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Comparative measurement and quantitative risk assessment of alcohol consumption through wastewater-based epidemiology: An international study in 20 cities

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HIGHLIGHTS
• First multi-city international study on alcohol consumption through WBE.
• WBE showed discrepancy in the level and pattern of alcohol use in 20 cities.
• MOE based on WBE was similar to that calculated from conventional prevalence data.
• All participating cities are exposed to a high risk in regard to alcohol consumption.
Quantitative measurement of drug consumption biomarkers in wastewater can provide objective information on community drug use patterns and trends. This study presents the measurement of alcohol consumption in 20 cities across 11 countries through the use of wastewater-based epidemiology (WBE), and reports the application of these data for the risk assessment of alcohol on a population scale using the margin of exposure (MOE) approach. Raw 24-h composite wastewater samples were collected over a one-week period from 20 cities following a common protocol. For each sample a specific and stable alcohol consumption biomarker, ethyl sulfate (EtS) was determined by liquid chromatography coupled to tandem mass spectrometry. The EtS concentrations were used for estimation of per capita alcohol consumption in each city, which was further compared with international reports and applied for risk assessment by MOE. The average per capita consumption in 20 cities ranged between 6.4 and 44.3 L/day/1000 inhabitants. An increase in alcohol consumption during the weekend occurred in all cities, however the level of this increase was found to differ. In contrast to conventional data (sales statistics and interviews), WBE revealed geographical differences in the level and pattern of actual alcohol consumption at an inter-city level. All the sampled cities were in the ‘high risk’ category (MOE < 10) and the average MOE for the whole population studied was 2.5. These results allowed direct comparisons of alcohol consumption levels, patterns and risks among the cities. This study shows that WBE can provide timely and complementary information on alcohol use and alcohol associated risks in terms of exposure at the community level.

1. Introduction

Alcoholic beverages are one of the most popular recreational substances in society and contain the psychoactive compound ethanol. It is well known that the misuse of alcohol is linked to various negative outcomes in terms of social, economic and health aspects (Room et al., 2005; Rehm et al., 2009). The degree of alcohol-related risks is not only determined by the amount of alcohol consumed, but also by drinking patterns (Rehm et al., 2003). Subsequently, average alcohol consumption per capita data are often presented together with the frequency of heavy drinking episodes and other indicators such as the type of alcohol beverage and level of abstainers (WHO; World Health Organization, 2014). Such information has been traditionally obtained from sales statistics and population surveys that require additional data due to their limits to cost- and time-effectiveness, representativeness and accuracy (Smith et al., 1990).

Drug-related harm has been extensively assessed to support public agencies designing health policies and allocating resources. A considerable amount of work has been conducted for the classification of drug harm based on the qualitative and quantitative analysis of multi-criteria (Nutt et al., 2007, 2010; Van Amsterdam et al., 2010; Taylor et al., 2012). Most of these studies categorise alcohol as one of the most harmful drugs and in turn reveal strong evidence that alcohol should be prioritised in terms of its effects on public health. Therefore, the accurate and timely assessment of alcohol consumption is necessary for maintaining and improving the quality of health care.

The quantitative analysis of specific biomarkers in wastewater, produced following the consumption and excretion of drugs, is a complementary approach for estimating the levels and trends of drug use by a population in a specific sewer catchment area (Castiglioni et al., 2006; Zuccato et al., 2008; Thomas et al., 2012; Kasprzyk-Hordern and Baker, 2012; Ort et al., 2014). Compared to sales figures and general population surveys, the data source for this approach (wastewater) is readily available and feasible to provide reliable information almost in real-time. Wastewater-based epidemiology (WBE) has been used to monitor drug use at local- (Zuccato et al., 2008; Van Nuijs et al., 2011; Reid et al., 2011a), national- (Van Nuijs et al., 2009; Van der Aa et al., 2013; Nefau et al., 2013; Kankaanpää et al., 2014; Östman et al., 2014; Du et al., 2015), and international- (Thomas et al., 2012; Ort et al., 2014) scales with time-frames ranging from weekly to yearly, identifying the spatial and temporal trends of drug use. WBE can therefore provide important information that complements existing epidemiological data, consequently resulting in a better understanding of the drug use situation. Furthermore, the drug use data obtained from WBE have been previously demonstrated to be suitable for the quantitative risk assessment of illicit drugs based on the margin of exposure (MOE) approach (Lachenmeier and Rehm, 2015). The MOE is defined as a reference dose (e.g. benchmark dose) divided by the estimated human exposure, and therefore the smaller the MOE the higher the risk to the human population (Benford et al., 2010; Cunningham et al., 2011; Lachenmeier et al., 2011; Lachenmeier and Rehm, 2015). The MOE values based on prevalence data and those based on wastewater analysis have shown similar figures in terms of the risk related to illicit drugs at the population level (Lachenmeier and Rehm, 2015). However, there have been no studies where WBE-derived alcohol consumption data have been used to estimate population risk based on the MOE approach.

The specific biomarker for estimating alcohol consumption is ethyl sulfate (EtS) and is typically quantified in daily composite wastewater samples (Reid et al., 2011b; Mastroianni et al., 2014; Rodríguez-Álvarez et al., 2014, 2015; Andrés-Costa et al., 2016; Boogaerts et al., 2016). EtS is a metabolite of ethanol that indicates the recent consumption of alcohol with detection times up to 48 h in healthy volunteers (Helander et al., 2009; Walsham and Sherwood, 2014) and is stable in wastewater (Reid et al., 2011b; Rodriguez-Álvarez et al., 2014). To date few WBE studies have been performed to estimate community alcohol consumption, although two studies have reported the comparative consumption of alcohol between different cities using 3 weekly samples taken annually over 3 years (Rodríguez-Álvarez et al., 2015; Boogaerts et al., 2016). There are no multi-city international reports on the comparative measurement of alcohol use through WBE.

Herein, we present an evaluation of alcohol consumption in several European, Australian and Canadian communities (20 cities in total) over a one-week period through the application of WBE. The resulting data were statistically tested for spatial and temporal (weekly) patterns and further compared with global statistics reported by WHO (2014) and European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) (2015). These population exposure data based on wastewater analysis were also used for an assessment of the risk posed by alcohol using the MOE approach.

2. Methods

2.1. Materials and chemicals

EtS and EtS-d5 (internal standard) were supplied by TCI Europe (Zwijndrecht, Belgium) and AthenaES (Baltimore, MD, USA),

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respectively. Ultrapure water was obtained from a Milli-Q water generator (Millipore, Billerica, MA, USA) and HPLC grade methanol was purchased from Rathburn Chemicals (Walkerburn, UK). Stock solutions of EtS and EtS-d5 were prepared in methanol and further diluted as required. Dihexylammonium acetate was prepared by adding acetic acid (Merck, Darmstadt, Germany) into equimolar amount of dihexylamine (Sigma-Aldrich, Steinheim, Germany).

2.2. Sample collection and analysis

Raw 24-h composite wastewater samples were collected following a common protocol (Thomas et al., 2012; Castiglioni et al., 2013; Ort et al., 2014) from 23 wastewater treatment plants (WWTPs) in 20 cities (4 WWTPs in Berlin) representing a combined population of 17 million people. Sample collection in each site was conducted over 7 consecutive days in March 2014, except Milan and Oslo (7 days in February 2015 and 8 days in March 2015, respectively). Malfuction of the autosampler in Eindhoven (12th March) caused a smaller number of samples (n = 6). Weekend samples in one of WWTPs in Berlin were not available (Berlin M, 15th–16th March). Each sampling period was found to be “normal week” without any special events in the area. Detailed information for sample collection in each WWTP is presented in Table S1. All samples, except those from Australia, Barcelona and Lugano were delivered frozen to Oslo for analysis. Each sample was spiked with EtS-d5 and cleaned up by simple centrifugation at 20,000 × g for 10 min before liquid chromatography-tandem mass spectrometry analysis as described by Reid et al. (2011b). The calibration curve was established by the analysis of standard solutions at 7 different concentrations (2–200 ng/mL). The concentration of EtS in wastewater sample was obtained by comparing the response (peak area) ratio of the EtS and EtS-d5 to its corresponding ratio in the calibration curve. The samples from Australia, Barcelona and Lugano were analysed in the country of origin using their in-house validated analytical methods. More details on the methods used in the present study can be found in the Supplementary Material or elsewhere (Reid et al., 2011b; Mastroianni et al., 2014).

2.3. Estimation of alcohol consumption rate

Daily mass loads of EtS were calculated by multiplying the concentration in the 24-h composite sample with corresponding daily flow of wastewater. Concentrations below limit of quantification (LOQ) were replaced with 0.5 × LOQ as described in previous studies (Ort et al., 2014; Salvatore et al., 2015). Subsequently, the urinary excretion rate (0.010–0.016, median 0.011% on a molar basis; Høiseth et al., 2008) and the density of alcohol (ρ = 0.789) were used for estimating the daily consumption of pure alcohol in volume. As the excretion rate has been reported with only a range and median, the mean and standard deviation (SD) have been estimated using equations (Eqs. (1) and (2)) suggested by Hozo et al. (2005). As a result, the mean excretion rate has been estimated as 0.012%, which coincides with the value used by Rodríguez-Álvarez et al. (2015) and Boogaerts et al. (2016). The resulting data were then normalised by the sewer catchment population to allow comparison with other cities and other data sources, such as those available from WHO (2014) and EMCDDA (2015). Since the WHO consumption rates were presented as yearly consumption per person aged 15 and older (L/year/person (15+)), they were converted into the daily per capita use for entire population (L/day/1000 inhabitants) by taking into account the proportion of the population aged 15 and older in each country. Furthermore the data were used for statistical analysis (Minitab 17, Minitab, Inc., State College, PA, USA) and Monte Carlo simulations (RISK version 7.0, Palisade Corporation, Ithaca, NY, USA).

\[
\text{estimated mean} = \frac{\text{high end of the range} + \text{low end of the range} + 2 \times \text{median}}{4} \quad (1)
\]

\[
\text{estimated SD} = \frac{1}{T2} \left( \frac{\text{high end of the range} + \text{low end of the range} - 2 \times \text{median}}{4} \right)^2 + \left( \frac{\text{high end of the range} - \text{low end of the range}}{2} \right)^2 \quad (2)
\]

2.4. Uncertainty assessment

The estimation of population normalised alcohol use by WBE is subject to various sources of uncertainty associated in each step of the backcalculation: sampling (U_s), chemical analysis (U_c), wastewater flow measurement (U_f), excretion rate (U_e) and population estimation (U_p). Since EtS is stable in wastewater (Reid et al., 2011b; Rodríguez-Álvarez et al., 2014), uncertainty related to biotransformation in sewers is not taken into account in this study. An estimate of 5% was made for U_s since sample collection was carried out following a “best practice protocol” which would provide 5 < U_s < 10% for illicit drug analysis in wastewater (Castiglioni et al., 2013). Our assumption is particularly conservative as the number of toilet flushes containing EtS is expected to be higher than that containing an illicit drug considering the prevalence, pharmacokinetics and pharmacodynamics of alcohol. U_c was determined from method validation parameters (i.e. RSD) and U_f was derived from the mean and SD of excretion rate estimated in Section 2.3. The other factors, U_e and U_p were assumed to be 20% as described in the previous study (Ort et al., 2014). Monte Carlo simulation has been used to avoid underestimation of the total uncertainty (U_t) (Ort et al., 2014; Lai et al., 2015). Further information for uncertainty assessment can be found in the Supplementary Material (Table S2).

2.5. Margin of exposure

The MOE value is a measure of the health risk by exposure to substances, which can be used for comparison of those compounds in terms of risk assessment (Lachenmeier et al., 2011; Lachenmeier and Rehm, 2015). In this work, the MOE for quantitative risk assessment of alcohol was calculated by Monte Carlo simulation. As described by Lachenmeier and Rehm (2015), the MOE was defined as the ratio between the lower confidence limit of the benchmark dose (BMDL) derived from median lethal dose (LD50) and the estimated population exposure based on wastewater analysis. The BMDL value used in the present study was BMDL10 (BMDL for a 10% incidence of health effect) obtained by extrapolating the animal LD50 (Gold et al., 2003). Using per capita consumption data from wastewater analysis, the population exposures were estimated for both individual cities and entire population included in this study. Detailed parameters used for Monte Carlo simulations are reported in Table S3.

3. Results and discussion

3.1. Alcohol consumption rates

The estimates of daily alcohol consumption in all participating cities are presented in Fig. 1. EtS was quantified in all of the samples collected, apart from a single day (Tuesday 18th March 2014) in Munich. The mean daily consumption of alcohol per 1000 inhabitants ranged from 6.4 (Milan) to 44.3 (Granby) L/day/1000 inhabitants. Granby, Copenhagen, Munich, Dresden and Montreal (in order of decreasing consumption) showed higher alcohol use (>29 L/day/1000 inhabitants) than the other cities (<24 L/day/1000 inhabitants). The daily alcohol consumption in 20 cities calculated as a population-weighted mean was 20.6 L/day/1000 inhabitants.

It was possible to compare the results from three cities (Oslo, Barcelona and Milan) with previous reports (Reid et al., 2011b; Mastroianni et al., 2014).
et al., 2014; Rodríguez-Álvarez et al., 2015) (Fig. S1). The levels of alcohol consumption in Oslo 2015 have not significantly changed (Wilcoxon, $\alpha = 0.05$) compared to the results from 2009. Barcelona also showed insignificant changes in alcohol consumption between 2013 and 2014 (Wilcoxon, $\alpha = 0.05$). In the case of Milan, the population normalised alcohol load in 2015 was higher than in 2012 and 2014 (Wilcoxon, $\alpha = 0.05$). Nevertheless, it should be noted that the data were obtained from a limited number of samples and longer sampling period is required for a more solid estimation of consumption trends. A recent study in Belgium reported that alcohol use was higher...

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**Fig. 1.** Estimated population-normalised daily alcohol consumption in 20 cities during the predefined sampling period (March 2014). Error bar indicates the combined uncertainty calculated (1σ). Dashed lines present the daily consumption (both recorded and unrecorded) in the corresponding country calculated based on the report by WHO (2014). *Sample of Tuesday 18th March < LOQ. *n = 26 (4 WWTPs). *Different sampling period (February 2015). *n = 6 (sample of Wednesday 12th March missing). *n = 8, different sampling period (March 2015).

**Fig. 2.** Day-to-day variation of per capita alcohol biomarker load in wastewater from 20 cities during the predefined sampling period. The boxes present median (line), 25th and 75th percentiles (bounds), and the whiskers extend to the minimum and maximum values. Outliers are indicated as dots. *Sample from Munich < LOQ, *Sample from Eindhoven missing, *Sample from Berlin M missing.
in more urbanised cities than in smaller towns (Boogaerts et al., 2016), however such a trend was not visible in this study. This may be due to the fact that all of the cities included in this study would be regarded as large urban areas in comparison to the smaller towns included in the Belgian national study.

It is also important to note that the excretion rate used in this work (0.012%) was based on 10 healthy males (Høiseth et al., 2008) and as such introduces an additional source of uncertainty. Excretion rates representative of a large population are essential in order to back calculate a reliable estimate of alcohol use from ETS loads in wastewater, however only few studies with a scarce number of subjects have to date been performed. Further studies should be carefully designed for pharmacokinetics of alcohol to re-evaluate the currently available excretion rates.

3.2. Weekly patterns of drinking

Fig. 2 shows the daily proportion of per capita alcohol biomarker load derived from all data. The load was highest on Saturday or Sunday in all cities, apart from Almada where the weekly peak was on Friday. Considering the excretion profile, diurnal cycle (sleep/awake) and wastewater residence time, alcohol biomarker loads captured in the morning indicate the consumptions occurred in the previous evening (Reid et al., 2011b; Boogaerts et al., 2016). In case of Almada, however, the residence time of wastewater ranged between 0.1 and 3.4 h and the sampling time started slightly later (09:30 AM) than the other cities. Therefore, it was assumed that the majority of alcohol biomarker load derived from the consumption on Friday evening was captured in the Friday sample. Taking into account such factors, Saturday and Sunday were defined as weekend for all cities except Almada (Friday and Saturday day as weekend). The average weekday and weekend consumptions in 20 cities (population-weighted) were 17.4 and 28.4 L/day/1000 inhabitants, respectively. There was a significant increase in weekend alcohol consumption (Wilcoxon, α = 0.05) when the uses over all cities were compared, which was in accordance with previous reports on weekly pattern of alcohol use during “normal week” (Reid et al., 2011b; Mastroianni et al., 2014; Rodríguez-Álvarez et al., 2014, 2015; Andrés-Costa et al., 2016; Boogaerts et al., 2016). The highest degree of increase was observed in Oslo followed by Dresden, Castellon, Dülmen, Copenhagen and Munich (in order of decreasing degree). The weekday and weekend alcohol consumptions in each city are plotted in Fig. S2.

3.3. Comparisons with conventional alcohol consumption statistics

The WBE results were compared with country specific alcohol consumption data (sum of recorded and unrecorded rates) based on the report for alcohol and health by WHO (2014) (Fig. 1). The authors are aware that WBE data from an individual city may not necessarily reflect the whole population of a country. Indeed, 11 out of 20 cities showed differences in estimated alcohol consumption higher than 34%, which is the maximum total uncertainty of the mean alcohol consumption in the present study. The per capita daily consumptions derived from WHO reports are relatively consistent among studied countries, however WBE results showed significant discrepancy in terms of daily consumption in the selected cities. It is shown that the mean alcohol consumptions obtained from WBE matched those from WHO data the most in Dresden, Munich, Amsterdam and Eindhoven (difference < 5%). Castellon and Oslo were also in good agreement (difference < 15%), while previous WBE data for Oslo, 2009 (Reid et al., 2011b) indicate better agreement with WHO surveillance data compared to the present study. It may be attributed to the longer period (4 weeks) of sampling in 2009 and the fact that WHO report is based on the sales statistics and interviews from the period of 2008–2010. The minimum period of sampling to reveal statistically valid pictures of alcohol consumption needs to be further investigated, however it is clear that reliable results can be obtained by WBE within much shorter period than general population data that requires at least several months.

3.4. Correlation with other recreational drugs

The recreational use of alcohol in combination with other licit or illicit drugs is well known (Windle, 1991; Leon et al., 2007; Goldstein et al., 2009), and therefore possible correlations between the daily loads of alcohol and those of selected illicit drugs in wastewaters of each city have also been investigated. This was performed by comparing the data for ETS in each city (present study) with those for selected illicit drugs in the same samples reported by EMCDDA (2015). The selected drugs were quantified by methods based on deuterated internal standard addition followed by solid phase extraction and LC-MS analysis. More details on the methods and wastewater analysis for illicit drugs can be found elsewhere (Thomas et al., 2012; Ort et al., 2014; EMCDDA, 2015). It is important to note that such comparisons are only meant as a crude assumption since specific biomarkers of co-consumption (such as cocaethylene) are required to verify the combined use of alcohol and other drugs. Especially, the excretion rates of drugs can be affected by co-administration of alcohol (Shimosato, 1988; Khan and Nicell, 2011), and therefore the direct comparisons of drug biomarker loads (present study) should be carefully interpreted.

 Spearman’s rho computed from the consumptions of alcohol and drug in each city are presented in Table 1. Cocaine showed the strongest correlation with alcohol from the 5 drugs evaluated (cocaine, amphetamine, methamphetamine, MDMA, cannabis), which is in accordance with previous findings (Grant and Harford, 1990; Goldstein et al., 2009; Mastroianni et al., 2014; Rodríguez-Álvarez et al., 2015). High levels of correlation (P < 0.05) between MDMA and alcohol were found in Toowoomba, Lugano, Dülmen, Copenhagen and London while those between amphetamine and alcohol were shown in Toowoomba, Granby, Munich and Berlin. This result is in contrast with the previous study carried out in Barcelona, 2013 (Mastroianni et al., 2014) that presented strong correlations of MDMA and amphetamine with alcohol consumption. Methamphetamine consumption levels were reported for only 7 cities that showed poor correlation with

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Spearman’s rank correlation between alcohol and illicit drug consumption measured by WBE.</th>
</tr>
</thead>
<tbody>
<tr>
<td>City</td>
<td>Cocaine</td>
</tr>
<tr>
<td>Canberra*a</td>
<td>0.82**</td>
</tr>
<tr>
<td>Toowoomba*b</td>
<td>0.96**</td>
</tr>
<tr>
<td>Montreal*b</td>
<td>0.00</td>
</tr>
<tr>
<td>Granby*b</td>
<td>0.61</td>
</tr>
<tr>
<td>Lugano*b</td>
<td>0.86</td>
</tr>
<tr>
<td>Dortmund</td>
<td>−0.36</td>
</tr>
<tr>
<td>Dülmen</td>
<td>0.93**</td>
</tr>
<tr>
<td>Dresden</td>
<td>−0.04</td>
</tr>
<tr>
<td>Munich</td>
<td>0.75</td>
</tr>
<tr>
<td>Berlin</td>
<td>0.89**</td>
</tr>
<tr>
<td>Copenhagen</td>
<td>0.89**</td>
</tr>
<tr>
<td>Barcelona</td>
<td>0.71</td>
</tr>
<tr>
<td>Castellon</td>
<td>0.79*</td>
</tr>
<tr>
<td>London</td>
<td>0.61</td>
</tr>
<tr>
<td>Milan*c</td>
<td>NA*</td>
</tr>
<tr>
<td>Amsterdam</td>
<td>0.71</td>
</tr>
<tr>
<td>Eindhoven</td>
<td>1.00**</td>
</tr>
<tr>
<td>Utrecht</td>
<td>0.64</td>
</tr>
<tr>
<td>Oslo*d</td>
<td>NA*</td>
</tr>
<tr>
<td>Almada</td>
<td>0.82</td>
</tr>
</tbody>
</table>

*a P < 0.05.
** P < 0.01.
† Data for the illicit drug consumption not available on EMCDDA (2015). See Supplementary Material for details (Tables S5).
* Data for the illicit drug consumption not available on EMCDDA (2015).
# No data reported.
# Data for the illicit drug consumption not available on EMCDDA (2015). See Supplementary Material for details (Tables S5).
* Smaller number of data (n = 6) reported for illicit drug consumption.
alcohol use except for Canberra. For cannabis that has no obvious weekly pattern (Thomas et al., 2012; Ort et al., 2014), correlations with alcohol consumption were observed in only two cities (Castellon and Utrecht), which were negative and positive, respectively.

3.5. Risk assessment by margin of exposure

The MOE values estimated from WBE data in the 20 cities were calculated using probabilistic Monte Carlo simulations as described by Lachenmeier and Rehm (2015). The MOE for each city, based on the data from wastewater analysis, is presented in Fig. 3, indicating the alcohol consumption in 20 cities would be categorised as “high risk” (MOE < 10) at the population level. As MOE is influenced mostly by human exposure (Lachenmeier and Rehm, 2015), the order of the MOE values in this study is generally the reverse order of mean per capita alcohol consumptions. The MOE for whole population was calculated as 2.5, which was very similar with the MOE value of 2 based on conventional prevalence data in Europe (Lachenmeier and Rehm, 2015). The results confirm previous research (Nutt et al., 2007; Van Amsterdam et al., 2010; Lachenmeier et al., 2011; Lachenmeier and Rehm, 2015) that emphasised requirement for appropriate assessment of alcohol use for public health.

4. Conclusion

An international study on the alcohol consumption through WBE in 20 cities with a combined population of 17 million people has been performed. WBE showed alcohol consumption levels and patterns in the studied cities, providing the most recent snapshot of actual alcohol use in these communities. Comparisons of data with international reports, such as global status on alcohol and health (WHO, 2014) and illicit drug estimation by wastewater analysis (EMCDDA, 2015) were also carried out, which showed WBE would be an important complement for the assessment of actual alcohol use at the community level. Such comparisons also highlighted the importance of further investigation particularly on the sampling design in WBE and possible coinadministration of alcohol with illicit drugs. A quantitative risk assessment by the MOE approach showed that all cities in this study were exposed to high risks regarding alcohol consumption at the population level.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2016.04.138.

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