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Investigation of agreement between wastewater-based epidemiology and survey data on alcohol and nicotine use in a community

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Abstract

Background/Aims
Alcohol and nicotine are the two most used substances world-wide and associated with increased burden of disease. Since surveys on substance use may be difficult due to response biases, wastewater-based epidemiology (WBE) was developed as a more objective measure of nicotine and alcohol use. This study compares estimates of nicotine and alcohol use from a wastewater sampling campaign in a medium-sized Belgian city with a concurrently executed population survey.

Methods
29,083 letters about participation in an online survey study on weekly alcohol and tobacco use were sent to the inhabitants of Lier, Belgium. Wastewater samples were collected from the associated treatment plant in four bi-weekly periods. Samples were analyzed using liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). Ethylsulfate was used as alcohol biomarker and cotinine and trans-3’-hydroxycotinine as nicotine biomarker.

Results
263 (1%) surveys were filled out on average per week. According to survey data, alcohol and nicotine were used less than in the rest of Belgium and this was matched by the wastewater data. Nicotine use, but not alcohol use, showed a significant variation over the sampling periods. Both nicotine and alcohol showed increase use during the weekend while only alcohol showed a different use pattern throughout the week.

Conclusion
No correlation between WBE and survey data could be demonstrated, possibly due to small sample sizes. However, this study shows that weekly trends in alcohol and nicotine use can be quickly detected from wastewater analysis and the occurrence of major events such as festivals can be identified.
Introduction

In the most recent data from the World Health Organization it is shown that 66% of European inhabitants older than 15 have drunk alcohol in the past 12 months. In Belgium, this number is 82% (World Health Organisation, 2014). For nicotine, the numbers are lower, but it is still the most used substance after alcohol (23% on average in Belgium, (World Health Organization, 2015a). This makes alcohol and nicotine the two most frequently used substances in the world. Both substances are significantly associated with increased burden of disease. For example, in Belgium 6% of all inhabitants >15 years of age drink at levels potentially dangerous to their health (Gisle and Demarest, 2014). Since there are considerable health-related and economic issues associated with the use of both tobacco and alcohol (i.e. lung cancer from tobacco smoke (Castaldelli-Maia et al., 2015) or liver disease, stroke etc. (Costin and Miles, 2014) in the case of alcohol), accurate measurements of their use are necessary for health care professionals, researchers and policy makers. However, research on substance use is often difficult. Several well-established methods have been developed to estimate substance use on a population level, but most of these methods rely on self-report surveys which are known to be subject to response biases. For example, overestimation or underestimation of alcohol or nicotine use may occur by users, leading to mistakes in reporting of substance use numbers.

A more objective measure of nicotine and alcohol use is wastewater-based epidemiology (WBE). Wastewater samples collected at the inlet of a wastewater treatment plant (WWTP) can be analyzed for the presence of biomarkers (parent compound and/or metabolites) of the substance under investigation (van Nuijs et al., 2011; Zuccato et al., 2008, 2005). Over the past 10 years, WBE has been refined to reliably evaluate spatio-temporal trends in the use of illicit drugs in wastewater (Castiglioni et al., 2013; Ort et al. 2014; Thomas et al., 2012; Salvatore et al., 2015) and its application has been extended to other (licit) substances like alcohol, nicotine or caffeine (Castiglioni et al., 2015; Reid et al., 2011a; Senta et al., 2015). However, few comparisons have been made so far between traditional epidemiological measures such as surveys and WBE on alcohol and nicotine use. Nevertheless, two studies on comparisons between WBE and other epidemiological measures showed good comparability for cocaine (Reid et al., 2012) and morphine (Been et al., 2015) use.

The current paper aims to investigate the agreement between concurrently executed population surveys and a wastewater sampling campaign in a medium-sized Belgian city. Samples were analyzed for the presence of alcohol and nicotine biomarkers and based on
these measurements, an estimation of the use of alcohol and nicotine in the community was made. These estimates were then compared to survey data on alcohol and nicotine use in the same region. Furthermore, comparisons were drawn between estimations of nicotine and alcohol use and national consumption data to see how wastewater analysis matches national drug use estimates.

Methods
Wastewater-based epidemiology

Sampling
During autumn 2014, a bi-weekly sampling campaign was set up in the WWTP of Lier, Belgium. The selected WWTP has a design capacity of 30,600 inhabitant equivalents (data from www.aquafin.be, accessed 23-01-2015) and serves the city of Lier, Belgium. The sampling campaign resulted in data from four two-week periods, spanning 01 September 2014 until 30 November 2014. For each two-week period, daily 24-hour composite samples were collected. The composite sampling was done in a time-proportional manner with 10-min time intervals. All samples were collected in high-density polyethylene containers and stored at -20 ºC until analysis.

Analytical methodology
The compounds of interest were nicotine and its metabolites (cotinine and trans-3'-hydroxycotinine) and ethyl sulfate (EtS), an ethanol metabolite which is excreted in urine after the ingestion of alcohol.

For the analysis of nicotine and its metabolites, samples were centrifuged at 2500 rpm for 5 minutes to remove particles. Following centrifugation, 3 mL were spiked with an internal standard mixture containing cotinine-D$_3$ (2 ng) and nicotine-D$_3$ (20 ng). This was followed by a solid-phase extraction (SPE) procedure using Oasis HLB cartridges (3 mL, 60 mg) which were acquired from Waters Corp. (New Bedford, MA, USA). Further details about sample preparation and method performance are described elsewhere (Senta et al., 2015).

For the analysis of EtS, a method previously developed in the Mario Negri Institute was adapted. 1 mL of influent wastewater was centrifuged at 8000 rpm for 5 min. Then an aliquot of 190 µL was spiked with 10 µL of the internal standard ethyl sulfate-D$_3$ (1 ng µL$^{-1}$). After mixing, the total volume was transferred to a centrifugal filter (0.2 µm) and centrifuged for 5 min at 8000 rpm. Finally, the extract was transferred into a glass vial for instrumental analysis.
For detection and quantification of nicotine, cotinine and trans-3’-hydroxycotinine, an API 5500 triple quadrupole equipped with a Turbo Ion Spray source (Applied Biosystems - Sciex, Thornhill, Ontario, Canada) and a 1200 Series pumps system (Agilent Technologies, Santa Clara, CA, USA) were used. These compounds were separated on 100 x 1 mm X-Terra C18 column from Waters Corp. using Milli-Q water with 10 mM ammonium acetate (eluent A) and acetonitrile (eluent B), with the following gradient program: initial condition 98% of eluent A, followed by a 10-min linear gradient to 100% of eluent B, 4-min isocratic elution and 1-min linear gradient back to 98% of eluent A, which was held for 13 minutes to equilibrate the column. The flow rate was maintained at 70 μL/min and the injection volume was 1 μL. Samples were ionized using electrospray ionization in positive polarity (Senta et al., 2015). To calculate the nicotine used, cotinine and trans-3’-hydroxycotinine mass loads were summed and then back-calculated using a method previously developed (Castiglioni et al., 2015). Briefly, the mass loads of nicotine metabolites were multiplied by a correction factor (CF = 1.35) developed taking into account the excretion rate of cotinine and trans-3’-hydroxycotinine. Further back-calculation was based on the average amount of nicotine absorbed systemically when smoking, the average number of cigarettes smoked per day and prevalence data in order to obtain the number of cigarettes and the number of smokers.

For the analysis of EtS, an Agilent 1200 series LC system with an Atlantis T3 2.1 mm x 150 mm, 3 μm column (Waters) was used. The column temperature was maintained at 20 ºC. The flow rate was 0.18 mL/min and the injection volume was optimized and set to 4 μL. Mobile phase consisted of Milli-Q water with 0.1% acetic acid (eluent A) and acetonitrile (eluent B). The percentage of eluent A changed as follows: 0 min, 98%; 10 min, 85%; 11 min, 5%; 13-20 min, 98% for column equilibration. The mass-spectrometry (MS) system was an Agilent 6460 triple quadrupole mass analyzer equipped with an electrospray interface operating in negative ionization mode. Specific MS parameters such as fragmentor voltage and collision energy were optimised. Quantitative analyses were performed in multiple reaction monitoring (MRM) mode, and the two most abundant fragmentation products (selected as quantifier and qualifier) were recorded. A correction factor for EtS (CF = 3047) was used to back-calculate the amount of alcohol consumed in Lier based on measured concentrations of EtS in wastewater, taking the excretion rate of alcohol as EtS into account (Rodríguez-Álvarez et al., 2015).
Surveys

Two weeks prior to the start of the study in September 2014, all inhabitants of the city of Lier above the age of 15 (N=29,083) received an introductory letter addressed to them personally to inform them about the study and how they could participate. In order to preserve the privacy of the participants, all the addresses were collected by employees of the city council and letters were sent using an external mailing company. At no point in the data collection did the researchers have access to personal information about the participants, except for those who chose to do so themselves. The procedures of the survey study are discussed in detail in van Wel et al (unpublished data).

A web-based survey was made available during the same period as the wastewater sampling campaign (i.e. September 2014-November 2014). Participants were asked, among other questions, to indicate if they had used alcohol or nicotine in the past week. If the answer was no, they could click ahead to the end of the questionnaire. If the answer was yes, a page opened with further questions on the use of the selected substance, such as the number of days they used, the amount of substance they used and in which way they had used it. In this way, the total duration of the questionnaire could vary between participants and between weeks, depending on the use pattern of each individual. Since this study was part of a larger investigation, the results from other questions dealing with the use of illicit substances are described in van Wel et al (unpublished data).

Ethical approval for the study was acquired from the Ethical Committee of the Social Sciences and Humanities of the University of Antwerp (approval number SHW_14_05_02). Prior to continuing to the actual survey, participants were required to give consent by agreeing to an informed consent statement on the website.

Statistics

All data were analyzed using IBM SPSS version 22.0 for Windows. The data on self-reported alcohol and nicotine use were summarized using means and standard deviation (SD). The calculated biomarker loads in wastewater (g/day) were identified in three ways: either as (1) belonging to one of four sampling sessions (period 1 from 1 - 14 September, period 2 from 18 September - 13 October 2014, period 3 from 28 October - 10 November 2014, period 4 from 25 November - 11 December), (2) as being a weekday (Monday through Friday) or weekend day (Saturday and Sunday) or (3) each day of the week separately. First, Shapiro-Wilk’s tests were done to check for normality of calculated drug consumption. Neither alcohol use nor
nicotine use were normally distributed for all variables. Thus, Kruskal-Wallis tests were used to calculate whether a significant difference existed for alcohol or nicotine over the 4 sampling sessions. If significant, post-hoc Dunn-Bonferroni testing was employed to test for differences between two separate sampling sessions. The same procedure was used for analyzing the differences between separate days of the week. For the week vs weekend analysis the independent samples Mann-Whitney U Test was used. P-values less than 0.05 were considered statistically significant.

In order to investigate the agreement between survey and wastewater data, the positive responses for each individual drug were summed per week and wastewater biomarker loads (g/day) were averaged for each week. Spearman rank correlations were then used to assess the relationship between survey and wastewater data.

Results
Wastewater results
The daily loads of each selected compound in wastewater were calculated by multiplying its concentration (ng/L) by the daily flow rate (L/day) measured during the sampling campaign. The results show that, on average, 290630 grams of alcohol were consumed daily in the catchment area of Lier WWTP. Considering that a standard glass of alcohol contains 10 grams alcohol, on average 29,063 glasses of alcohol were drunk daily. Further, an estimated average of 171g of nicotine was used daily. When taken into account that the amount of nicotine absorbed from each cigarette smoked is approximately 1.25 mg (Castiglioni et al., 2015), that means that in general, 136,457 cigarettes were smoked daily in Lier. If taking into account that an average smoker smokes 15 cigarettes per day (Stichting tegen Kanker, 2014) this would mean that roughly 9097 smokers were present in the catchment area daily.

The Kruskal-Wallis test showed significant differences between the four sample sessions for nicotine ($\chi^2(3)=19.21$, p<.01), but not for alcohol. Additional post-hoc comparisons between sessions showed that this significant difference occurred between sample session 2 and sample session 1 and 4 (all p’s <.01) (fig. 1).

Mann-Whitney U tests showed significant differences between weekdays and weekend scores for both nicotine (U=164, p<.01) and alcohol (U=37, p<.01). Thus, there was a higher load of nicotine and alcohol in wastewater at the weekend than on weekdays. Additional Kruskal-Wallis analyses on weekday differences for nicotine showed no differences between separate days of the week ($\chi^2(6)=10.93$, p=.09) whereas alcohol consumption in wastewater was significantly different ($\chi^2(6)=37.59$, p<.001). Further Dunn’s post-hoc tests showed an
increase in alcohol load in wastewater on Sunday vs. Tuesday, Wednesday, Thursday and Friday (all p’s < .01) and Saturday vs Wednesday (p < .05) and Thursday (p < .01) (fig.1).

Surveys
A total of 3,425 questionnaires were collected over the 12 week survey period. This amounted to an average of 263 unique participants each week (total response rate 1%). The average age of participants was 42.8, and the mean male:female ratio was 1:2. Each week, on average 61% of all responders indicated having used any alcohol. The numbers for nicotine use were much lower, with only 13% indicating using tobacco in the past week. In week 10 a sharp decline occurred in the number of participants. During this week, the program used to send automatic e-mails did not work properly and therefore less people received reminders to fill out the questionnaire.

Survey data vs wastewater analyses
The correlation between alcohol use estimates from wastewater and survey was not significant (σ = -.64, p = .12). Neither was the correlation between nicotine (represented by using cotinine and trans-3’-hydroxycotinine) and the survey responses for smoking (σ = -.14, p = .76).

Discussion
In this paper, simultaneously collected survey data and wastewater data on alcohol and nicotine use were compared. No relationship was found between alcohol and nicotine use reported in the survey and the data from WBE. This is at odds with what we expected, since we hypothesized that we would see an increase or decrease in positive answers on the questionnaire to match increases or decreases of the substance in wastewater. These non-matching results might be due to the low number of responders to the survey. A response rate of 1% per week makes it difficult to provide a completely representative population sample. While the average age of the participants is approximately the same as that for the Belgian population (42.8 in our survey vs 41.2 in Belgium), the male:female ratio in the survey is about 1:2 while in Lier it is roughly 1:1 (Statistics Belgium, accessed 01-10-2015). Men are more likely than women to use substances (Drieskens et al., 2013; World Health Organization, 2015b), thus influencing the comparisons with wastewater data. However, it is
also possible that the wastewater data do not accurately reflect the amount of substances used by the community in the catchment area of the WWTP, but reflects an increase in the concentration of nicotine and alcohol in wastewater due to people coming into the city for particular events. Nevertheless, the city of Lier was chosen because it was not expected to have big fluctuations in the number of persons present. Still, as shown in the data, two large events took place in period 2 with an associated increase in tourists which might have influenced the results. We do not know of such events causing large increases or decreases in population size in other periods, but it might explain part of the lack of agreement between survey and wastewater results. For nicotine results, the lack of agreement between the two sources of data may be explained by two major factors, issues with survey responding and issues with wastewater sampling. Smokers might underreport their use because, despite the legal status of nicotine, they viewed it as a sensitive question (Tourangeau and Yan, 2007) and declined to answer truthfully. At the same time, wastewater results might overestimate the use because the nicotine metabolites used for estimating the number of smokers in the population through wastewater analysis are not formed exclusively through smoking tobacco (Castiglioni et al., 2015). The consumption of other nicotine-containing products such as gum and patches (used to quit smoking), e-cigarettes or snuff-tobacco can also cause an increase of the concentration of nicotine in wastewater, but the use of these products was not investigated in the survey. Finally, non-response bias also might have influenced the results, considering that the questionnaire reminders were always on Monday. Since our results point towards a negative correlation between the two methods, it seems to be the case that the persons that used more alcohol and tobacco during the weekend were less likely to answer the survey on the following Monday. Thus, while the lack of agreement between survey and wastewater data is noteworthy, it is difficult to pinpoint its cause. The use of other triangulation sources, such as sales data would have been useful as an additional way of estimating use, apart from the wastewater and survey data. Unfortunately, these data were not available on a city level and therefore not specific enough to use in this instance. Based on our results, a number of recommendations can be made for future studies in this direction. First, a more reliable sampling method for estimating the population served by the WWTP should be used and survey participation should be made as easy as possible. For example, an app could be made available for those who decide to participate to the study. This app could prompt for answers more frequently, potentially reducing non-response bias. Secondly, a place and period of sampling should be chosen in which population fluctuations are as small as possible to prevent any confounding. Finally, it would be helpful if either a metabolite more specific to
smoking tobacco would be used, or if a survey could take into account the various products which cause the excretion of nicotine. Examples of more tobacco-specific metabolites are anabasine or anabatine (Tscharke et al., 2015), although these are also present in dried tobacco and thus levels in wastewater might reflect thrown away cigarette butts as well as smoked tobacco.

The results shown for alcohol, 134,448 L per year, correspond to a per capita consumption of alcohol among the adult population (above 15 years of age) of 4.3 L pure alcohol per year. This is below the Belgium average of 11 L alcohol per person per year as presented by the World Health Organization (WHO) in 2014 (World Health Organisation, 2014). However, the data from the WHO is based on official statistics on the production, import, export and sale of alcohol. This might give an overestimation of use since the act of buying alcohol does not mean that it is actually consumed. However, the discrepancy between official estimates of alcohol use and wastewater results was also found in a recent study for Italian and Spanish cities (but not in Norway) (Rodríguez-Álvarez et al., 2015). Further research is needed to see if this discrepancy is due to more or less accurate reporting of sales figures in different countries or whether there are some methodological reasons for the low estimations of alcohol use. Patterns of alcohol use in Lier match the pattern seen for illicit party drugs such as cocaine and MDMA, with heavier use during the weekend (Huerta-Fontela et al., 2008; Reid et al., 2011b; van Nuijs et al., 2009; unpublished data by van Wel et al). This is in accordance with survey data, who indicate using (more) alcohol during the weekend (see, for example, (Reich et al., 2015)). Interestingly, some carry-over effects from alcohol use on Sunday can be detected in wastewater data on Monday, suggesting that people drink more alcohol on Sunday evening than on other evenings during the week.

Analysis of the nicotine data resulted in an estimation of on average 136,457 cigarettes smoked per day in Lier, which comes down to 9,097 smokers (25% of the total census population of 37,236) if an average of 15 cigarettes per smoker per day (data taken from Stichting tegen Kanker, 2014) is considered. This is similar to the Belgian average of 26% (Stichting tegen Kanker, 2014) which indicates that the method of estimating nicotine use with WBE is in good accordance with numbers on smoking prevalence from traditional epidemiological methods and thus might be used in the future to provide accurate data on nicotine use. Nicotine consumption was generally stable over the week with no differences between days of the week although when considering weekdays and weekend days, more nicotine was used in the weekend. This was in agreement with previous WBE studies on nicotine (Tscharke et al., 2015), although these authors used different nicotine biomarkers.
Although WBE has shown to reliably measure substance use, including licit drugs such as alcohol and nicotine, there are still some limitations for this method which should be taken into consideration. As mentioned before, the nicotine metabolites used to estimate nicotine use are not selective to cigarettes (Castiglioni et al., 2015) but may reflect use of other nicotine-containing products as well. This may lead to overestimations of smoking if one does not take this into account. However, up until now, cotinine and trans-3’-hydroxycotinine are the best, most stable metabolites available for analysis. Additionally, the data show good accordance between estimates of smoking from the wastewater method and those from official smoking estimates, providing evidence that the use of these metabolites is warranted. Furthermore, it has to be noted that EtS, while selective for the human metabolism of alcohol and stable in wastewater, is excreted at very low rates and it was assessed in very few studies (0.010-0.016% of all consumed alcohol) (Reid et al., 2011a). From this excretion rate, a correction factor is calculated that transforms raw wastewater data into data on the actual use of alcohol (CF 3047), (Rodríguez-Álvarez et al., 2015). Since this study shows that alcohol use estimates per year differ strongly between wastewater data and data from the WHO, the possibility should be considered that the correction factor used is imprecise in estimating alcohol use. Thus, calculating a more refined correction factor for alcohol is a point of concern for future studies. However, in order to do this, the knowledge of EtS excretion rates in human urine after alcohol ingestion should be explored further. Finally, it was surprising that no clear variation between the days of the week was seen for nicotine use, especially since there was a distinct weekend vs weekdays use pattern for this substance.

Conclusions

This study illustrates the added value of WBE to study alcohol and nicotine use. It proves that while survey research can be difficult to carry out and is dependent on the willingness of inhabitants to fill out a questionnaire honestly and completely, WBE can independently and objectively give timely information on substance use within a community. Furthermore, the current study illustrates that trends in substance use and the occurrence of specific events can be detected from WBE studies, as was the case here where a festival was taking place during the second sampling session and higher nicotine loads were detected in wastewater. In conclusion, WBE seems to be reliable for a wide number of substances, including legal drugs such as alcohol and nicotine which have a widespread use.
References


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Figure 1: Boxplot A and B showing differences between sample sessions for A) nicotine (using cotinine and trans-3’-hydroxycotinine loads measured in wastewater) and B) alcohol (using EtS) and C and D showing variations between days of the week in use of C) nicotine (using cotinine and trans-3’-hydroxycotinine loads measured in wastewater) and D) alcohol (using EtS)