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A comparison between wastewater-based drug data and an illicit drug use survey in a selected community

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Abstract

Background

Estimations of drug use are mostly based on population surveys that can suffer from response biases. The current study evaluates using wastewater-based epidemiology (WBE) for assessing illicit drug use by comparing wastewater data with that from a population survey.

Methods

Introductory letters (29,083) were sent to inhabitants of Lier, Belgium, asking them to participate in an online survey study. Participants were asked to indicate their drug use in the past week for a 12-week period (September-November 2014). Concomitant wastewater samples were collected from the associated wastewater treatment plant in four bi-weekly periods. Samples were analyzed using solid-phase extraction and liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS).

Results

On average, 263 (1%) inhabitants filled out the questionnaire each week. According to the survey results, cannabis was the most used drug, followed by amphetamine, cocaine and methylenedioxymethamphetamine (MDMA). Wastewater data corroborated these results. Cocaine, amphetamine and MDMA showed a significant difference between days of the week. The four sampling periods differed significantly from each other for cocaine, amphetamine and methadone.

Conclusion

Observed drug consumption patterns from survey and wastewater data match national and international data. Wastewater analyses confirm that WBE can be reliably used to confirm patterns and trends in drug use. Future studies should focus on identifying the most opportune sampling period giving the most reliable estimates of drug use and use smaller, contained communities such as festivals or prisons if methodology allows.
Introduction

Based on survey studies an estimated 5.3% of European adults have used cannabis in the past year (EMCDDA, 2014). However, it has been questioned how reliable these results are since survey studies suffer from a number of methodological issues. Firstly, drug users are in general a challenging population to work with when it comes to survey research. The overall low number of current drug users decreases the chances of including them in a general population survey, thereby gathering too little data to make a reliable statement about drug use in that population. Also, drug users as a population may be less likely to fill out the surveys, for example because they are afraid of judicial consequences or because they live in a situation where they cannot be reached (i.e. not having a postal address). Furthermore, due to the work-intensive nature of population surveys, it can take several weeks to months from the starting point of a survey study until the results of a survey can be communicated.

Considering the dynamic character of the drug market, use patterns could have changed during that time and new drugs and trends may have emerged, thereby decreasing the validity of the survey (Griffiths & Mounteney, 2010). However, one of the greatest issues with survey research is the possibility of reporting errors on a certain topic due to its sensitive nature, as is the case with questions on drug use (Tourangeau & Yan, 2007). This makes population surveys vulnerable to response biases since users may either under- or over-report their drug use.

There are a number of options to either circumvent these issues concerning collecting data on drug use or to supplement the information collected from survey research. These include extrapolating from registered traffic accidents, hospital admissions or admission to addiction clinics as well as looking at police data on drug seizures and trafficking. However, none of these methods can give the full picture of actual drug consumption. Thus, there is a lack of data on current drug use in the general population and methods complementary to traditional studies are necessary. These methods should not only complement current measures of drug use in the population, but may also make it possible to combine both subjective and objective measures of drug use and thereby increase the accuracy of drug use epidemiology significantly.

One of these potentially useful new approaches is analyzing wastewater to assess the use of illicit drugs in an area served by a wastewater treatment plant (WWTP). Hereby, wastewater is analyzed for the presence of drug target residues (DTRs): parent compounds and/or metabolites. DTRs end up in wastewater after drug use, metabolism and subsequent excretion. In the past 10-15 years, the field of wastewater-based epidemiology (WBE) has
seen important improvements. Since its first application in 2005 (Zuccato et al., 2005), wastewater analysis techniques for DTRs have continued to be refined and extended and protocols for the correct handling and storage of wastewater samples have been developed (Castiglioni, Bijlsma, et al., 2013). WBE has a number of advantages over traditional survey methods of estimating drug use. The presence of DTRs can be measured in near real-time as time from sampling to data reporting takes approximately two weeks (anecdotal evidence). Thus, trends and changes in drug use can be detected faster and more accurately than with traditional survey-based techniques. Furthermore, since WBE is performed on the combined wastewater from a large number of households (i.e. the catchment area of a WWTP), none of DTRs in the wastewater can be traced back to a certain individual. This makes the method truly anonymous and, if done in large enough samples, without major ethical issues (Hall et al., 2012; Prichard, Hall, de Voogt, & Zuccato, 2014). Finally, the use of DTRs as objective indicators of drug use could eliminate the need for subjective reporting from the population if the goal is performing a quantitative measurement of drug use. However, as mentioned by Castiglioni, Thomas, Kaspryzk-Hordern, Vandam, & Griffiths (2013), research making a direct comparison between WBE data and traditional epidemiological indicators has been scarce. This is necessary in order to promote the use of WBE either as the sole or as an additional method for monitoring drug use in the general population. Previous research on combining WBE with other epidemiological methods has highlighted the need for using comparable populations while performing these studies (e.g. a WWTP and survey covering the same population) (Reid et al., 2012). This would require a rigorous approach whereby WBE and population surveys are conducted simultaneously. Therefore, in the study described here the aim was to compare the usefulness of WBE for assessing illicit drug use in a community by comparing the results of wastewater analysis with those from a concomitantly administered population survey.

Methods

Wastewater samples

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 around 35,000 inhabitants. The city of Lier was chosen for this study because the WWTP covered only the city of Lier, so that the data obtained from the wastewater study and the data from the survey study would cover the
The sampling campaign resulted in data from four two-week periods (called sampling sessions), spanning 01 September 2014 until 30 November 2014. For each two-week period, 24-h composite wastewater samples were collected daily. 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 °C until analysis.

**Analytical methodology**

Wastewater samples were analysed according to previously validated and published methods (van Nuijs et al., 2009; van Nuijs et al., 2013; Kinyua et al., in press). Samples were first filtered through a glass filter (0.7 µm retention capacity) to remove solid particles. This was followed by a solid-phase extraction (SPE) procedure on Oasis MCX and Oasis HLB cartridges to concentrate analytes and remove interferences. Resulting extracts were analyzed by liquid chromatography coupled to tandem mass spectrometry. The DTRs of interest were cocaine, benzoylecgonine (BE, being the main human metabolite of cocaine (Jufer, Walsh, & Cone, 1998)), amphetamine, methamphetamine, methylenedioxymethamphetamine (MDMA), 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP, as the specific metabolite of methadone), ketamine, 6-monoacetylmorphine (6-MAM, as the specific human metabolite of heroin), and 11-nor-9-carboxy-delta-9-tetrahydrocannabinol (THC-COOH, as the specific metabolite of cannabis). Further details about sample preparation, analysis and quality control are described elsewhere (van Nuijs et al., 2009; van Nuijs et al., 2013; Kinyua et al., in press).

Measured concentrations (in ng/L) were multiplied by the flow rate of the sample (in L/day) to obtain mass loads (expressed in mg/day) for all DTRs. Correction factors (Castiglioni, Bijlsma, et al., 2013; Ort et al., 2014) were then applied to the mass loads for each DTR in order to calculate actual drug use and correct for differences in excretion patterns of illicit drugs (see table SI-1). This results in a value referred to as ‘drug consumption’ (expressed in mg/day).

**Surveys**

In August 2014, 29,083 introductory letters were sent out to the inhabitants of Lier above the age of 15 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 data...
collection did the researchers have access to personal information about the participants, except that which they chose to divulge themselves.

In the introductory letter, it was explained that a web-based survey would be made available during the same period as the wastewater sampling campaign (i.e. September 2014-November 2014) and the addressee was asked to fill out this questionnaire. Furthermore, it was made clear that although the weekly completion of the survey was preferred, every type of participation was allowed (e.g. once, twice or more times). Participants could choose to leave their e-mail address through which they would receive a reminder to fill out the questionnaire each week. While the focus of the survey was on the past-week use of illicit drugs (cannabis, cocaine, (meth)amphetamines, heroin, MDMA, ketamine, new psychoactive substances (NPS) or mephedrone), participants were also asked to indicate if they had used alcohol, tobacco or a number of pharmaceutical drugs such as codeine, methadone, dextroamphetamine (Dexedrine®) or methylphenidate (Concerta®, Ritalin®) in the past week. If the answer was “no” for all substances, 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 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. The questions were partly taken from the validated Belgian Health Interview Survey and comply with guidelines for drug use questionnaires as mentioned in (Bühringer & Sassen, 2010).

Ethical approval for the study was acquired from the Ethical Committee of the Social Sciences and Humanities of the University of Antwerp. Participants were offered the chance to win a prize if they participated in the study. This was checked by them filling out their e-mail address at the start of the questionnaire. In order to prevent persons who did not want to leave their personal data from not participating, this was not a required field for continuing to the actual survey. All participants were required to give consent by agreeing to an informed consent statement on the website prior to continuing to the actual survey.

Statistics
All data were analyzed using IBM SPSS version 22.0 for Windows. The data on self-reported drug use were summarized using means and standard deviation (SD). The calculated drug consumption (mg/day) from the wastewater data was identified in two ways: as (1) belonging to one of four sampling sessions or (2) as separate weekdays. In order to do this, drug consumption was averaged over sampling sessions and days of the week respectively (e.g. all
data belonging to Monday were averaged and compared with all averaged data from Tuesdays. First, Shapiro-Wilk’s tests were done to check for normality of calculated illicit drug consumption. None of the tested substances were normally distributed or homogenous. Thus, separate Kruskal-Wallis tests were used to calculate whether a significant difference existed in drug consumption over the four sampling sessions or if there was a significant difference between weekdays. If significant, post-hoc Mann-Whitney U-tests would be employed to test for differences between two separate sampling sessions or between two separate weekday pairs. The α-criterion was set to 0.05.

In order to correlate the survey data with the wastewater data, per week the yes-responses for each individual drug were summed and drug consumption (mg/day) was averaged for each week. Spearman rank correlations were then done to assess the relationship between survey and wastewater data.

Results
Surveys
A total of 3425 questionnaires were collected over the 12 week survey period. This amounted to an average of 263 unique participants each week (response rate approximately 1%). A summary of responses (in percentages) can be found in Table 1. In week 10, there was a sharp decline 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. The average age of participants was 42.8 years. The overall male/female ratio was about 1:2. Over 95% of participants had completed at least high school education, which was expected since high school is compulsory in Belgium.

Wastewater analyses
Shown in Figure 1 are the log_{10}-transformed results of the calculated use of amphetamine (AMP), cocaine (using BE, its main human metabolite), methadone (using its main metabolite EDDP), MDMA and cannabis (using its main metabolite THC-COOH). No ketamine, methamphetamine or 6-MAM were detected in the samples and were not included in the statistical analyses. Cannabis is the most used drug in the city of Lier, followed by the stimulants amphetamine and cocaine. The level of MDMA was intermediate, while EDDP could be detected in the samples only at low levels.
The Kruskal-Wallis test showed significant differences between the four sample sessions for cocaine ($\chi^2(3)=9.55$, $p<.05$), amphetamine ($\chi^2(3)=12.62$, $p<.05$) and methadone ($\chi^2(3)=16.85$, $p<.05$). There were no differences between sample sessions for MDMA ($\chi^2(3)=4.24$, $p=.24$) or cannabis ($\chi^2(3)=6.93$, $p=.07$). Additional post-hoc comparisons between sessions showed that for cocaine a significant difference was seen between sample session 2 and sample session 4 ($U=17.53$, $p<.05$), for amphetamine between sample session 2 and both sample sessions 3 ($U=17.4$, $p<.05$) and 4 ($U=20.40$, $p<.05$), and for methadone between sample sessions 1 and 2 ($U=-16.83$, $p=.05$) and 1 and 4 ($U=-25.13$, $p<.05$) (see also Figure SI-1).

Kruskal-Wallis tests showed significant differences between weekday scores for cocaine ($\chi^2(6)=35.63$, $p<.001$), MDMA ($\chi^2(6)=38.43$, $p<.001$), amphetamine ($\chi^2(6)=13.11$, $p<.05$) and cannabis ($\chi^2(6)=14.42$, $p<.05$), but not for methadone ($\chi^2(3)=7.45$, $p=.28$). For cannabis, these differences showed only in the comparison between Monday and Thursday ($U=25.44$, $p<.05$, adj.). Cocaine, amphetamine and MDMA showed a typical party-drug profile, where on weekend days more was used than on weekdays (see Figure 2). Although initial Kruskal-Wallis testing was significant for amphetamine, post-hoc analyses did not show a difference between weekdays for this drug. There were significant differences between Sunday and Tuesday ($U=-33.74$, $p=.001$) Wednesday ($U=-33.74$, $p=.001$), Thursday ($U=-37.25$, $p<.001$) and Friday ($U=-33.5$, $p<.05$) for cocaine. For MDMA, significant differences were seen between Sunday and Wednesday ($U=-35.82$, $p<.001$), Thursday ($U=-34.88$, $p=.001$) and Friday ($U=-30$, $p=.01$), between Friday and Monday ($U=29.18$, $p=.01$) and between Monday and Wednesday ($U=35$, $p<.001$) and Thursday ($U=34.06$, $p=.001$).

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Survey data vs wastewater analyses
Spearman’s rank correlation analyses were executed between all DTRs targeted in the wastewater analysis and the relevant survey items. There were no significant correlations between any of the calculated drug consumption values and the numbers of persons indicating having used that drug on the survey. However, a trend towards a negative correlation was found for MDMA ($r_s=-.72$, $p=-.07$). No Spearman’s rank correlations could be calculated for methadone, heroin or ketamine, since either no one indicated having used these drugs in the surveys (methadone) or drug levels were non-existent or below the limit of quantification (ketamine and 6-MAM).
Discussion

The main goal of this study was to simultaneously conduct WBE and a population survey in order to perform correlation analyses on the data. In the survey part of the study, only a few people indicated they used any type of drug in the past week. The most prevalent illicit drug according to the survey results was cannabis, followed by cocaine, amphetamine and MDMA. None of the investigated correlations between drug use as measured by WBE and the population survey data were significant. Interestingly, the correlation between MDMA in wastewater and the indicated use in the survey approached significance in a negative direction. Thus, when an increase in the use of MDMA was seen through wastewater analysis, a decrease in the amount of responses on the questionnaires occurred. A possible explanation for this phenomenon could be that persons who used a certain drug were experiencing a hangover or Monday ‘low’. This might prevent them from filling out the survey the day(s) following use, thus leading to underreporting. Although there was no direct correlation between the survey data and wastewater data, the distribution of use patterns in the survey matches what was found in the wastewater. This reiterates that drug use patterns investigated through traditional methods match those found in wastewater. Potentially, WBE might be a better method than survey research to estimate the amount of drug use since it does not depend on response rates or honest responding. However, since wastewater can only give absolute numbers on drug use (i.e. how many mg of drug was used) and not on subjective data (how much or in what way someone uses), population surveys are still preferable if more in-depth knowledge is needed.

Analyses of the wastewater data provided four interesting results, which will be discussed below.

First, results from the current study are in agreement with the results from national drug use surveys. As is the case in the rest of Belgium, cannabis is the most used drug, followed by amphetamine, cocaine and MDMA (Scientific Institute of Public Health, 2013). The levels of heroin, methamphetamine and ketamine were below the limit of quantification, thus their consumption in Lier is low to negligible. Results are further in accordance with those reported from other countries of the European Union as reported by the European Monitoring Centre for Drugs and Drug Abuse (EMCDDA, 2014), with the exception of cocaine and amphetamine, which are inverted. This might be due to differences in availability of both drugs in Belgium compared to that of the European Union in general.

Second, it was demonstrated that drugs showed a different pattern of use throughout the week. Cocaine and MDMA use increased during the weekend, while cannabis and
methadone did not show a week/weekend variation. Such differences between week and weekend days are consistent with previous data (Huerta-Fontela, Galceran, Martin-Alonso, & Ventura, 2008; Jaroslav et al., 2014; Reid, Langford, Mørland, & Thomas, 2011; van Nuijs et al., 2009). Further, these results confirm findings from sociological studies on drug use (Curran & Travill, 1997; Parrott, Lock, Conner, Kissling, & Thome, 2008; Verheyden, Hadfield, Calin, & Curran, 2002). As mentioned above, cannabis and methadone do not show a week vs. weekend pattern of use. This is not surprising, since both cannabis and methadone are drugs typically taken multiple times per week (Douaihy, Kelly, & Sullivan, 2013; Perkonigg et al., 1999).

Third, the current study also illustrates one of the weaknesses of WBE. From the results shown here, it can be seen that patterns of drug use can differ significantly over the course of several weeks. Since WBE aims to be able to give accurate descriptions of drug use in a population, variations in drug levels as a consequence of uncontrollable variables should be taken into account. In this study, conclusions on overall drug use based on choosing one sampling session over the other could differ significantly. Thus, this study illustrates the point made by Ort et al. (2014) that for future sampling campaigns it is wiser not to pick a random week of the year, but instead use at least 56 random stratified samples. Future studies should focus on what the most advantageous method of sampling is with regard to the timeframe in which sampling takes place as well as how long of a period it should be. For example, using continuous sampling on a certain day of the week could reduce uncertainty about the results from WBE by 5-10%, according to Castiglioni et al. (2013). Nevertheless, large variations between weeks can also be indicative of true variations in drug use thus results such as these should not be disregarded too quickly.

Related to the above, a fourth point that this study illustrates is that WBE is sensitive enough to detect abnormal events. Previous studies have shown that increasing levels of illicit drugs during and after a big event are picked up by wastewater analyses (Bijlsma, Serrano, Ferrer, Tormos, & Hernández, 2014; Jiang, Lee, Fang, Tu, & Liang, 2015). However, those studies were always carried out with the aim of finding such differences and might therefore be more sensitive to finding them. In trying to explain the increase in the use of amphetamine and cocaine during sampling session 2 we discovered that at the same time, two significant events were taking place (a reunion party held in a club and a regional event). This could explain why the weekend peaks during sampling session 2 were higher than during the other weeks. Thus, caution should be exerted in choosing the period of analysis or data collection since a large event occurring at the same time as the sampling period might cause
misinterpretation of data on illicit drug use among the inhabitants of a certain region. An increase in measured drug consumption could have three reasons; 1) an increase in drug consumption by the inhabitants, 2) an increase in persons who consume drugs in the region (i.e. an increased number of visitors) or 3) a combination of the two. Therefore, researchers should be careful interpreting drug consumption in a population from data corrected using solid population estimates. Senta et al (Senta, Gracia-Lor, Borsotti, Zuccato, & Castiglioni, 2015) have been working on a method to assess fluctuating population sizes which would solve this issue.

Unfortunately, response rates to the surveys were very low and as such are a major limitation to interpreting the survey data alone as well as the comparison between WBE and survey data. Despite applying a number of proven techniques to improve survey response such as including incentives for participation and sending out personalized missives (Cook, Heath, & Thompson, 2000), only a small number of people could be motivated to participate in the survey (approximately 1% of the eligible population). Our data are in agreement with the national data on drug use in that the percentage of users is quite low and cannabis is the most used drug. Numbers of recent drug use in Belgium in adults aged 15-64 range from 2.6% for cannabis (in the past 30 days) to 0.8% for any drug other than cannabis (in the past year) (Scientific Institute of Public Health, 2013) and this matches our results. However, the small amount of participants remains a limitation to the interpretation of the data from the survey study.

This study is the first attempt to combine WBE and survey-based epidemiology using the same timeline. Due to the low number of completed population surveys, the comparison of wastewater data to population survey data proved to be difficult. This is a major limitation to the comparison between wastewater results and population surveys in this study. A possible reason for the low response rate might be that this study employed an online questionnaire. However, it is unlikely that the used medium is to blame since recent studies have shown online surveys to have similar response rates to paper-and-pen surveys (see (Greenlaw & Brown-welty, 2009; van Gelder, Bretveld, & Roeleveeld, 2010) for a review). Worldwide a declining trend has been seen in the willingness of persons to participate in survey research and this might also be at work here (McCluskey & Topping, 2011). Furthermore, it might be possible that when inhabitants were informed of the participation of their city in the wastewater study, they felt unwilling to give out more information through surveys than that which was already gathered through wastewater. Concern about the use of wastewater research has been reported previously (Hall et al., 2012) and, in our case, might have led to
non-participation. Therefore, future studies combining wastewater and survey research should be careful about exactly what to communicate to participating cities about the study to make sure such bias does not occur. A way to possibly increase response rates in a future study could be to embed the questions about drug use within a study on general health, where the topic might become less threatening to talk about (Tourangeau & Yan, 2007). This adds the advantage of personalized interviewing, which usually gathers a higher response rate.

While it is important to combine WBE and survey research to make a good estimation of their quality and accuracy, the low response rates indicate that it might be too difficult to do so in a relatively small city (population ±35,000) such as the one used in this study. One solution might be to perform a similar study in smaller communities such as festivals or schools. However, several ethical considerations should then be considered because the smaller the catchment area gets, the more difficult it is to guarantee anonymity and thus the chances of adverse consequences for the inhabitants of a small community increase (Hall et al., 2012; Prichard et al., 2014). Furthermore, the technology behind WBE has not progressed far enough to make a reliable estimate of drug use when drug use in a sample is low and populations small (Ort, Lawrence, Rieckermann, & Joss, 2010), leading to unreliable results.

The current study also illustrates how a body of government or a research institute could use WBE to make an assessment of drug use in the general population. For example, from our results, it can be deduced that the opioid replacement program probably is effective since no heroin could be found in the wastewater while methadone use was detected. Policy makers could potentially use this information to instigate more focused prevention programs or health care interventions or evaluate their current programs. Another possible use of WBE could be as an evaluation tool for drug policy. This could be done by performing repeated wastewater sampling, and thereby investigating whether following a policy alteration, a change in drug use as reflected in the amount of DTR present in wastewater occurs.

In conclusion, the study discussed in this paper is instrumental in providing support for the usefulness of WBE in the estimation of illicit drug use. Declining response rates to population surveys might lead to decreases in the reliability of such surveys, especially in regard to sensitive topics such as drug use. The data on illicit drug consumption through wastewater analysis indicate that WBE can be reliably used to confirm patterns and trends in drug use. Future studies on the methodology of WBE should focus on identifying the most ideal timeframes in which sampling should take place in order to use the most opportune period giving the most reliable estimates of drug use. In order to carry out rigorous ecological comparisons between WBE data and survey data on illicit drug use, research should focus on
smaller, contained communities such as festivals or prisons while keeping in mind several ethical considerations and methodological constrictions. This study also illustrates how WBE can be used to inform policy makers about drug use in the general population.

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Supplementary information is available
The supplementary information contains one figure and table.
References


**Figure 1:** Drug consumption per drug in mg/day (log$_{10}$ transformed). Grey, vertical lines indicate weekends.

**Figure 2:** Boxplots showing differences between different days of the week for A) Used MDMA (mg/day), B) Used cocaine (mg/day), C) Used amphetamine (mg/day) and D) Used cannabis (mg/day)