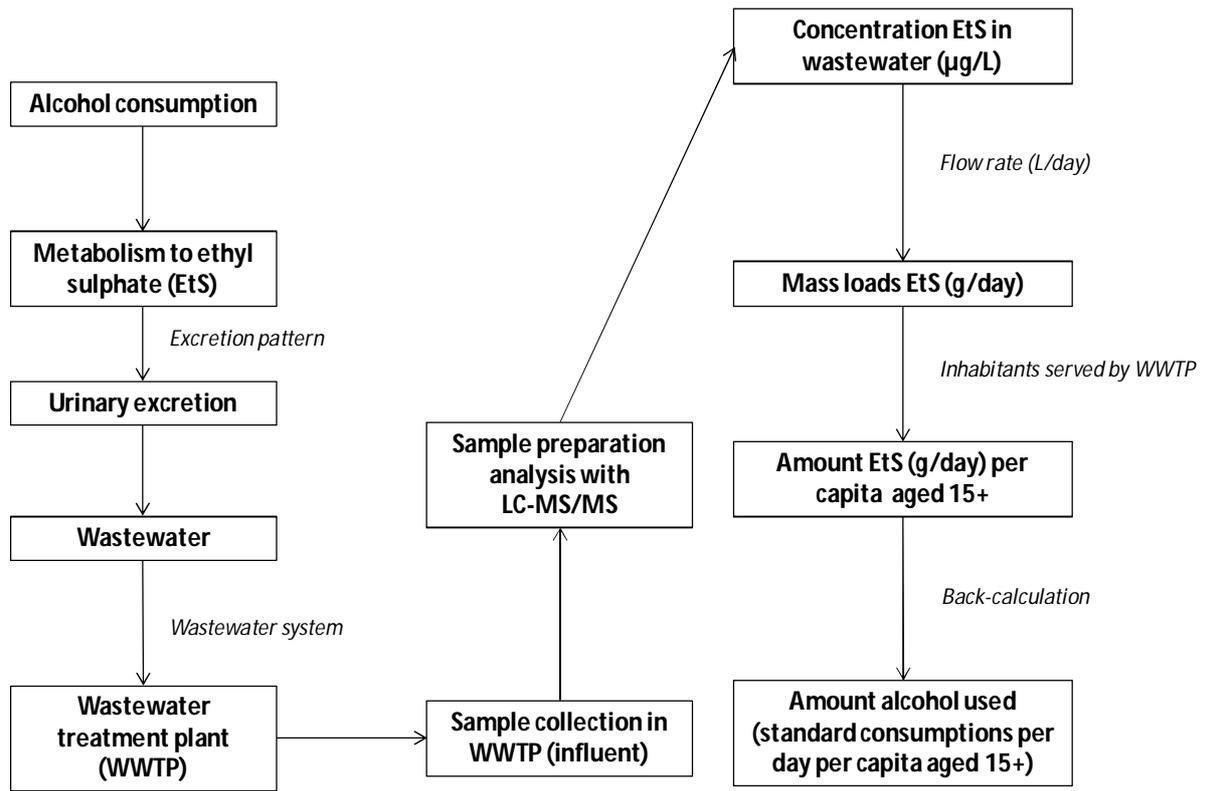
414 **Figure captions**

415 Figure 1. Wastewater-based epidemiology approach to assess the use of alcohol in communities

416 Figure 2. Spatial variations in alcohol consumption for seven locations in Belgium. The symbols (\*; §)  
417 represent no statistical difference.

418 Figure 3. Weekly pattern in alcohol consumption. The graph represents per day of the week the  
419 average value of all investigated locations in all investigated years. The error bars represent the  
420 variation between the different locations.

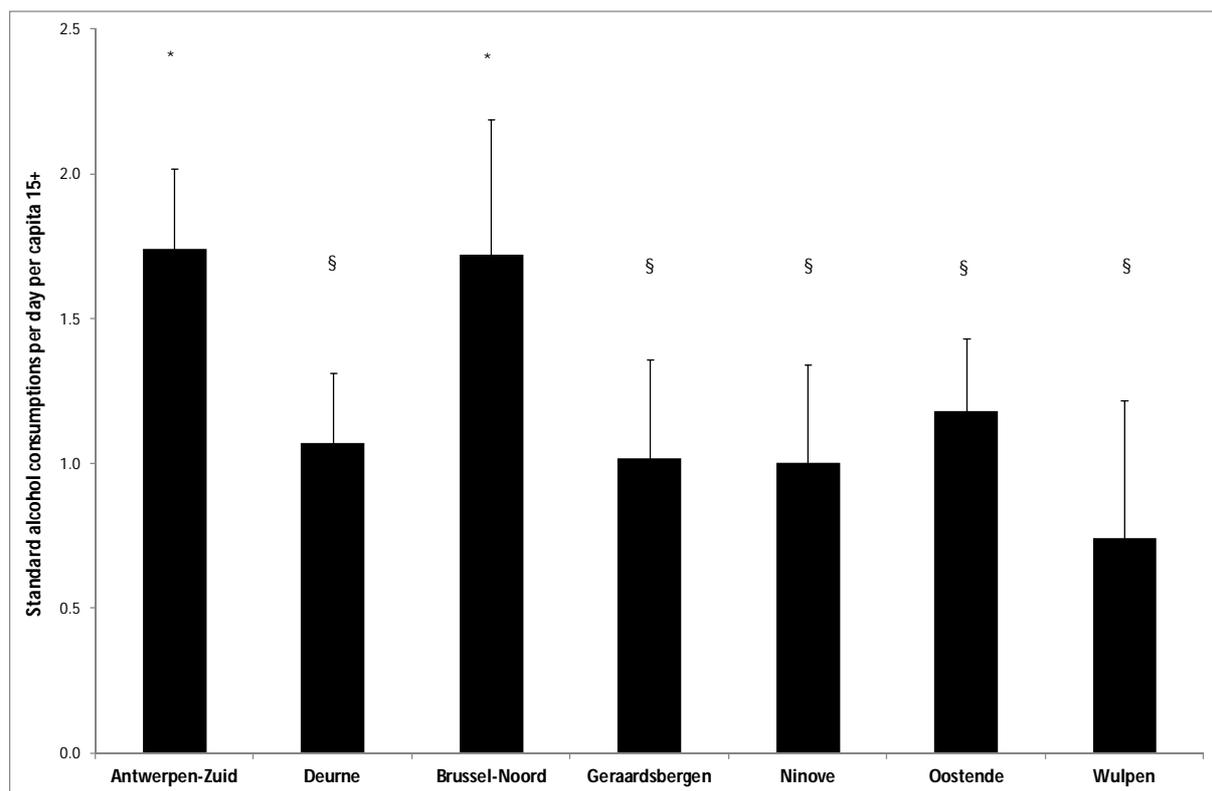
421 Figure 4. Annual variation in ethanol consumption for three locations in Belgium and their average.  
422 The symbol (§) in the average represent statistically significant difference.



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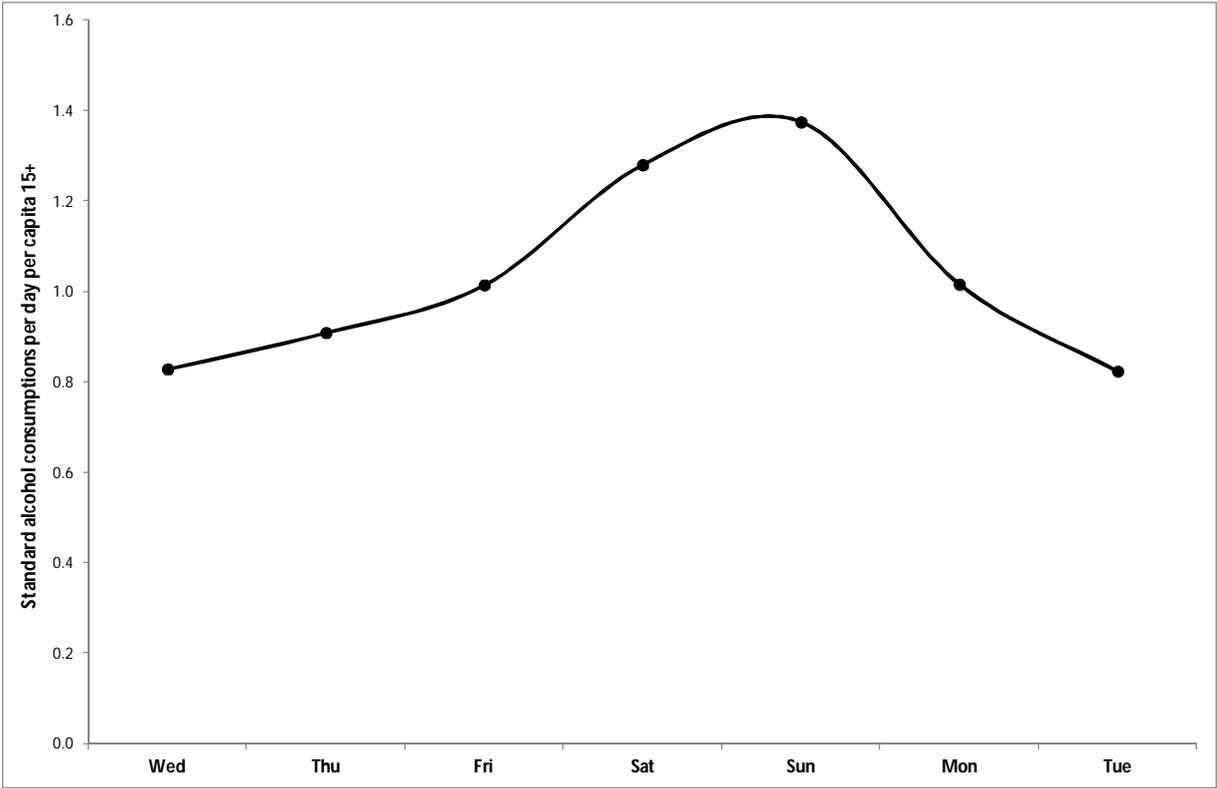
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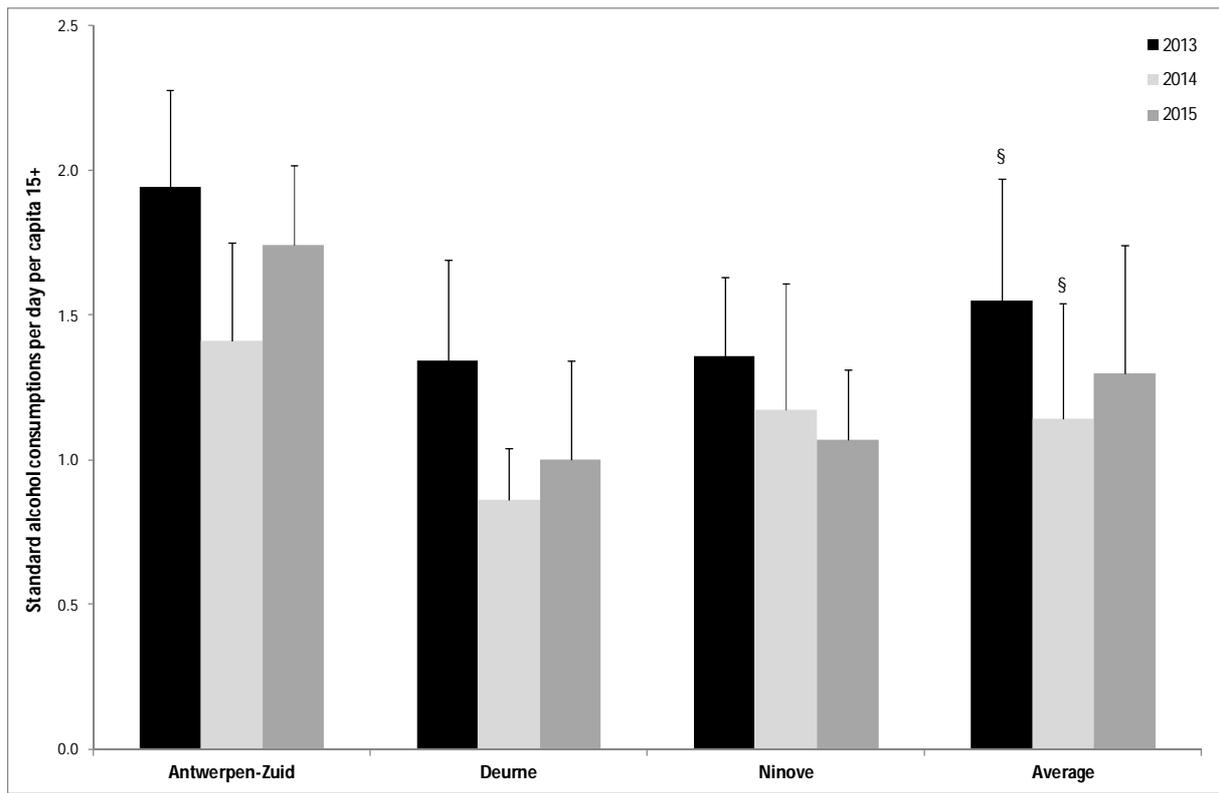
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