Estimating community drug use through wastewater-based epidemiology


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Assessing illicit drugs in wastewater: advances in wastewater-based drug epidemiology

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A stepwise approach

The wastewater-based epidemiology approach relies on the principle that traces of almost everything we consume are excreted, unchanged or as a mixture of metabolites, in urine and faeces, and ultimately end up in the sewer network. Thus, measuring target drug metabolic residues in raw wastewater allows the identification of the use of specific substances by a population. To date, the most popular application of this approach is for the estimation of illicit drug use in a community.

The method consists of several consecutive steps that allow researchers to identify and quantify target metabolic residues of illicit drugs in raw wastewater and back-calculate the amount of the corresponding illicit drugs that would have been consumed by the population served by the wastewater treatment plant. The general scheme for this approach is outlined in Figure 1.1. First, representative composite samples of raw wastewater are collected and analysed for the selected substances. The back-calculation of drug consumption is performed by (1) calculating the daily sewer loads of target residues (g/day) by multiplying the concentrations of the measured target residues (ng/l) by the daily flow rates of sewage (m³/day); (2) estimating the total consumption by applying a specific correction factor, which takes into account the average excretion rate of a given drug residue and the molecular mass ratio of the parent drug to its metabolite (Zuccato et al., 2008; van Nuijs et al., 2011); (3) normalising consumption by dividing daily values by the number of people in order to facilitate comparison among cities (mg/day/1,000 population); and (4) assuming a mean dose to obtain a value in doses/day/1,000 population.

Between 2005 and 2010, an increasing number of research groups applied their own methods to assess the use of illicit drugs, at local and national levels, in several countries, demonstrating the potential of the approach for quantifying illicit drug use at a community level. Unfortunately, it is difficult to compare the results of these early studies because of the lack of common procedures with regard to the approaches used for sampling the wastewater and for the back-calculation of illicit drug consumption. Therefore, it was essential to establish some practical guidelines to ensure the proper application of the wastewater-based epidemiology approach. In 2010, a group of researchers working in this field established the Sewage Analysis CORE group Europe (SCORE) network to harmonise the approach and to coordinate international studies through the establishment of a common protocol of action. The first activity organised by the SCORE group was a Europe-wide investigation, performed in 2011 in 19 European cities, which allowed the first ever wastewater study on the regional differences in illicit drug use in Europe (Thomas et al., 2012). This study also included the first intercalibration exercise for the evaluation of the quality of the analytical data and allowed a comprehensive characterisation of the major uncertainties of the approach (Castiglioni et al., 2013).

This chapter first presents an overview of the most relevant areas of uncertainty related to wastewater-based epidemiology. This is followed by an overview of the wastewater-based epidemiology stepwise approach, describing the SCORE best-practice protocol, which represents the most comprehensive and up-to-date information available on this topic. The latest research on sampling, chemical analyses, stability of target residues
some research involving annual monitoring campaigns that include specific quality control tests is being performed in order to evaluate the reliability of sampling, sample storage and chemical analysis (Ort et al., 2014b).

Lai et al. (2011) assessed, for the first time, the uncertainties associated with sampling, through the optimisation of the sampling method, and with back-calculation of the per-capita drug consumption, through a refined estimation of the number of people contributing to the wastewater. The uncertainties related to the sampling and chemical analysis of cocaine and its main metabolite benzoylecgonine were also assessed (Mathieu et al., 2011).

More recently, data collected from the first Europe-wide monitoring study (Thomas et al., 2012) and from other available literature were used to comprehensively address the uncertainty associated with all of the steps of the wastewater-based epidemiology approach (Castiglioni et al., 2013).

### Areas of uncertainty

The areas of uncertainty related to the wastewater-based epidemiology approach were identified soon after its first applications (Zuccato et al., 2008), and some of these have already been mentioned in a previous EMCDDA publication (EMCDDA, 2008). These uncertainties are mostly associated with the main steps used to estimate community drug consumption through wastewater analysis (see Figure 1.1), namely sampling, chemical analysis, the stability of drug biomarkers in wastewater, the back-calculation of drug use and the estimation of the population size in a catchment area. Several efforts have been made in recent years to address these uncertainty factors in order to improve the reliability of the method. Nevertheless, the need to improve the comparability of data is still a priority, and some research involving annual monitoring campaigns that include specific quality control tests is being performed in order to evaluate the reliability of sampling, sample storage and chemical analysis (Ort et al., 2014b).

### Sampling of raw wastewater

Collecting representative composite samples of untreated wastewater is essential if the results of chemical analysis of wastewater are to give reliable figures for use in wastewater epidemiology, as demonstrated by Ort et al. (2010a). To improve the quality of the data, the sampling protocols were evaluated by analysing information collected using standardised questionnaires from all wastewater treatment plants involved in the first Europe-wide study. Data on sampling set-up (particularly sampling mode and frequency) and on catchment characteristics were gathered and the biases related to each sampling mode were evaluated. Based on these analyses and the expert judgement of a group of sewage engineers, some best-practice requirements were proposed, with the aim of greatly reducing sampling artefacts — which can range from ‘non-significant’ to ‘100 % or more’ resulting in overinterpretation of measured data — and minimising the uncertainty related to sample collection (< 10 %) (Castiglioni et al., 2013).

### Biomarker analysis

Laboratories performing chemical analysis of wastewater typically use their own in-house analytical methods. Despite the application of properly validated procedures, the employment of different analytical methods can produce results that are affected by bias, thus making the comparison of results difficult. Because
of the prohibitive costs of additional analytical materials and instruments, it is not possible to ask researchers to change their in-house methods and adopt the same technique or the same equipment as other laboratories; however, several common quality control criteria can be adopted to reduce the potential errors associated with sample manipulation and storage, and to ensure similar evaluations of method performance. For instance, the use of reference standards for each compound was proposed in order to compensate for matrix effects during analysis and some guidelines have been established to coordinate the estimation of realistic limits of quantification and a common procedure for confirming positive results has been adopted in accordance with international standards (UNIDO, 2009).

An interlaboratory study was organised during the first EU-wide campaign to provide information on the variability resulting from the analytical measurements made by each of the participating laboratories. Two vials containing known concentrations of the selected analytes in methanol were prepared by one group and sent blind to each participating laboratory. Each laboratory was asked to determine the analyte concentrations in each vial, by quantitatively analysing three independent replicates of each solution, and to report the mean value of the triplicate measurements. The analytical performance of the laboratories was evaluated by calculating the variability from the mean (z-scores) for each laboratory. This is an internationally accepted measure for evaluating the performance of an individual laboratory with regard to a group average and it was a useful tool for evaluating the results of this interlaboratory study (Castiglioni et al., 2013). During successive analytical campaigns, intercalibration studies were conducted by also sending real wastewater samples spiked with different amounts of the selected analytes blind to each participating laboratory.

### Biomarker stability

The stability of the illicit drugs and metabolites normally chosen for monitoring in wastewater has been evaluated in sewer systems and during sampling, storage and analysis of samples by collecting the available information from the literature. In this way, it was possible to identify the most stable compounds that can be safely used as target residues to estimate drug use (see ‘Target drug residues in wastewater’ in Chapter 2 for detailed results). For instance, benzoylecgonine was found to be the most suitable metabolite for estimating cocaine use, because of its relatively high stability in sewer systems. This information is essential for choosing a proper target residue and eventually identifying the degree of uncertainty that may arise from the biotransformation of a substance in a sewer and during sample handling.

### Back-calculation of drug use

There is also uncertainty associated with the correction factors used for back-calculating drug use from the levels of target residues. Normally, the correction factors are developed using the average excretion percentages of a target residue (Zuccato et al., 2008), which are obtained from a limited number of studies based on a very small sample of healthy volunteers. Moreover, excretion can vary according to the route of administration and the frequency of use of a substance. In order to reduce the uncertainty related to these variable factors, a systematic review of all pharmacokinetic data available for a substance was recently performed for cocaine, as a case study (Castiglioni et al., 2013). In this study, after sample collection, the excretion percentage of benzoylecgonine, which is the metabolite selected for estimating cocaine use, was weighted by the number of subjects involved in the pharmacokinetic studies and by the frequency of use of different routes of administration. This approach reduced the variability of the average benzoylecgonine excretion rate, from 42 %, before the refinement of data, to 26 % (Castiglioni et al., 2013), thus allowing refinement of the correction factor used to back-calculate cocaine consumption. Similar results were obtained by using the Monte Carlo simulation approach in order to consider back-calculation of cocaine use in a formal statistical framework (Jones et al., 2014).

### Estimation of population size

If a comparison between different geographical areas is desired, drug estimates should be normalised to population size and, therefore, some measure of population size is needed. This is not an easy task, and a high degree of variability can be introduced in these calculations, as recently demonstrated during the analysis of data collected in 19 European cities (Castiglioni et al., 2013). Several methods based on measuring hydrochemical parameters in wastewater and collecting census data are currently used to estimate the population using a given sewer network. Additional methods, currently under development, use specific substances, such as creatinine, cotinine, pharmaceuticals, coprostanol and hormones, as anthropogenic markers in order to estimate population size and reduce associated uncertainties (see ‘Estimation of population size’, page 28 for details).
The established common protocol of action, which was tested during the first European study, was later adopted by two successive studies conducted in 2012, in 25 cities, and 2013, in 43 cities (Ort et al., 2014b). The concerted effort to produce comparable results allowed the generation of the most useful wastewater-based information on illicit drug use in Europe to date, and the first ever quantitative measurements of illicit drug use in certain European countries.

### Optimisation of sampling and monitoring: challenges and alternatives

The first step to estimate drug use through wastewater analysis is the collection of ‘representative samples’ that should contain the entire amount of a substance discharged daily into wastewater from a defined community. Proper procedures should be therefore adopted to collect such samples from untreated wastewater at the point of inflow to wastewater treatment plants. Deciding upon the intensity of the monitoring effort entails weighing up the costs and benefits of possible sampling and analytical regimes. In simple terms, if information is to be of use for policymaking, it will need to have a relatively low level of uncertainty. Achieving this may imply relatively intensive sampling and analytical efforts, which may be costly. In practice, this means that an optimum level of research conditions for sample handling during storage and analyses.

### Best-practice protocol

Several efforts have been made in recent years to address the uncertainty factors mentioned above in order to improve the reliability of the entire method. Knowledge of the proper procedures that should be adopted when implementing wastewater-based epidemiology has greatly improved, and specific guidelines are available as a best-practice protocol. The main aims of establishing this best-practice protocol were (1) to produce homogeneous and comparable data at different sites and (2) to provide the most reliable estimates of drug use to complement existing epidemiological studies consistently.

In view of the enormous potential of the wastewater-based epidemiology approach and its wide application by different research groups, it is now highly recommended that all groups working in this field follow a common procedure while implementing the approach.

The best-practice protocol consists of several guidelines that address sample collection, storage and chemical analyses (see Table 1.1 for a summary of the main points). The protocol was established and formally agreed at a meeting held at Dublin City University (Ireland) on 14 December 2010. Later, the protocol was revised and improved after new expertise was gained during the successive analytical campaigns conducted in Europe. During these campaigns, sewer engineers were involved in evaluating the influence of different sewer designs and sampling procedures on the data generated, and analytical chemists were involved in establishing common procedures for evaluating the quality of analytical results and identifying the best conditions for sample handling during storage and analyses.

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### Chemical analysis — quality control

- **Substances investigated:** cocaine, benzoylecgonine, amphetamine, methamphetamine, MDMA (3,4-methylenedioxymethamphetamine), 11-nor-9-carboxy-delta-9-tetrahydrocannabinol (THC-COOH)
- **Internal quality control:** use of labelled analytical standards for each compound
- **External quality control:** analysis of methanol standards and influent samples as prepared by one laboratory

**NB:** BOD, biological oxygen demand; COD, chemical oxygen demand; N, nitrogen; P, phosphorus; PET, polyethylene terephthalate. The protocol is available at [www.emcdda.europa.eu/waste-water-analysis](http://www.emcdda.europa.eu/waste-water-analysis).

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**TABLE 1.1**

Summary of the main procedures described by the best-practice protocol currently adopted by Europe-wide studies

<table>
<thead>
<tr>
<th>Phase of the approach</th>
<th>Agreed procedures</th>
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| **Sampling and sample handling** | **Sampling point:** wastewater treatment plant influent  
**Sample type:** 24-hour flow-weighted composite  
**Sampling container:** PET or glass container  
**Questionnaire:** developed to collect information on sewer systems, sampling mode and additional parameters such as BOD, COD, N, P, flow data, type of sewage influent, temperature, pH |
| **Storage treatment during sampling** | **During sampling:** < 4 °C  
**After sampling — two possible options:**  
1. Process the sample for analysis within 12 hours  
2. Freeze the samples immediately after collection |
| **Chemical analysis — quality control** | **Substances investigated:** cocaine, benzoylecgonine, amphetamine, methamphetamine, MDMA (3,4-methylenedioxymethamphetamine), 11-nor-9-carboxy-delta-9-tetrahydrocannabinol (THC-COOH)  
**Internal quality control:** use of labelled analytical standards for each compound  
**External quality control:** analysis of methanol standards and influent samples as prepared by one laboratory |

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effort must be found, the so-called fit-for-purpose uncertainty level (Ramsey and Thompson, 2007). However, without a good understanding of the cost–benefit relations of drug policy, it is difficult to establish the optimum uncertainty level for wastewater-based epidemiology.

It is worth noting that wastewater-based epidemiology can use the existing infrastructure and that, other than the logistics involved, samples can be obtained at almost no cost (Banta-Green and Field, 2011). As there have been no relevant changes related to the sampling techniques described in the previous EMCDDA Insights on wastewater (Rieckermann, 2008), the aim of this section is to elucidate the scientific advances made since 2008 in order to answer the following three questions:

1) What level of uncertainty could be achieved with the existing sampling equipment and the routinely applied sampling modes and frequencies?
2) Are there situations that require particular attention?
3) Are there alternative sampling technologies that could apply to raw wastewater?

### Fluctuations of illicit drugs in sewers

The statement that ‘almost everything that is worth analysing is actually or potentially heterogeneous’ (Thompson, 1999) also applies to illicit drugs in sewers. Targeted high-frequency sampling campaigns have revealed high temporal fluctuations in the concentrations of illicit drugs and pharmaceuticals. These fluctuations are caused by substances entering wastewater in toilet flushes or pump stations lifting and transporting wastewater from entire sub-catchments intermittently to wastewater treatment plants. Specifically tailored sampling proficiency tests have demonstrated that inadequate sampling modes (e.g. grab samples or time-proportional composite sampling) and frequencies (i.e. intervals longer than 1 hour) can lead to substantial sampling artefacts, which can result in both over- and underestimation of results. In these cases, sampling errors can be larger than errors associated with chemical analysis (Ort et al., 2010a, b).

### Collecting 24-hour composite samples

For various practical reasons, 24-hour composite samples of raw wastewater from the influent of wastewater treatment plants are normally collected (Ort, 2014). Thus, daily samples are the unit for analysis. Studies focusing on relatively large catchment areas and frequently used substances have concluded that sampling uncertainty can be kept below 10 % (relative standard deviation, RSD; also known as the coefficient of variation) (Mathieu et al., 2011; Thomas et al., 2012; Castiglioni et al., 2013). Because of systematic diurnal variations of wastewater flows and drug loads (Brewer et al., 2012; Lai et al., 2013), samples need to be collected in a flow- or volume-proportional manner (Ort et al., 2010a, b) to avoid incorrectly weighted samples and biased results. Furthermore, because of potential short-term fluctuations, it is recommended that sampling intervals do not exceed 5–10 minutes. This would result in approximately 100–200 individual samples being collected over a 24-hour period. However, it should be noted that all of these samples are pooled before analysis and, therefore, this high sampling frequency does not necessarily increase analytical effort. A questionnaire to aid the collection of details relevant to the estimation and minimisation of sampling uncertainty in wastewater-based epidemiology is provided in the supporting information of Castiglioni et al. (2013), and free open-source software can also be found at www.eawag.ch/spg.

### Estimating annual averages

The estimation of annual averages is a suitable approach for wastewater-based epidemiology, as the resulting annual estimates of drug consumption by a population can be compared with several existing established drug epidemiology datasets and indicators (e.g. the self-reported annual prevalence of drug use among the general population and annual drug seizure incidences). Specific efforts are now being directed towards finding the best procedures for estimating annual averages, as these cannot be directly obtained by analysing a few samples but need to be determined from a sufficient number of 24-hour composite samples collected throughout a year. This sample number is highly dependent on weekly and seasonal variations — for which we have limited information — and the desired level of accuracy. To date, only five studies, summarised in Ort et al. (2014a), investigated daily loads of illicit drugs over a 1-month period or more. Figure 1.2 shows the observed variations of daily drug loads expressed as coefficients of variation (CV). For benzoylcegonine, which was measured in all studies, load variations decreased with increasing population size. For other substances, such expected decreases could not be confirmed for various reasons. The high variation in 3,4-methylenedioxymethamphetamine (MDMA) loads was mainly attributed to the high consumption of this drug at weekends and the fairly low (or non-detectable) consumption on working days. The number of samples (n) required to stay below a certain level of uncertainty...
Assessing illicit drugs in wastewater: Advances in wastewater-based drug epidemiology

proportional sampling at even higher frequencies (i.e. frequencies of 1 per minute to 1 per 5 minutes) than the sampling frequency required for influents to large wastewater treatment plants. This would be even more pronounced for effluent from individual premises, such as schools or prisons. Another challenge is the assessment of the accuracy of flow measurements (Rieckermann, 2008). This can be partly resolved by estimating the population size used to calculate population-normalised drug loads from wastewater parameters (Lai et al., 2011; O’Brien et al., 2014) (see also Estimation of population size, page 28). An alternative to active sampling technology is passive sampling. This involves the placement of a device (passive sampler) in the wastewater, where it accumulates chemicals through diffusive processes over time. Such technologies offer practical and economical advantages for gathering long-term, or geographically broad, data. For example, they have been used to estimate drug use in Oslo, Norway, for a 1-year period (Harman et al., 2011). It should be noted that there are several challenges involved with applying these techniques, including those associated with calibration and quantification, knowledge of kinetics, and the correction for different exposure scenarios (Harman et al., 2012).

The sampling procedures normally used in wastewater-based epidemiology are sufficiently robust and reliable, (U) can be calculated by \( n = \frac{(CV/U)^2}{U} \). This means that site- and substance-specific coefficients of variation need to be calculated, which is a laborious task. Based on the limited data available to date, it seems that a coefficient of variation of 75 % is exceeded in only rare cases and, therefore, this could be considered a reasonable value. For certain substances, coefficients of variation can be substantially lower than this, implying that, for such substances, a smaller number of samples is required for the same level of accuracy. However, most samples are analysed for multiple substances and, therefore, the substance with the highest coefficient of variation will dictate the number of samples required. If the uncertainty of an annual mean does not exceed 20 %, 14 samples randomly distributed over a year would be required (or for \( U = 10 \% \), 56 samples would be required).

### Challenges and alternatives

Future wastewater-based epidemiology may require sampling from small wastewater treatment plants, but these are often not equipped with sampling devices for the collection of raw influent wastewater. Furthermore, the concentrations of illicit drugs in wastewater and flows from small catchment areas can be subject to much higher fluctuations, which would require flow- or volume-

![FIGURE 1.2 Variability of daily drug loads expressed as coefficients of variation (CV, standard deviation/mean) for five long-term studies](image)

NB: Population sizes (P) and the number of subsequent monitoring days (d) for the five studies were as follows: P = 7 160, d = 1 369; P = 278 000, d = 311; P = 557 000, d = 28; P = 1.1 million, d = 239; P = 1.3 million, d = 28 and d = 35 (references and details in Ort et al., 2014a). EDDP, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine; MDMA, 3,4-methylenedioxyamphetamine.
except in settings where the target residues’ dynamics are extraordinarily high because of (1) a small absolute number of wastewater pulses containing the substances of interest (i.e. searching for ‘a needle in a haystack’) and (2) sampling locations close to the source. The latter is the case for effluent from individual premises or influents to small wastewater treatment plants. This is because toilet flushes are not attenuated by dispersion effects to the same extent over short distances as they are over longer distances: a toilet flush may extend over only a couple of seconds directly outside a house, depending on the sanitary installations and hydraulic conditions of the house connection. For high-prevalence drugs in large catchment areas, current best practice for sampling is expected to result in uncertainties that are smaller than or in the same range as other components of uncertainty (Castiglioni et al., 2013, Ort et al., 2014a).

### Chemical analysis and quality control

The estimation of community drug use through wastewater analysis requires accurate and sensitive quantification of illicit target drug residues (usually the unaltered drug, or the drug’s main metabolite, excreted in urine). Reliable data are the basis of subsequent calculations of drug loads in wastewater and drug consumption. The principal difficulties associated with the quantitative analysis of illicit drugs relate to their very low concentrations in combination with the complexity and unknown composition of wastewater. The concentrations of illicit drugs in wastewater are generally around a thousand-fold lower than in human biological fluids. Furthermore, the presence of a large number of other substances in the sample matrix may hamper sensitive quantification and reliable identification. Hence, detection technologies should be both sufficiently specific and sensitive.

Modern analytical chemistry offers the solution to this challenging task. The use of advanced analytical techniques and the expertise of analysts are essential for obtaining accurate data with regard to drug residues in wastewater at trace levels (ng/l or parts per trillion) (Castiglioni et al., 2008; Postigo et al., 2008b). The medium-high polarity and low volatility of these compounds makes liquid chromatography coupled to mass spectrometry the technique of choice, particularly when using tandem mass spectrometry (Castiglioni et al., 2013). Liquid chromatography–tandem mass spectrometry allows the simultaneous quantification and identification of the target compounds in complex matrices, thanks to its excellent sensitivity and selectivity. In the process, the substances are ionised and fragmented and are subsequently detected by monitoring for specific ion mass-to-charge ratios (m/z) for each compound. Typically, two transitions are acquired by selecting the precursor ions and the fragmented ions characteristic of the compound under study: one of these transitions, usually the most intense, is used for quantification (Q), and the other is used for confirmation (q). As an example, Figure 1.3 illustrates the detection and identification of cocaine and its major metabolite benzoylecgonine on the basis of two transitions (Q and q) acquired for each compound. For cocaine, the fragments with mass-to-charge ratios of 182 and 82, from the precursor ion with a mass-to-charge ratio of 304, were selected (Figure 1.3, left panel), and for benzoylecgonine, the fragments with mass-to-charge ratios of 168 and 82, from the precursor ion with a mass-to-charge ratio of 290, were used (Figure 1.3, right panel). By considering the

### FIGURE 1.3

Identification and quantification of cocaine (382 ng/l) and its major metabolite benzoylecgonine (931 ng/l) in wastewater by liquid chromatography coupled to tandem mass spectrometry

**NB:** Q, quantification transition; q, confirmation transition.
peak area of the quantitative transition (Q) in the sample and comparing it with that obtained for the reference standard, it is possible to calculate the concentration of each substance. The acquisition of two transitions, together with retention time data and the measurement of ion intensity ratios between recorded transitions in standards and samples, permits a reliable identification of the compound detected, even at very low concentrations (see quantification-to-confirmation-transition ratios in Figure 1.3 and the deviation values, which are within the permitted maximum tolerance level) (UNIDO, 2009).

Despite the strong potential of liquid chromatography–tandem mass spectrometry for wastewater analysis, other compounds present in the sample may interfere and compete with the target residues during the ionisation process; this is known as the ‘matrix effect’. One of the key aspects of this analytical methodology that must be addressed, in order to ensure accurate quantification and reliable identification, is the removal, minimisation or correction of such matrix effects. Although the sensitivity of modern instruments is excellent, a sample treatment step is necessary to concentrate the analytes and clean up the sample. Solid-phase extraction is widely used for this sample treatment step. Other alternative sample treatment procedures, such as on-line solid-phase extraction (Postigo et al., 2008a) and large-volume injection (Chiaia et al., 2008; Berset et al., 2010), open up possibilities for fully automated analysis. In the near future, new and even more sensitive liquid chromatography–tandem mass spectrometry instruments may help to improve the performance of these methods, and will allow the extra dilution of sample extracts or reduce the need to concentrate the samples, thereby helping to minimise matrix effects.

Most of the reported methodologies use internal standards, which are added to the samples as surrogates (i.e. before sample treatment) for more accurate quantification. Reference standards, preferably an isotope-labelled analyte for each target compound, are commonly added to compensate for matrix effects and to ensure the satisfactory correction for analytical errors associated with sample manipulation and storage.

Nowadays, liquid chromatography–tandem mass spectrometry is widely recognised and accepted as an accurate method for the quantification of target drug residues in wastewater. However, high-resolution mass spectrometry provides new perspectives for this analytical field because of the powerful information provided by this technique (accurate-mass full-spectrum mass data). When using liquid chromatography–tandem mass spectrometry, identification and quantification are directed towards specific compounds that have previously been selected, and, therefore, this technique is limited to substances for which the method has been developed. Consequently, compounds other than the target compounds may be ignored in the analyses. High-resolution mass spectrometry transcends this limitation and shows strong potential for target and non-target screening. Another important possible use of this technique is for the investigation of the transformation products that can form in water. Liquid chromatography–high-resolution mass spectrometry has been limited to mainly qualitative screening (i.e. the detection and identification of compounds); however, recent improvements have also allowed its use for accurate quantification (Gonzalez-Marino et al., 2012; Bijlsma et al., 2013).

Any analytical methodology should comply with strict quality requirements in order to generate reliable data. Quantitative method validation is obviously required, but the application of updated criteria based on the acquisition of several transitions, considering their specificity, or based on mass accuracy measurement is also necessary. Furthermore, the analysis of internal quality controls in each sample sequence ensures quality and tests for daily variations. However, another key aspect of the analytical methodologies used for wastewater-based epidemiology is that they generate data that are comparable among different laboratories. Therefore, the performance of interlaboratory exercises, in which the same sample is analysed by all participants, is necessary. The results obtained by such analyses provide an indication of the accuracy and performance of each laboratory, and the presence (or absence) of systematic errors.

Until now, most research in this field has been aimed at estimating the use level of established illicit drugs such as amphetamine, cannabis, cocaine, MDMA and methamphetamine. However, the advanced analytical techniques now available allow the presence of other compounds in wastewater, such as new psychoactive substances, which regularly appear on the market (Reid et al., 2014; van Nuijs et al., 2014, Chapter 4 of this Insight), to be investigated. In this regard, non-target high-resolution mass spectrometry is especially attractive, because of the lack of reference standards in many cases for new psychoactive substances, and the lack of available information on the metabolism of these substances (Ibáñez et al., 2014).

<table>
<thead>
<tr>
<th>Enantiomeric profiling of illicit drugs</th>
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<td>When the presence of illicit drugs in wastewater is monitored regularly using a frequent sampling protocol, a baseline for daily drug loads resulting from consumption</td>
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in the corresponding community can be estimated. In some cases, however, aberrantly high loads may be observed in a sewer which could not possibly correspond to the actual level of drugs consumed by that specific community. These abnormally high loads may result from the direct disposal of unused drugs or production waste from, for example, illegal manufacturing facilities; these factors make the epidemiological estimation of community-wide drug use via wastewater analysis difficult and potentially unreliable. Therefore, it is of the utmost importance that new approaches are introduced to distinguish between drug loads in wastewater that result from consumption and those that result from the direct disposal of unused drugs (Emke et al., 2014). Enantiomeric profiling of drugs in wastewater by chiral chromatography coupled with mass spectrometry could be a viable option to solve these problems.

A chiral molecule usually has at least one chiral centre (e.g. an asymmetric carbon atom); as a result of this, it shows optical activity. Chiral molecules exist as two enantiomers (if only one chiral centre is present), which are non-superimposable mirror images of each other (Figure 1.4). Many of the popular psychoactive illicit and new drugs (e.g. cocaine, amphetamines and cathinones) contain one or more asymmetric carbon atoms (Emke et al., 2014). Enantiomers of the same compound exhibit the same physicochemical properties, but they differ in their biological properties: in their distribution in the body, their metabolism and their excretion from the body, as one enantiomer will be favoured over the other. This results from the fact that enantiomers react stereoselectively, for example with enzymes, in biological systems (Kasprzyk-Hordern, 2010). Two enantiomers of the same drug can also exhibit different potencies; for example, S(+)-MDMA is known to be more amphetamine-like than the R(−) enantiomer of this drug, R(−)-MDMA is known to be more hallucinogenic than the S(+) enantiomer, and S(+)amphetamine has a two-fold higher stimulant activity than R(−)-amphetamine (Kasprzyk-Hordern and Baker, 2012b). The chemical synthesis of compounds with one asymmetric centre will generally lead to equal amounts of the two corresponding enantiomers (a racemic mixture) in the product synthesised (e.g. the synthesis of MDMA usually produces equal amounts of the S(+) and R(−) enantiomers). The ratio of the concentration of one enantiomer to the sum of the R(−) and S(+) forms, that is R(−):(R(−) + S(+)), can be defined as the enantiomeric fraction; therefore, a racemic mixture will have an enantiomeric fraction of 0.5. It should, however, be emphasised that certain illicit drugs are synthesised via stereoselective routes (subject to the availability of substrates). For example, more potent S(+)-methamphetamine is usually synthesised in clandestine laboratories by the reduction of 1R,2S(−)-ephedrine or 1S,2S(+)-pseudoephedrine (naturally produced by the ephedra plant) (Kasprzyk-Hordern and Baker, 2012b).

The human metabolism of a product containing a racemic mixture of enantiomers will change the enantiomeric ratio as a result of differences in the metabolic conversion rates of different enantiomers (Emke et al., 2014). For example, S(+)-amphetamine is metabolised preferentially over R(−)-amphetamine, leading to a relative enrichment of the R(−) enantiomer in urine (Kasprzyk-Hordern and Baker, 2012b). Furthermore, the enantiomeric ratio can be influenced by microbial activity during sewage water transport in the catchment area and also by active sludge in the sewage water treatment plant, leading to, for example, further enrichment of the R(−) enantiomers of amphetamine and MDMA (Kasprzyk-Hordern and Baker, 2012a).

An example of enantiomeric profiling: analysing MDMA in wastewater

Many synthetic routes for producing MDMA start with piperonyl methyl ketone (PMK) and use either the
Leuckart route or various reductive amination reactions (Renton et al., 1993). All of these methods produce racemic MDMA. S(+)-MDMA is, however, metabolised in preference to R(−)-MDMA, which leads to the relative enrichment of the MDMA R(−)-enantiomer and the preferential formation of S(+)-3,4-methylenedioxyamphetamine (MDA) (Moore et al., 1996). Moore et al. (1996) also observed that in both bile and urine, which are the primary routes of MDMA excretion in humans, R(−)-MDMA was present at a higher concentration than S(−)-MDMA (an enantiomeric fraction of 0.57, based on autopsy findings). These fluids also contained a two-fold higher concentration of S(+)-MDA than the R(−)-enantiomer of MDA (enantiomeric fraction of 0.37, based on autopsy findings). This information is very important with regard to the verification of whether residues of a chiral drug present in wastewater result from its actual consumption (i.e. if the enantiomeric fraction is not equal to 0.5) or from its direct disposal (i.e. if the enantiomeric fraction is 0.5). As MDMA does not currently have medical applications, its presence in biological specimens is believed to result from illicit use (Emke et al., 2014). Indeed, Kasprzyk-Hordern and Baker (2012b) reported, in the first study of its kind, that wastewater was enriched with the R(−)-MDMA enantiomer because of the preferential metabolism of S(+)-MDMA in humans. Furthermore, the majority of MDA identified was the S(+)-enantiomer, which suggests that its presence is associated with MDMA consumption and subsequent metabolism into S(+)-MDA and not intentional MDA consumption (if the latter were true, there would be more of the R(−)-enantiomer of MDA in wastewater).

In 2011, anomalously high mass loads of MDMA were observed in wastewater from the city of Utrecht in the Netherlands. These loads deviated greatly from the loads observed during the previous monitoring campaign in 2010 (the average load in 2011 was 20-fold higher than the average load in 2010) (Bijlsma et al., 2012). To determine whether or not the MDMA in the wastewater had been consumed by humans, enantiomeric profiling of these sewage water samples was undertaken. It was shown (Figure 1.5) that the average enantiomeric fraction of MDMA was 0.54 for the 2011 sampling week. This indicates that the MDMA quantified in wastewater during this sampling week was a racemic mixture, which indicates that it resulted from the direct disposal of MDMA into the sewage system and therefore explains the high loads of MDMA found in Utrecht wastewater during the 2011 sampling week.

The relatively slow decrease in the MDMA load after the assumed disposal (red line in Figure 1.5) can be explained by the characteristics of the wastewater treatment plant in Utrecht, in which effluent is partly recirculated (one-third on a dry day) into the influent. This direct disposal could have been the result of a police raid on an illegal production facility that took place 2 days before the monitoring had started: the police estimated that 30 kg of raw MDMA or tablets had been disposed of in response to the raid. In contrast, the samples from 2010 (green line in Figure 1.5) showed an average enantiomeric fraction of 0.65, which corresponds to excretion profiles in urine after MDMA consumption (Emke et al., 2014).

Until now, it has been difficult to determine if mass loads of studied drugs originated from consumption, the disposal of unused drugs or production waste. This uncertainty in the route by which drugs enter wastewater should not be underestimated when applying wastewater-based epidemiology. In this regard, enantiomeric profiling of wastewater is a new and very promising approach to solving this problem.

**FIGURE 1.5**
MDMA loads detected in samples from the wastewater treatment plant of Utrecht, the Netherlands, collected in 1-week sampling periods in 2010 and 2011, and their corresponding enantiomeric fractions
Stability of drug residues in urban wastewater

The stability of drug residues in wastewater is a property that has to be evaluated with care, as it can lead to significant under- or overestimations when calculating drug use in wastewater-based epidemiology. Therefore, it is imperative that knowledge is gathered on the behaviour of the target drug residues in sewer systems (i.e. the in-sewer stability of drug residues from the place of excretion to the place of sample collection) and the stability of these compounds in the sample matrix during the collection and storage of wastewater.

The transformation of drug residues in wastewater from the place of excretion to the place of sample collection in the wastewater treatment plant (in-sewer biotransformation) has been assessed in a number of studies. In all of these studies (Table 1.2), the amphetamine-like stimulants under investigation were amphetamine, methamphetamine and MDMA, and these substances showed negligible transformation in wastewater after 12 hours (or even up to 24 hours) at room temperature. At the lower temperature of 4 °C, these amphetamine-like stimulants were stable for up to 3 days. These three compounds have also been found to be stable in urine at 37 °C for 3 days and longer. Experiments have also been performed to assess the stability of cocaine and its major metabolites, benzoylecgonine and ecgonine methyl ester, in wastewater. Benzoylecgonine was found to be the most stable cocaine residue in wastewater, with less than 20 % biotransformation after 24 hours, at pH 7.5 and room temperature (Table 1.2). The observed increase in benzoylecgonine concentrations over time was due to a partial degradation (hydrolysis) of cocaine to benzoylecgonine, a process that was also observed in blood and urine. The two other residues under investigation, cocaine and ecgonine methyl ester, were significantly less stable in wastewater than benzoylecgonine, with losses of up to 60 % and 40 %, respectively, after 12 hours, at pH 7.5 and room temperature. However, some inconsistencies in the degradation rates of these two compounds were observed among various studies, probably because of differences in the experimental set-ups, such as the sample matrix (i.e. differing characteristics of the wastewater used) and spiking concentrations.

Experiments to assess the stability of 11-nor-9-carboxy-delta-9-tetrahydrocannabinol (THC-COOH), the most abundant residue in wastewater resulting from cannabis use, demonstrated that this compound is stable under relevant conditions (24 hours, pH 7.5 and 20 °C). By contrast, significant losses were observed, under these conditions, for the transformation product of heroin consumption, namely 6-monoacetylmorphine.

### TABLE 1.2

<table>
<thead>
<tr>
<th>Reference</th>
<th>Time (hours)</th>
<th>Temperature (°C)</th>
<th>pH</th>
<th>Cocaine, %</th>
<th>Benzoylecgonine, %</th>
<th>Ecgonine, %</th>
<th>Amphetamine, %</th>
<th>Methamphetamine, %</th>
<th>MDMA, %</th>
<th>THC-COOH, %</th>
<th>6-MAM, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Castiglioni et al., 2006</td>
<td>72</td>
<td>4</td>
<td>7.5</td>
<td>−36</td>
<td>14</td>
<td>NA</td>
<td>5</td>
<td>0</td>
<td>1</td>
<td>−8</td>
<td>−14</td>
</tr>
<tr>
<td>Gonzalez-Marino et al., 2010</td>
<td>24</td>
<td>4</td>
<td>7.5</td>
<td>−7</td>
<td>7</td>
<td>NA</td>
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<td>NA</td>
<td>NA</td>
<td>2</td>
<td>NA</td>
</tr>
<tr>
<td>Bisceglia, 2010; Bisceglia and Lippa, 2014</td>
<td>12</td>
<td>23</td>
<td>7.4</td>
<td>−50</td>
<td>10−14</td>
<td>−40</td>
<td>−15</td>
<td>0</td>
<td>0</td>
<td>NA</td>
<td>−15</td>
</tr>
<tr>
<td>Baker and Kasprzyk-Hordern, 2011</td>
<td>12</td>
<td>19</td>
<td>7.4</td>
<td>−8</td>
<td>7</td>
<td>NA</td>
<td>47</td>
<td>8</td>
<td>1</td>
<td>NA</td>
<td>−42</td>
</tr>
<tr>
<td>Castiglioni et al., 2011</td>
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<td>−25</td>
<td>20</td>
<td>−50</td>
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<td>NA</td>
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<tr>
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<td>7.5</td>
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<td>6</td>
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<td>3</td>
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<td>3</td>
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<td>−20</td>
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<tr>
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<td>7.4</td>
<td>−60</td>
<td>18</td>
<td>−29</td>
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</tr>
<tr>
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<td>20</td>
<td>7.5</td>
<td>−20</td>
<td>14</td>
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<td>NA</td>
<td>0</td>
<td>0</td>
<td>NA</td>
<td>−25</td>
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<tr>
<td>Chen et al., 2013</td>
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<td>7.0</td>
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<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>−5</td>
<td>1</td>
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<td>−53</td>
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<tr>
<td>Senta et al., 2014</td>
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<td>7.5</td>
<td>−35</td>
<td>15</td>
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<td>−5</td>
<td>−10</td>
<td>−10</td>
<td>0</td>
<td>−15</td>
</tr>
</tbody>
</table>

6-MAM, 6-monoacetylmorphine; MDMA, 3,4-methylenedioxymethamphetamine; NA, not applicable; THC-COOH, 11-nor-9-carboxy-delta-9-tetrahydrocannabinol.
Considering that typical in-sewer residence times are less than 10 hours, this means that transformation (or degradation) is generally lower than 10% for amphetamine, methamphetamine, MDMA, benzoylecgonine and THC-COOH. In-sewer degradation will, therefore, have negligible influence on wastewater analysis results if these compounds are used in back-calculations. However, if 6-monoacetylmorphine is used for back-calculation of heroin consumption, actual heroin use is likely to be underestimated because of the considerable losses of the residue due to in-sewer transformation.

Clearly, better designed and more sophisticated research in this area is necessary to assess other factors that could influence in-sewer losses and transformation, such as adsorption to solid matter, formation of biofilms and deconjugation processes. Moreover, most of these experiments have been conducted only in the laboratory, mimicking ‘real conditions’ for temperature and sewage composition. Only one modelling study addressing drug stability in wastewater has been conducted to date (Plosz et al., 2013); thus, it is important that in-sewer experiments are designed and additional modelling studies are performed to further investigate the in-sewer biotransformation of target residues and to confirm the current data.

In addition to assessing in-sewer transformation, it is important to evaluate the stability of drug residues in wastewater during sampling (typically 24-hour composite sampling) and sample storage. Upon collection, samples are typically cooled to 4°C and stored at that temperature (Table 1.1). The experiments summarised in Table 1.2 demonstrate that cocaine, ecgonine methyl ester and 6-monoacetylmorphine are not stable at 4°C and pH 7.5. In addition, the concentration of benzoylecgonine in composite samples could possibly increase by as much as 20% over a 24-hour period at 4°C if cocaine is present. This would result in overestimations of cocaine use in wastewater-based epidemiology. Acidification efficiently prevents the ‘formation’ of benzoylecgonine from cocaine during 24-hour composite sampling. For the other investigated drug residues, bringing the samples to refrigerator temperatures is sufficient to prevent transformation. After sampling, drug residues need to be stable in wastewater until the actual analysis can be performed. The two most commonly applied strategies described in the literature are as follows: (1) samples are directly frozen (at −20°C) after collection or (2) samples are processed using solid-phase extraction cartridges within 12 hours of collection (Table 1.1). These conditions prevent the degradation of the drug residues in the collected wastewater. It should be noted that, if passive samplers are used, analytes are extracted from the wastewater in situ, which should overcome some of these stability issues. However, this assumption has yet to be tested.

In view of the above-mentioned findings, if the proper procedures are adopted, the degradation processes that occur during in-sewer transport, sampling and storage can be expected to make a negligible contribution to the total uncertainty of the results of wastewater-based epidemiology for several of the most commonly used illicit drugs.

### Estimation of population size

To compare results from different sites, it is essential to know the size of the population that contributes to the sampled wastewater (Figure 1.1). Different methods have been proposed for the collection of information on population size and fluctuations thereof. Because of the different kinds of potential bias related to each of these various methods, it is not recommended that only one particular method is relied upon. Currently, population size can be estimated by measuring different hydrochemical parameters, such as biological oxygen demand, chemical oxygen demand, and nitrogen and phosphorus levels, and by using specific loads for these parameters (i.e. per-capita loads from domestic activity) to calculate the number of people contributing to the sampled wastewater (Andreottola et al., 1994). Recently, Been and co-authors (2014) tested the possibility of normalising population size using ammonium levels, and their method appears to be able to detect fluctuations in the size of a population over long periods or during major events. Another option for estimating population size is to collect census data for the area under investigation. A comparison of the population estimates obtained from these different methods has been performed using data collected from 19 European cities and the variability was shown to range from 7% to 55% (RSD) (Castiglioni et al., 2013). The reliability of these estimates depends on factors that cannot easily be controlled, such as the composition of the sewage (e.g. industrial, domestic or mixed), which can influence the hydrochemical parameters, the reliability of census data, the quality of the measured flow data and the method used to calculate population equivalents. Moreover, in the case of large cities, the number of commuters should also be evaluated. Therefore, it is not deemed appropriate to use a mean value of the population estimates calculated using the different methods described because of the large amount of bias that could be introduced into the final calculations of drug use estimates. So far, the best
option available, even if not ideal, is to compile estimates based on different methods and to choose the most reliable one using the expert judgement of wastewater treatment plant personnel. This was the procedure adopted in several recent European monitoring campaigns (Thomas et al., 2012; Ort et al., 2014b).

An interesting possibility would be to find specific substances that, once measured in wastewater, could indicate unequivocally the number of people served by a wastewater treatment plant. Such substances would have to fulfil several requirements; for example, they would have to be excreted in urine in known amounts, be detectable and stable in wastewater, and originate from only human metabolism (see Chapter 2 for further details). Several potential candidates, such as creatinine, coprostanol, caffeine, pharmaceuticals, biocides and food additives, have been proposed for further investigation (Daughton, 2012), and some studies tested the viability of these substances as population biomarkers in the 2 years prior to the publication of this EMCDDA Insight.

Because of the relatively homogeneous spatiotemporal use of certain pharmaceuticals, measuring pharmaceutical loads was suggested as a means of estimating the number of people that contribute to sampled wastewater (Lai et al., 2011). Unfortunately, methodological challenges related to the availability of reliable prescription data on pharmaceuticals, data on actual consumption (which depends on patients’ compliance), data on excretion rates and the estimation of associated uncertainties remain. However, expanding this approach from single to multiple substances is considered very promising.

Creatinine was used as a qualitative biomarker to normalise the loads of several illicit and licit drugs, and this allowed the study of diurnal and between-day trends by taking into account changes in population (Brewer et al., 2012). Nevertheless, the stability of creatinine in such studies should be established, since there is evidence that the degradability of creatinine in sewer conditions can affect its potential for use as a biomarker and could, therefore, introduce further bias to the estimation of population size (Chen et al., 2014; Thai et al., 2014).

Seven substances, comprising those already proposed (creatinine, cholesterol, coprostanol and cotinine) and three new compounds (cortisol, androstenedione and 5-hydroxyindoleacetic acid), were screened as potential population biomarkers using five different criteria. These criteria, namely quantification methods, affinity to particulates, stability in wastewater, constancy of interday excretion and correlation with census population data, were fully investigated for the first time by Chen et al. (2014). The results of this study suggest that cotinine and 5-hydroxyindoleacetic acid are the most suitable compounds (Chen et al., 2014).

Some years ago, the concentrations of the principal metabolites of nicotine, cotinine and trans-3′-hydroxycotinine, were found to correlate with the population in the catchment areas of several Swiss lakes, and were proposed as anthropogenic markers (Buerge et al., 2008). These substances were recently measured in raw wastewater from eight wastewater treatment plants in Italy and were assessed for their potential to be population biomarkers. They were shown to have a defined urinary metabolism in humans, and to be easily detectable and stable in wastewater; thus, it was possible to back-calculate nicotine consumption using specific correction factors. The prevalences calculated through the analyses of these substances in wastewater were very similar to those obtained from epidemiological surveys (Castiglioni et al., 2015). Similar results were obtained by a study in Lisbon, Portugal, in which only cotinine was measured in three wastewater treatment plants and was used to back-calculate nicotine consumption; the results of this study were in line with the findings of a European survey (Lopes et al., 2014). This suggests that the levels of nicotine metabolites measured in wastewater reflect the number of smokers within a population. Therefore, by considering this information and the average number of cigarettes smoked per day according to epidemiological surveys, it is possible to use nicotine metabolites to estimate the population size served by a wastewater treatment plant. Further investigations are now required to confirm these preliminary results.

To reduce and quantify the uncertainty of population estimates, it seems reasonable to combine multiple, unbiased indicators of population size measured in wastewater. One option — applying Bayesian inference — was recently developed by O’Brien et al. (2014). The results, based on multiple pharmaceuticals, were validated with the de facto population size, enumerated on census day through a georeferenced analysis (in Australia, both de facto and de jure population sizes are determined on census day). Therefore, no information on pharmaceutical sales was needed. This approach is able to produce accurate estimates of population sizes for large cities, while further research is needed to improve estimates for smaller populations. Most importantly, this approach provides a reliable indication of the uncertainty of the population estimate, implicitly including the spatiotemporal variability of indicators. This cannot be obtained in the same manner with other
methods. A methodological advantage of estimating population size from parameters measured in wastewater is the fact that the potential bias from flow measurements is cancelled out in the back-calculation (Lai et al., 2011). This is advantageous as it is usually very difficult to assess the bias resulting from flow measurements.

**Ethical aspects of wastewater-based epidemiology**

Because of the novelty of this field of investigation, no ethical rules are yet available for researchers applying wastewater-based epidemiology, but some general considerations have recently been provided (Hall et al., 2012; Prichard et al., 2014). Hall et al. (2012) analysed the ethical principles that are often used for assessing the ethics of biomedical and epidemiological research: the respect for autonomy (the informed and voluntary consent of participants, and the maintenance of confidentiality and privacy), non-maleficence (the avoidance of harm or risks for participants), beneficence (the benefits from the research should outweigh any burdens or risks) and distributive justice (the equitable distribution of burdens and benefits among groups of participants). The application of wastewater-based epidemiology in the general population does not generally give rise to notable ethical issues, mainly because wastewater is collected as a composite sample which has been contributed to by a large number of people, and individuals are not identifiable (Hall et al., 2012). Moreover, such studies are likely to satisfy the principle of beneficence, since the results may potentially improve public health and the health of illicit drug users. There is a possibility of indirect harm caused by the stigmatisation of a particular community with respect to others, but this risk would normally be remote because of the dimensions of the catchment areas being investigated, which are likely to include at least 10,000 people. This risk will be highly influenced by how the media communicate research results to the public: accurate communication can highlight the benefits to society, while erroneous communications may result in sensationalism and stigmatisation of vulnerable groups (Prichard et al., 2014). Particular attention should therefore be paid to media communication, even if predicting the outcomes of the media coverage of an emotive topic such as illicit drug use is particularly difficult.

By contrast, there are greater ethical concerns, which require careful consideration, with regard to smaller communities (e.g. workplaces, schools, prisons, city districts and entertainment venues). The ethical concerns regarding such settings are mainly related to the possible identification or stigmatisation of a particular group. In the case of prisons and entertainment venues, risks are also related to the policies that authorities may apply in response to wastewater-based epidemiological findings, which may lead to a reduction of drug supply and demand that could adversely affect all occupants of such premises (Prichard et al., 2014). These risks could be prevented by introducing rigorous procedures into the study design to protect the anonymity of sample members and by not identifying the location of study sites. Moreover, particular care must be taken when sampling in small communities, as artefacts can easily occur because of the very limited number of people using illicit drugs and the specific design of small sewer systems.

The two available studies that deal with ethical issues on wastewater-based epidemiology suggest that the development of ethics guidelines that retain the scientific rigour of the research while protecting the anonymity of smaller or disadvantaged populations, such as those of prisons, schools, workplaces or marginalised residential districts, is required. This would entail some consideration of how findings should be interpreted within the socio-political context of the research, how media coverage might misrepresent findings and how policymakers may respond. Special care is suggested in three areas: (1) the study design; (2) the management of relationships with research partners, such as prison or forensic authorities; and (3) how information is communicated to the media (Prichard et al., 2014).

**Conclusions**

Concerted efforts have been made in recent years to improve the wastewater-based epidemiology approach and to reduce the uncertainties related to community drug use estimates. These efforts have resulted in a good knowledge of the critical steps of the wastewater-based epidemiology approach and the actions required for improvements, as reported extensively within this chapter. This was made possible through the establishment of a European network (SCORE group) and the collaboration of different experts, including analytical chemists, drug toxicologists and sewer engineers. The final goal is now to start a close collaboration with drug epidemiologists in order to further discuss the opportunities for bringing together wastewater-based epidemiology and drug epidemiology.
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