Abstract

Population size is crucial when estimating population-normalized drug consumption (PNDC) from wastewater-based drug epidemiology (WBDE). Three conceptually different population estimates can be used: *de jure* (common census, residence), *de facto* (all persons within a sewer catchment), and chemical loads (contributors to the sampled wastewater). *De facto* and chemical loads will be the same where all households contribute to a central sewer system without wastewater loss. This study explored the feasibility of determining a *de facto* population and its effect on estimating PNDC in an urban community over an extended period. Drugs and other chemicals were analyzed in 311 daily composite wastewater samples. The daily estimated *de facto* population (using chemical loads) was on average 32% higher than the *de jure* population. Consequently, using the latter would systematically overestimate PNDC by 22%. However, the relative day-to-day pattern of drug consumption was similar regardless of the type of normalization as daily illicit drug loads appeared to vary substantially more than the population. Using chemical loads population, we objectively quantified the total methodological uncertainty of PNDC and reduced it by a factor of 2. Our study illustrated the potential benefits of using chemical loads population for obtaining more robust PNDC data in WBDE.
Introduction

Measuring drug residues in raw sewage—subsequently referred to as wastewater-based drug epidemiology (WBDE)—has become an important tool to estimate illicit drug use worldwide. The final estimate is typically presented on a basis of per capita consumption (i.e., consumed mass of a drug per capita per day). The back-estimation methodology of WBDE relies on a number of parameters, including concentrations of drug residues, wastewater volumes, excretion fractions, and population size. There is uncertainty in each of these parameters. One key component concerns the number of people in a catchment, which can substantially affect the accuracy of the final estimate and comparisons of data across different communities. The variability and accuracy of this parameter cannot be easily estimated.

In most WBDE studies, the population parameter has been obtained either from the design capacity of wastewater treatment plants (WWTPs) or the most recently available census data. Design capacity usually refers to population equivalents including industrial pollutant loads and also refers to a certain (long-term) planning horizon. For these two reasons, usually less people contribute to the wastewater than indicated by design capacity. In exceptional circumstances, WWTPs may also operate above design capacity to receive wastewater from more people than expected. However, this is difficult to assess and quantify objectively. Census data, taken on a specific day, provide only a single estimate of a population size; typically, it refers to a de jure population, which is counted according to home address, but does not provide important information on whether people are actually within the WWTP catchment under investigation or elsewhere on a specific day. Relying on such a fixed population size may not be practical to WBDE.

Researchers have recommended that attempts should be made to estimate the number of people effectively contributing to a wastewater sample; e.g., see refs 1, 3, and 4. A few indicators have been proposed to estimate de facto populations by means of chemical loads measured in wastewater. Theoretically, if all households in the catchment of a WWTP are connected to a central sewer system, and assuming that no wastewater losses occur—e.g., leaky sewers, wastewater bypassing the WWTP during rainy periods, or abnormal operational situations—the population estimated from chemical loads in wastewater is a fair approximation of de facto population. Therefore, de facto subsequently refers to both de facto population and population estimated from chemical loads.

For practical applicability in WBDE it appears important that (i) selected chemicals are human-specific; (ii) the method is applied over an extended period of consecutive days (to reveal day-to-day variation); (iii) methodological uncertainties can be quantified objectively; and (iv) the relevance of the parameter populations size is determined in the context of the overall methodological uncertainty of WBDE.

Human-specific chemicals were proposed—but not validated—in five studies, namely, creatinine,(5, 6) coprostanol,(4) cholesterol, cotinine, and a neurotransmitter metabolite, 5-hydroxyindoleacetic acid,(7) and commonly prescribed pharmaceuticals and artificial sweeteners.(8) Only two studies were found in which the measurement was over an extended period: one covered 235 days in a 1 year period using hydrochemical parameters (nitrogen, phosphorus, biological oxygen demand, and chemical oxygen demand), and another one covered 13 days in an 8 month period using ammonium; those selected chemicals are not human-exclusive. Only one recent publication provides an approach to quantify objectively the methodological uncertainty of the population.
estimates. Lastly, the two studies addressing population size estimation in the context of overall uncertainty were review-type reports that did not provide new methods. In summary, to the best of our knowledge, no study to date has addressed all four aspects listed previously in a comprehensive manner that is pertinent to their applicability in WBDE. Furthermore, most studies rely on a day-specific excretion fraction by one person, which does not allow an objective quantification of associated uncertainty if these values are either missing or unreliable.

Recently, a multisubstance model to estimate de facto population was developed and calibrated with wastewater samples collected on a census day. Applying this model facilitates the examination of the variation in daily de facto populations over time in large catchments and consequently its effect on the estimation of population-normalized illicit drug loads in WBDE—particularly when compared to using a constant population number such as that from census data. The aims of our study were to (a) estimate a day-specific de facto population using the recently developed model in a large catchment on consecutive days over an extended period, (b) compare the estimated de facto populations with the de jure population and the effects of using the different population estimates on temporal patterns and levels of illicit drug use, and (c) evaluate whether the overall methodological uncertainty of WBDE can be reduced and better quantified using day-specific estimated de facto populations.

2 Materials and Methods

2.1 Wastewater Sampling

The sampling was set up at the inlet of a wastewater treatment plant that served a mainly urban catchment in South East Queensland (Australia) with a de jure population of 211,340 people and a de facto population of 230,117 on census day (Aug. 11, 2011) according to the Australian Bureau of Statistics. Samples were collected between June 2011 and June 2012 (n = 311 days; see Table S1 in the Supporting Information for missing data (n = 33 days) due to logistical or technical reasons). A continuous flow-proportional sampling technique was applied to ensure collection of representative daily composite samples (from 6 AM to 6 AM the next day). The samples were refrigerated at 4 °C during collection, acidified on site to pH 2 using 2 M hydrochloric acid, and then frozen until analysis. This preservation method has been commonly used and can stabilize the targeted residues of illicit drugs in wastewater during storage; e.g., see refs 5 and 16. Data on daily wastewater volumes were recorded by the WWTP.

2.2 Analysis of Targeted Compounds

Samples were analyzed for the targeted illicit drug residues and high-use chemicals using liquid chromatography (Shimadzu, Nexera UHPLC system, Kyoto, Japan) coupled with tandem mass spectrometry (AB SCIEX QTRAP5500, Ontario, Canada) (LC-MS/MS). The analytical method applied in this study has been validated and described previously. Briefly, an electronic robot (Tecan Genesis Workstation 200, Australia) was used to transfer filtered samples into a vial which were then spiked with mixtures of carbon-labeled and deuterium-labeled chemical standards (Table S2 of the Supporting Information) for compensating potential instrumental variability and matrix effects during analysis. Separation of the targeted analytes was performed on a C18 LC analytical column using gradient mobile phases (Table S2 of the Supporting Information). Together with the calibration standards, concentrations of the targeted analytes were measured using mass spectrometry with a
multireaction monitoring (MRM) scheme. Two MRM transitions were used for identification and quantification of each analyte.\(^{(8,11)}\) Concentrations of the targeted analytes have taken the recovery of the spiked mass-labeled standards into account (i.e., isotope dilution method).

In every batch of analysis, Milli-Q water samples (i.e., procedural blanks), duplicate samples, and samples spiked with native chemicals were included as quality assurance and control (QA/QC) of the analysis. The QA/QC results are summarized in Table S3 of the Supporting Information. Briefly, no contamination was found in the blank samples. The difference (coefficient of variance, CV (%)) between duplicate samples was on average 4.8–7.9% for the illicit drug residues and 4.2–9.4% for the high-use chemicals. Recovery of the native chemicals spiked in the samples was 75–81% for the illicit drug residues and 99–113% for the high-use chemicals. Interday variation across 3 days was 2.3–11% for the illicit drug residues and 4.7–21% for the high-use chemicals.

2.3 Estimating Daily \textit{de facto} Populations

Day-specific \textit{de facto} populations and confidence intervals were estimated with the multisubstance model.\(^{(11)}\) Details on the calculation (calibration, Bayesian inference, and validation) can be found in O’Brien et al.\(^{(11)}\) Briefly, we calibrated the model with mass loads of 14 chemicals in the influent of 10 WWTPs (catchment sizes ranging from approximately 3,500 to 500,000 people) and \textit{de facto} populations, both determined on or around the last Australian census day. It should be noted that normally census data refer to \textit{de jure} populations; however, in Australia, \textit{de facto} populations are also determined on census day.\(^{(11)}\) In this study, we only used eight chemicals that can be readily measured in the samples without preconcentration, consistent with the measurement of the targeted illicit drug residues. These eight high-use chemicals included acesulfame, atenolol, caffeine, carbamazepine, codeine, hydrochlorothiazide, naproxen, and salicylic acid. Model calculations indicated that the use of eight chemicals provided \textit{de facto} population estimates consistent with using 14 chemicals (Table S4 of the Supporting Information). It should be noted that any variability that is unknown or cannot be quantified explicitly was implicitly accounted for when calibrating the model with mass loads of the high-use chemicals in wastewater from various catchments and accurate population sizes. The uncertainties encompass systematic or random effects due to, e.g., disposals of unused chemicals, unknown absolute excretion rates, day-to-day variations of actual consumption, and transformations of chemicals in sewer systems, sampling, and storage (see O’Brien et al. for more explanation).\(^{(11)}\) It is a fair assumption that the system under investigation (e.g., catchment and sewers), and the relevant processes (e.g., average consumption habits, pharmacokinetics, and transformation in sewers) did not change substantially between the census day and our monitoring period.

2.4 Population-Normalized Drug Consumption

The back-estimation method follows the previously proposed equation (see Supporting Information).\(^{(1)}\) Briefly, the estimation involves three main steps: (a) the mass loads (mg/day) of drug residues are obtained by multiplying concentrations (μg/L) with total wastewater flow (ML/day); (b) the estimated mass loads are then extrapolated to the consumed amount with a correction factor taking the average excretion rate and the ratio of molecular weight between the parent drug to its metabolite into account (Table S5 of the Supporting Information); and (c) the consumed amount is normalized to a population size to result in the collective consumption of the population (mg/day)/(1000 people)). We chose, when analytically possible, two drug residues (i.e., dual tracers, the unchanged parent drug and metabolite) to back-estimate consumption of the parent drug (Table S3 of the Supporting Information): cocaine and benzoylecgonine, respectively, for estimation of cocaine; methamphetamine and amphetamine, respectively, for estimation of
methamphetamine (as illicit amphetamine use is rare in Australia);(17) and only MDMA (3,4-(methylenedioxy)methamphetamine) itself for estimation of MDMA. These targeted residues have been demonstrated to be adequate for back-estimating consumption of the corresponding parent drug; see, e.g., refs 1, 3, and 18–20. Consistent patterns and consumption rates obtained from the two residues allow verification of the reliability of the estimate of the parent drug.(8)

2.5 Uncertainty Analysis

Previous studies have revealed the uncertainty components associated with the back-estimation methodology of WBDE.(-2, 8) Five components were considered for contribution to the overall uncertainty (U_{tot}) for the final estimate of population-normalized drug consumption (PNDC; (mg/day)/(1000 people)); we refer to the U_{tot}(de jure) as the total uncertainty of the de jure-PNDC and U_{tot}(de facto) as the total uncertainty of the de facto-PNDC. These encompass uncertainties related to sampling (US), chemical analysis (UC), flow measurements (UF), excretion rates (UE), and population size (UP). The magnitude of each uncertainty component is presented as relative standard deviations (RSD, %)(8) as determined from modeling results for US and UP, interday variability of instrumental analysis for UC, literature data for UE, and estimates from WWTP operators for UF (Table 1). These uncertainty components are independent from each other, because they arise from individual aspects of studies and the methods of calculating them. As the primary metabolite chosen for back-estimating consumption of the three illicit drugs is relatively stable under typical sewer conditions,(21) we therefore consider the uncertainty of in-sewer chemical biodegradation as negligible. This study used Monte Carlo simulation to estimate U_{tot} as described previously.(22, 23) Linear error propagation as suggested earlier, see, e.g., ref 8, only provides a good approximation of U_{tot} if UP is small and also other uncertainty components do not exceed a certain value. Otherwise the linear error propagation tends to systematically underestimate U_{tot}.

2.6 Statistics

Nonparametric Mann–Whitney tests (unpaired) were used to examine significant differences between weekdays and weekends for the estimated de facto populations and for illicit drug consumption. Nonparametric Wilcoxon tests (paired) were used to assess significant differences between population-normalized consumption based on de jure and de facto populations. The statistics were performed using GraphPad Prism (version 6.00, GraphPad Software Inc.).

3 Results and Discussion

3.1 Estimated Day-Specific de facto Populations

The de facto population for individual days was estimated in a range between 96,400 and 466,000 (minimum–maximum; 90% interquantile, 96,400–304,000 (25% of all days were outside this range)), with an average of 280,000 persons and a variation (CV) of 15.6% throughout 311 monitoring days (Figure 1A). The variation of our data is similar to that reported in Brussels over 235 days (CV, 18%; 90% interquantile, 495,000–1,040,000 (25% of all days outside this range)).(9) We observed a mild seasonal variation in the estimated de facto population in the catchment: it gradually increased from approximately 264,000 to 284,000 persons (monthly average; Table S6 of the Supporting Information) between July and September, remained at about 300,000 persons from October to December 2011, was recorded as the highest estimate of 310,000 persons in January 2012, and then
slowly decreased from 294,000 persons in February to 243,000 persons in June 2012. The yearly average de facto population on the weekends (∼280,000 persons) was similar to that during the weekdays (∼278,000 persons) over the study period, as were most of the months (Figure 1A). The variation (CV) of the estimated de facto population among individual week days was relatively lower for Monday (13%), Friday (14%), and Sunday (13%) but slightly higher for Saturday (18%) and from Tuesday to Thursday (16–17%). There was also a significant difference in the estimated de facto populations between Sundays and Wednesdays–Saturdays (p = 0.005–0.03) and between Mondays and Tuesdays–Saturdays (p < 0.0001–0.005).

3.2 Comparison between Two Different Population Estimates

The estimated de facto population (∼280,000 people) is on average about 32% higher than the de jure population (i.e., ∼211,000 people on the census day) (Figure 1B). For almost all days, the estimated de facto population was higher than the de jure population (Figure 1A). The difference ranged from +2.1 to +120% (Figure 1B). On only 17 days was the estimated de facto population lower than the de jure population, ranging from −2.8 to −54% (Figure 1B). The overall differences between the estimated de facto population and the de jure population appear reasonable because the studied catchment is one of the most urbanised areas in South East Queensland and it is a popular destination for domestic and international visitors with many resorts, music festivals, theme parks, and tourist attractions that operate throughout the year. High commuting flow is thus common in the catchment area.

While the difference between the estimated de facto population and the de jure population is in a range of 22–44% (25–75 percentile) for half of the monitoring days (Figure S2 of the Supporting Information), there were some days on which there was a substantial increase or decrease in the estimated de facto population compared to the de jure population (Figure 1B). For example, an elevated de facto population was estimated on Nov. 28, 2011 (+101%, Figure 1B), Jan. 26, 2012 (+120%, Figure 1B) and Jan. 30, 2012 (+94%, Figure 1B). We noticed a coincidence between a high rainfall level recorded from Jan. 23, 2012 to Jan. 30, 2012 (peak rainfall on Jan. 25, 2012 with ∼230 mm)(24) and an increase in the de facto population on Jan. 26, 2012 and Jan. 30, 2012. However, it should be noted that the de facto population, estimated based on the daily mass loads of the high-use chemicals, cannot be driven by the increase in wastewater volumes and is independent of the dilution factor, as long as the lowered concentrations of the chemicals are still above the quantification limits. This can be seen from the data, for example, on Mar. 4, 2012 and Mar. 29, 2012, when increased wastewater volumes due to rainfall around these days (Figure S3A) did not result in an increase in estimated de facto population (Figure1A,B). Higher de facto population estimates may reflect either an increase in per capita consumption of the high-use chemicals or more people being present who consume the usual per capita amount of the chemicals. The observed elevated de facto population appears reasonable because (a) every November is a special period when young teenagers gather in this urban catchment to celebrate their completion of secondary school and (b) the 26th of January is the Australia Day public holiday, so the catchment attracts a lot of people for holidays and celebration activities. On some of the monitoring days, the estimated de facto population was substantially lower than the de jure population (Figure 1A), for example, the data on Jan. 24, 2012 (−48%, Figure 1B) and Apr. 28, 2012 (−54%, Figure 1B). Heavy rainfall events were recorded in the catchment around those 2 days. This may have led to a diversion of wastewater (combined sewer overflows), resulting in substantially decreased mass loads of high-use chemicals entering the WWTP. However, PNDC is still thought to be unbiased as explained at the end of the next section.

3.3 Effects on PNDC Using Different Population Estimates
The deviation between the *de facto*-PNDC and the *de jure*-PNDC is calculated as the following:

\[
\text{deviation/\%} = \left( \frac{\text{drug loads}_{\text{de facto}} - \text{drug loads}_{\text{de jure}}}{\text{drug loads}_{\text{de jure}}} \right) \times 100
\]

This gives negative deviations (−2 to −54.6%) for most of the monitoring days (Figure 1C) since the PNDC is reduced when the drug load is normalized to the estimated *de facto* population which is greater than the *de jure* population. By contrast, the deviation becomes positive (+2.9 to +119%) when the drug load is normalized to an estimated *de facto* population that is smaller than the *de jure* population (Figure 1C). There were significant differences (p < 10−4) between the *de facto* population normalized and *de jure* population normalized consumption of the three illicit drugs. Over the monitoring period, the PNDC based on the estimated *de facto* populations was systematically lower by 22% (Figure 1C) because the average *de facto* population was 32% higher than the *de jure* population (Figure 1B). This indicates that the model of estimating the *de facto* populations allows for the assessment of how systematic differences in the population drug consumption are related to changes in the number of people who contributed in the sampled wastewaters in the studied catchment.

While there were absolute differences in the PNDC estimated using the two different population estimates, the relative day-to-day pattern of consumption for the three illicit drugs did not significantly change over the monitoring period (Figure S4 of the Supporting Information). The variation of PNDC was very similar, regardless of whether one used the estimated *de facto* or *de jure* population (*de facto/de jure*: for cocaine, 55%/59%; for methamphetamine, 38%/37%; for MDMA, 132%/138%) (CV, in Table S7 of the Supporting Information). The same was true for variations between weekdays and weekends over the year (Table S7 of the Supporting Information). Our data are in line with those of a recent study(23) which summarized and reported that only a small variation in a PNDC was observed between estimates made using *de jure* and *de facto* populations. For example, in Brussels, using hydrochemical parameters for *de facto* populations and census for *de jure* populations (census = 1.1 million people), the CVs for PNDC differed only slightly (*de facto/de jure*: for cocaine, 33%/31%; for methamphetamine, 40%/41%; for MDMA, 180%/186%).(9) Our data also follow the result of another recent study which revealed consistent patterns of per capita illicit drug use between using census data (*de jure* 220,000 persons) and estimated population numbers using ammonium ions in the urban area of Lausanne, Switzerland.(10) These coherent findings among international studies in large catchments indicate that the consumed mass of illicit drugs varies more substantially than the population, and consequently, relative temporal changes of population drug consumption usually persist irrespective of the type of population estimates used for normalization. This may not be the case for small catchments and days with special events.

Despite the absence of large variations in the overall day-to-day patterns, we observed 19 days with substantially higher or lower differences between the population’s drug consumption using the two different population estimates (Figure S5 of the Supporting Information). Those exceptional days indicate that a day-specific *de facto* population estimate is essential when estimating a PNDC on a single day. Our data suggest that it is hard to predict when substantial variations in *de facto* populations happen since those exceptional days were randomly detected and not simply related to specific events in the catchment. The deviations in population drug consumption on these 19 days may have been due to (a) the “normal number” of people increasing or decreasing their overall consumption of the eight high-use chemicals, (b) a substantial increase or a decrease in the number of people consuming the “average per capita amount” of the high-use chemicals, or (c)
unusual operational conditions in the sewer network affecting influent loads in the WWTP. Unfortunately, it is not possible to differentiate reasons (a) and (b). However, the values observed outside the “normal range of de facto population” indicate that the results of these few days should be interpreted cautiously. When using the de jure population, such “caution indicators” are missing. An example for reason (c) with “unusual operational conditions” was observed by van Nuijs et al.:(9) a de facto population which was less than 10% of the “normal” de facto population was estimated because an unreported amount of wastewater did not enter the influent of the WWTP where samples were taken. This does not imply that only 10% of the population were in the catchment but simply that only 10% of the normal wastewater volume was sampled and used to calculate a de facto population. Since the illicit drug loads were also quantified for 10% of the wastewater only, the PNDC was not affected. However, if the de jure population had been used, a substantial underestimation during the days with unusual operational conditions would have resulted; unless one had corrected for the missing wastewater volume, assuming that the nonsampled wastewater volumes showed similar concentrations (for both illicit drugs and substances to estimate de facto population). In a similar way, faulty flow measurements do not affect PNDC when the de facto population is estimated from chemical loads based on the same sample and wastewater volumes as illicit drug residues.(8)

3.4 Temporal Variations

3.4.1 Cocaine

Both cocaine and its major urinary metabolite, benzoylecgonine, were measured in all samples and used to back-estimate cocaine consumption in the catchment. The yearly average consumption (25–75 percentile; median) of cocaine was estimated at 264 (mg/day)/(1000 people) (162–315; 230) using cocaine itself and 193 (mg/day)/(1000 people) (108–249; 167) using benzoylecgonine. This is equal to, on average, approximately two doses in a day among 1000 people, assuming that the reference dose of cocaine is 100 mg.(25) Benzoylecgonine has been recommended as the key biomarker for back-estimating cocaine consumption because it is exclusively excreted by humans and more persistent in wastewater than cocaine.(16, 21, 26, 27) We thus used benzoylecgonine to assess the temporal pattern of cocaine consumption in the studied catchment. The overall variation of cocaine consumption in this study (55%, Table S7 of the Supporting Information) was about twice that reported in Brussels.(9) Cocaine consumption showed a gradual increasing pattern over the monitoring period (Figure 2A, Table S6 of the Supporting Information). Epidemiological indicators of cocaine use during this period are scant but generally suggest that there was limited use and stable markets since there was little change in hospital admissions, information calls, or self-reported substance use (among injecting drug and club drug consumers) in surveillance studies.(28-31) However, analyses of the small number of cocaine seizures suggested that purity increased during April–June 2012 compared with previous months in this period.(17)

The weekly pattern of cocaine consumption was consistent across the time period (Figure 2A). The average consumption of cocaine on the weekends (299 (mg/day)/(1000 people)) was approximately two times higher than that during the weekdays (149 (mg/day)/(1000 people)) (p< 10–4). This implies that, on the weekends, there was either a larger amount of cocaine consumed by the same number of regular users and/or an increase in the number of consumers who used a similar amount/dose within the catchment. Drug purity was irrelevant to the weekly pattern because it is highly unlikely that there would be marked variations in drug purity within the week and the weekly pattern is clear regardless of the general level of consumption. Given the urban location of the studied site, there were a number of specific events, such as car races, sport competitions, music concerts/festivals and New Year celebrations, during the monitoring period. Elevated consumption
of cocaine coincided with some of the major events in the catchment (Figure 2A). The effect of the major events on cocaine consumption was more pronounced than the weekend effect; for example, the level of cocaine use on the second-last weekend of October 2011 and on the 2012 New Year’s Day (Sunday) was well above the levels seen for weekends in most of the months (Figure 2A). This is also observed for another sampling day which was Sunday on the first weekend of May 2012.

It is noteworthy that the overall patterns of cocaine consumption estimated from cocaine itself and benzoylecgonine were very similar, except for a spike in cocaine consumption estimated from cocaine itself in one Thursday sample in February 2012 and one Tuesday sample in May 2012 (Figure S6A of the Supporting Information). This suggests that there may have been direct release of cocaine into the wastewater system on these 2 days. Environmental inputs of cocaine can arise from, for example, disposal of cocaine, which provides amounts of cocaine in sewage systems well above those from human consumption and results in overestimation of cocaine consumption using wastewater samples. e.g., (6, 26) This can also potentially explain the observed difference (an average relative difference of 30%) between two estimates in this study (higher consumption when using cocaine itself rather than benzoylecgonine) and also slightly higher cocaine/benzoylecgonine ratio in the wastewater samples (an average of 0.34) than that in human pharmacokinetic data (0.21 from 7.5%/35%; see Table S5 of the Supporting Information). Variations in metabolism among individuals and mode of administration can also lead to changes in excretion profiles of cocaine, and therefore affect the difference in the cocaine/benzoylecgonine ratio between the population in the studied catchment and that in pharmacokinetic studies; see, e.g., refs 32 and 33. As noted elsewhere, (34) the use of such a dual tracer approach (i.e., both parent drugs and metabolites) gives additional confidence in the results and may serve as a quality-control check providing information about potential breakdown/transformation of the compounds in the sewers (i.e., higher estimate from metabolites) or releases of unconsumed drugs (i.e., higher estimates using the parent compound).

### 3.4.2 Methamphetamine

As the primarily excreted metabolite, methamphetamine itself was chosen to back-estimate methamphetamine consumption. The average yearly consumption (25–75 percentile; median) of methamphetamine was estimated at 440 (mg/day)/(1000 people) (312–549; 391). This is equivalent to approximately 14 (doses/day)/(1000 people), assuming that a reference dose of methamphetamine of 30.5 mg. (25) The use of methamphetamine appeared more prevalent than that of cocaine in the catchment, and the variation in methamphetamine consumption (38%) was smaller than that of cocaine consumption over the whole monitoring period (Table S7 of the Supporting Information).

The yearly consumption pattern of methamphetamine (Figure S7A and Table S6 of the Supporting Information) was similar to that of cocaine (Figure 2A). Most epidemiological data in relation to methamphetamine are only compiled in annual aggregates but are generally in keeping with these trends: police seizures increased during 2011/12 in both number and weight, as did the number of clandestine laboratories identified; (17) self-reported frequency of use among club drug consumers increased and so did the number of calls made about methamphetamine to information lines and hospital admissions. (30, 31) Analysis of police seizures demonstrated steady increases in purity over the first three-quarters of the 2011/12 period. (17)

There was higher consumption of methamphetamine on the weekends (average, 523 (mg/day)/(1000 people)) than the weekdays (406 (mg/day)/(1000 people)) throughout the year (Figure 2B) (p < 10–4); the average difference was only about 1.3 times which is less than that for
cocaine consumption. A few days of elevated methamphetamine consumption were captured around specific events in the catchment (Figure S7A of the Supporting Information).

As for cocaine consumption, we also analyzed amphetamine as another drug residue for the estimation of methamphetamine consumption. Both amphetamine and methamphetamine itself estimated a similar pattern of methamphetamine consumption (an average relative difference of 17%) over the monitoring period (Figure S6B of the Supporting Information). The consistency of estimating methamphetamine consumption from both amphetamine and methamphetamine itself supports other evidence that illicit use of amphetamine is negligible in Australia; hence, amphetamine measured in the samples was primarily from the metabolism of methamphetamine consumption.

3.4.3 MDMA

For MDMA, only one excreted residue can be analytically identified with confidence; thus, estimating MDMA consumption relies solely on MDMA itself as described in the literature e.g. (35). The average consumption (25–75 percentile; median) of MDMA was estimated at about 229 (mg/day)/(1000 people) (75–277; 145) over the year. This is equivalent to an average of three doses a day per 1000 people, assuming a reference dose of 80.5 mg. (25) Two discrete rising consumption periods of MDMA were observed: June 2011–January 2012 and February–May 2012 (Figure S7B and Table S6 of the Supporting Information). Epidemiological indicators of MDMA consumption increased in this period, with self-reported frequency of use among club drug consumers increasing along with perceived purity. (30, 31) Analysis of police seizures demonstrated that purity increased between the third and fourth quarters of 2011, declining in the first quarter of 2012 and increasing in the second quarter of 2012. (17) While these purity data are based on a restricted number of seizures, the general pattern is consistent with the fluctuations detected in the wastewater samples in this study (Figure S7B of the Supporting Information).

A strong weekend effect was observed on MDMA consumption similar to that for cocaine. It was approximately two times higher on weekends (yearly average, 347 (mg/day)/(1000 people); Table S6 of the Supporting Information) than on weekdays (181 (mg/day)/(1000 people)) (p < 10–4). This pattern of use is consistent with trends reported in consumer studies. (30, 31, 36) A few spikes were identified in the quantities of MDMA consumed over the study period (Figure S7B of the Supporting Information). Some of these spikes coincided with major events in the catchment, confirming previous findings of links between MDMA use and musical entertainment events. (37, 38)

3.5 Overall Methodological Uncertainty

Three different evaluations were performed to calculate the total uncertainty (Utot) of PNDC (Table 1). First, the components UC, US, UF, and UE are typically considered for Utot. Often, UP is unfortunately excluded because it is unknown and obtaining a realistic, independent, and site-specific estimate is difficult. Applying Monte Carlo simulation, the average Utot was estimated at approximately 22% for the three illicit drugs over the monitoring period (see Table 1 for details and range), in which UF is the most dominant uncertainty component. This estimate was similar to our previous finding for this urban catchment. (8) Second, when considering a conservative value of 50% (range 7–55%, according to Castiglioni et al.)(12) for UP—analogue to the uncertain knowledge of population (wide prior) used for Bayesian updating in the population estimation model(11)—Utot (de jure) would be approximately 57% for the three illicit drugs, in which UP becomes the dominating uncertainty factor. Third, as mentioned previously, the daily de facto population is estimated from chemical mass loads which are calculated with the same daily flow data as the daily illicit drug loads.
Thus, UF affects both daily *de facto* population estimates and illicit drug loads in the same way and cancels out when calculating PNDC. For calculating *de facto*-PNDC, the Utot(*de facto*) is, therefore, based on US, UC, UE, and UP. For UP, the multisubstance model estimated an average of 3.5% over the monitoring period (posterior of population estimation(11)). The average Utot(*de facto*) is then estimated at approximately 10% for all compounds (Table 1).

Table 1. Evaluation of the Total Methodological Uncertainty (RSD %) for Estimating Illicit Drug Consumption between Normalization to the *de jure* Population and the Estimated *de facto* Population Using Monte Carlo Simulation

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</tr>
<tr>
<td>UE</td>
<td>1.9</td>
<td>1.9</td>
<td>0.82</td>
</tr>
<tr>
<td>UP</td>
<td>n.a.a</td>
<td>50</td>
<td>n.a.</td>
</tr>
<tr>
<td>Utot</td>
<td>22 (22–23)</td>
<td>57 (54–61)</td>
<td>21 (21–22)</td>
</tr>
<tr>
<td>with <em>de facto</em> populations</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>US</td>
<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td>UC</td>
<td>7.1</td>
<td>5.1</td>
<td>9.8</td>
</tr>
</tbody>
</table>
There was a distinctive difference among a typical Utot, Utot(*de jure*), and Utot(*de facto*) for the three illicit drugs over the monitoring period (Figure 2 and Figures S8 and S9 of the Supporting Information). The levels of Utot for estimating cocaine consumption was clearly diminished in a decreasing order as follows: Utot(*de jure*) (Figure 2C) > a typical Utot (Figure 2B) > Utot(*de facto*) (Figure 2A). The same holds true for estimating methamphetamine and MDMA consumption (Figures S8 and S9 of the Supporting Information). Our data clearly revealed a substantial reduction in the Utot when a day-specific *de facto* population was estimated from chemicals in the same sample as used to measure illicit drug residues. A European-wide study estimated about 26% (a typical Utot) as the uncertainty of estimating cocaine consumption.(12) A similar level was also estimated in our study, but our data reflected a much lower level when using the daily estimated *de facto* populations.

This study showed an objective quantification of the total methodological uncertainty and achieved a substantial reduction thereof. In our case, the total uncertainty of the *de facto*-PNDC was reduced by a factor of 2 because of (i) eliminating the uncertainty of flow measurements and (ii) performing a unique assessment of uncertainty related to the population size. Whether or not the effort to reduce the total methodological uncertainty from about 20% to 10% for a daily value is justified, there remains an open discussion and it depends on the specific application and setting. However, it appears that avoiding a systematic over- or underestimation of PNDC—which normally remains undiscovered—is highly desirable, particularly when comparing consumption data across different locations. With a dataset that covers a much shorter duration and does not cover consecutive days, we could have not derived these findings and substantiated our conclusions. This also holds true for the identification of monitoring days that require more attention for interpretation. In combination with previously summarized long-term studies,(23) our study offers guidance to optimize future
monitoring campaigns where financial or logistic reasons limit the numbers of sampling days for evaluation. A remaining challenge is to interpret an estimated population of, e.g., 100,000 people, as different settings could have led to this result: (1) 100,000 people all being present in a catchment over 24 h and having used the toilet an average of five times; or (2) 500,000 people in transit through the catchment area using the toilet an average of only once in the day, as an extreme opposite. Both situations would result in approximately 500,000 toilet flushes.

Supporting Information

Text describing calculation information for back-estimation of illicit drug consumption, tables listing dates of missing representative samples, a summary of LC-MS/MS analysis data, quality assurance, and control samples for illicit drug residues and high-use chemicals, estimated *de facto* populations, pharmacokinetic data of three conventional illicit drugs, population and consumption data for cocaine, methamphetamines, and MDMA, and CVs of consumption between *de jure* and estimated *de facto* populations, and figures showing Mann–Whitney test comparison between weekends and weekdays for estimated *de facto* populations and drug consumption, percentage differences between estimated *de facto* and *de jure* populations, daily concentrations of high-use chemicals for estimating *de facto* population and wastewater volume, high-use chemical concentrations vs flow rate, and various drug consumption comparisons. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.

Acknowledgment

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References


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Figures
Figure 1. (A) Estimated daily *de facto* populations from the wastewater samples over the monitoring period (orange solid line, the *de jure* population of 211,340 persons). (B) Percentage differences between the daily *de facto* population and the *de jure* population (i.e., differences of the two estimates divided by the *de jure* population). (C) Deviations (percentage differences) of per capita consumption rates between normalization to the estimated *de facto* population and the *de jure* population. This panel is different from that of panel B due to the fact that the population estimate influences the population-normalized estimates by \((\text{de jure}/\text{de facto} - 1)\) (see section 3.3 for details). Black bars along the X-axis = missing date (see Table S1 of the Supporting Information); triangles along the X-axis = days with special dates/events. Figure S1 of the Supporting Information shows a direct comparison of data between the weekends and weekdays for panel A (see Table S6 of the Supporting Information for the numerical data of the monthly average and the average over the entire monitoring period).
Figure 2. Estimated population-normalized consumption for cocaine considering different population estimates and uncertainty components: (A) de facto population normalized consumption with $U_{tot}(de \, facto) = US + UC + UE + UP(de \, facto)$; (B) de jure population normalized consumption with a typical $U_{tot} = US + UC + UE + UF$; (C) de jure population normalized consumption with $U_{tot}(de \, jure) = US + UC + UE + UF + UP(de \, jure)$. Black bars along the X-axis = missing dates (see Table S1 of the Supporting Information); triangles along the X-axis = days with special dates/events. Figure S1 of the Supporting Information shows a direct comparison of data between the weekends and weekdays for A (see Table S6 of the Supporting Information for the numerical data of the monthly average and the average over the entire monitoring period).