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

Tim Boogaerts[a], Adrian Covaci[a], Juliet Kinyua[a], Hugo Neels[a,b], Alexander L.N. van Nuijs[a,*]

[a] Toxicological Centre, Department of Pharmaceutical Sciences, University of Antwerp (UA), Universiteitsplein 1, 2610 Antwerp, Belgium
[b] Laboratory of Toxicology, ZNA Stuivenberg, Lange Beeldekensstraat 267, 2060, Antwerp, Belgium

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ABSTRACT

Background: In recent years, scientific evidence has emerged that wastewater-based epidemiology can deliver complementary information concerning the use of different substances of abuse. In this study, the potential of wastewater-based epidemiology in monitoring spatial and temporal trends in alcohol consumption in different populations in Belgium has been examined.
Methods: Concentrations of ethyl sulphate, a minor Phase-II metabolite of ethanol, in 163 influent wastewater samples from eight wastewater treatment plants in Belgium in the period 2013–2015 were measured with liquid chromatography coupled to tandem mass spectrometry and used to estimate alcohol consumption.
Results: The highest levels of alcohol consumption were detected in the metropoles Antwerp and Brussels compared to smaller villages. Annual variations were detected, with a higher alcohol consumption measured in 2013 compared with 2014. The weekly pattern showed a clear week and weekend difference in alcohol use, with intermediate levels on Monday and Friday. The results were extrapolated and a use of 5.6 L pure alcohol per year per inhabitant aged 15+ has been estimated in Belgium. The comparison with available information on drinking habits of the Belgian population further demonstrated the usefulness of the wastewater-based epidemiology approach.
Conclusions: This is the largest wastewater-based epidemiology study monitoring alcohol consumption to date, demonstrating that objective and quick information on spatio-temporal trends in alcohol consumption on a local and (inter)national scale can be obtained.

1. Introduction

Alcohol (ethanol, CH_3CH_2OH) use is the most common form of substance abuse and has a serious impact on societies with a higher incidence of traffic accidents and criminality, economic damage and health treatment costs (WHO, 2014). Actually, abuse of alcoholic beverages and in particular binge drinking occurs so often that it has become a sociological phenomenon all over the world (Courtney and Polich, 2009). Therefore, a concern about concomitant health problems is manifesting in different countries, which is demonstrated by the fact that four percent of all deaths on a global scale are caused by the (ab) use of alcoholic beverages (WHO, 2014). Because of this serious impact on human health in general, it is important to gather information on the average alcohol use in a population, which can assist authorities to take measures to diminish the morbidity and mortality due to alcohol usage. The findings of the World Health Organization (WHO) suggest a worldwide annual intake of 6.2 L pure ethanol per capita aged 15+ in 2010, which implies the consumption of a daily dose of 1.4 standard consumptions per capita aged 15+ (NIAAA, 2010; WHO, 2014). For Belgium, the WHO suggests an annual consumption of 11 L pure ethanol per capita aged 15+ in 2010, which corresponds to 2.4 standard consumptions per day (WHO, 2014). This value is considerably higher than the finding of 1.5 standard consumption per day per capita aged 15+, reported by the Belgian Scientific Institute of Public Health, 2013 (WIV-ISP, 2013).

In order to better evaluate these discrepancies and to have up-to-date and timely numbers on alcohol consumption, there is a need for quick, objective and complementary approaches to estimate the amount of consumed alcohol within communities. Currently, alcohol consumption is measured mostly based on indi-

* Corresponding author at: Toxicological Centre, University of Antwerp, Universiteitsplein 1, 2610 Wilrijk, Belgium. Fax: +32 3 265 27 22.
E-mail address: alexander.vannuijs@uantwerpen.be (A.L.N. van Nuijs).

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vidual surveys or sales statistics, but these approaches are not always reliable (NIAAA, 2003). Sales numbers are often inaccurate because they are only an indirect measure of alcohol consumption and they do not take international purchases and wastage or stock-piling into account. Interview- and questionnaire-based information on a large population scale suffers from bias since these methods rely on the self-report of the users themselves (NIAAA, 2003; Smith et al., 1990).

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

Alcohol is mainly metabolized through oxidation, which involves alcohol dehydrogenase and aldehyde dehydrogenase to form acetaldehyde and acetic acid. However, a small fraction of alcohol undergoes Phase-II metabolism to form ethyl sulphate (EtS) and ethyl glucuronide (EtG) in which sulphotransferase and UDP-glucuronosyltransferase enzymes play a key role (Helander and Beck, 2005; Kurogi et al., 2012; Schwab and Skopp, 2014; Walsham and Sherwood, 2014). EtS and EtG are commonly used in clinical and forensic toxicology to monitor individual alcohol consumption via the analysis of hair, urine and blood (Crunelle et al., 2014; Walsham and Sherwood, 2012, 2014). Based on this scientific evidence, both EtS and EtG form potential biomarkers that could be targeted in influent wastewater samples to determine the amount of alcohol used by a certain population. Previous studies have already proven the stability of EtS in this matrix and used it successfully on samples that originated from Italian, Spanish and Norwegian populations (Mastroianni et al., 2014; Reid et al., 2011; Rodríguez-Álvarez et al., 2014, 2015). Unlike EtS, EtG was not suitable as an alcohol biomarker in wastewater due to its marked instability in wastewater (Reid et al., 2011).

The present study reports on the application of WBE to evaluate spatial and temporal trends in alcohol consumption in eight different communities in Belgium through the analysis of EtS in wastewater samples and to compare our findings with other figures obtained through the classical approaches. The rationale of the WBE approach can be found in Fig. 1.

2. Materials and methods

2.1. Reagents and materials

LC-grade methanol (MeOH), acetonitrile (ACN), and acetic acid (CH_3COOH) were purchased from Merck (Darmstadt, Germany). Ultrapure water was obtained by purifying demineralized water in an Elga LabWater Purelab Flex system (Veolia Water Solutions and Technologies Belgium, Tienen, Belgium). Analytical standards of EtS and EtS-D_5 were acquired from Athena Enzyme Systems (Baltimore MD, USA) in neat powder. Dilutions of the reference standards were prepared in MeOH. Safe-Lock tubes (1.5 mL and 2 mL) were obtained from Eppendorf (Rotselaar, Belgium) and centrifugal filters with a nylon membrane with pore size 0.2 μm were purchased from VWR (Leuven, Belgium).

2.2. Sampling

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

2.3. Analysis

2.3.1. Sample preparation. Prior to the analysis, samples were thawed at room temperature and afterwards vigorously shaken. One mL of influent wastewater was transferred to a 2 mL Eppendorf tube which was centrifuged at 8000 rpm for 5 min. Thereafter, 190 μL supernatant was transferred to a 1.5 mL Eppendorf tube and 10 μL of the internal standard EtS-D_5 at a concentration of 1 ng/μL was added. The sample was vortexed for 1 min, subsequently brought to a centrifugal filter (0.2 μm) and centrifuged for 5 min at 8000 rpm. The resulting filtrate was transferred to a vial for determination with liquid chromatography coupled to tandem mass spectrometry (LC–MS/MS).

2.3.2. Liquid chromatography-tandem mass spectrometry. The LC system consisted of an Agilent 1290 series binary pump and an autosampler module. Separation was achieved with an Atlantis T3 column (150 mm × 2.1 mm, 3 μm) maintained at 30 °C and a mobile phase composed of (A) Ultrapure water with 0.1% CH_3COOH and (B) ACN using a gradient as follows: 0–10 min: 2–15% B; 10–11 min: 15–95% B; 11–12 min: 95% B; 12–13 min: 95–2% B; 13–20 min: 2% B for column equilibration. The flow rate was 0.18 mL/min and the injection volume was set at 4 μL. EtS and EtS-D_5 eluted at 4.80 and 4.75 min, respectively.

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

A curve containing seven calibration points was constructed with increasing concentrations of EtS (range: 1.5–100 μg/L) and a fixed amount of EtS-D_5 (50 μg/L). The calibration was considered as satisfactory when a coefficient of determination (R^2) >0.99 was obtained and when the back-calculated concentrations of the calibrators had an accuracy within 85–115% (EMA, 2011). The precision of the method could be determined based on these calibrators and was <10% relative standard deviation. The limit of quantification of the method was 1.5 μg/L and was determined as the lowest point of the calibration curve with an accuracy of the back-calculated concentration between 80% and 120% and a precision <20% relative standard deviation (EMA, 2011).

2.4. Calculations and statistical analysis

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

Secondly, back-calculations were made to transform to obtained population-normalized mass loads of EtS into a consumed amount of alcohol (Fig. 1, see Table S1 for the all raw data). Therefore, the excretion rate (0.012%) and the molar mass ratio of EtS and alcohol (0.369) were considered, resulting in a correction factor of 3047 (Hoiseth et al., 2008; Rodríguez-Álvarez et al., 2015). Note that when the volume of pure alcohol is desired, the density (ρ = 789 g/L) needs to be included. In order to report an amount of consumed alcohol doses per day per capita aged 15+, the value for a standard dose of alcohol needs to be known (9.86 g pure alcohol; NIAAA, 2010).

Statistical analysis was performed with IBM SPSS Statistics 22 software (IBM, Armonk, USA). A One-Sample Kolmogorov-Smirnov normality test was applied to determine if parametric or non-parametric tests should be used. To investigate if there were any daily differences in alcohol consumption, paired samples *T*-test or Wilcoxon signed pair rank test were used depending on the normal distribution. Variations between years and locations were evaluated by using the parametric One-Way Anova test, followed by a Bonferroni post-hoc test after validating the normality of these data. The limit for significance in all cases was set at $p < 0.05$.