European Monitoring Centre
for Drugs and Drug Addiction

# Assessing illicit drugs in wastewater

## Advances in wastewater-based drug epidemiology

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# Contents

## Foreword

The problem of measuring drug use, a complex, hidden and often highly stigmatised behaviour, is a central component of the work carried out by the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA). No single measure provides a full picture of the drug situation, and our overall strategy has been to adopt a multi-indicator approach. A number of specific information sources have been developed for this purpose, each of which highlights a particular aspect of the phenomenon, and by combining these we can build up a more comprehensive analysis. Nonetheless, the challenges in this area remain considerable, and thus the advantage of adding another tool to the epidemiological toolkit cannot be overestimated.

To be useful for policy, information needs to be technically robust and timely. Given the dynamic nature of the drug situation, equally dynamic monitoring responses should be available. However, a common problem of established monitoring tools is that they are time-consuming and complex, and thus require the investment of considerable resources if they are to produce reliable results. And in some areas, such as national surveys, the intervals between successive measurements will often be measured in years. In contrast, a relatively new approach, based on the analysis of municipal wastewater for drugs and drug residues, provides us with the opportunity to obtain more timely information on geographical and temporal drug use patterns.

In 2007, when the wastewater analysis approach was still in its infancy, the EMCDDA recognised that this fast-developing discipline had the potential to complement and extend the existing epidemiological tools for estimating illicit drug use. In that year, the Centre organised the first expert meeting on 'Assessing drugs in wastewater', and followed this up in 2008 by publishing an EMCDDA Insights on the topic. Later, in 2012, the EMCDDA was the driving force behind the first European multicity project to investigate the potential of wastewater analysis for estimating drug use at the level of the community. Using a common sampling approach, the project generated comparable data from over 25 European cities. The following year, in collaboration with the SEWPROF project, the EMCDDA organised 'Testing the waters', the first international multidisciplinary conference on detecting illicit drugs in wastewater; a second conference was held in October 2015. These initiatives brought together experts from a diverse range of disciplines to discuss future opportunities for integrating wastewater analysis into drug epidemiology. Indeed, a multidisciplinary approach is a central requirement for developing this new field, and researchers working in areas as diverse as chemistry, physiology, sewage engineering, statistics and drug epidemiology, to name a few, are contributing to our knowledge.

Wastewater-based epidemiology has now demonstrated that it has the potential to become an important adjunct to established drug monitoring tools. Its ability to deliver near-real-time data is particularly relevant to the mercurial nature of today's drug problem. By being able to detect changes in drug use patterns over time and as they occur, wastewater analysis can help health and treatment services in a number of ways. Alerting hospitals to the identities of new psychoactive substances being used in nightlife settings and predicting changes in treatment needs based on longer-term monitoring are but two potential examples.

The considerable methodological developments that have occurred in wastewater-based epidemiology over the past 8 years have both highlighted that the EMCDDA's interest in this area of study was not misplaced and underlined the importance of the original Insights publication *Assessing illicit drugs in wastewater: Potential and limitations of a new monitoring approach*. At the same time, they have rendered that original study obsolete: outdated and in need of replacement. To fill this need, the EMCDDA commissioned the

present publication, which I am proud to introduce. Based on the contributions to the most recent 'Testing the waters' conference, this publication presents a comprehensive review of the state of the art in wastewater-based drug epidemiology in Europe.

**Alexis Goosdeel**
Director, EMCDDA

# Executive summary

## Background

Monitoring illicit drug use is difficult because of the hidden and complex nature of drug-using behaviours. 'Wastewater analysis', or 'wastewater-based epidemiology', holds promise for complementing established methods of drug use measurement. Wastewater in municipal water treatment plants contains traces of chemicals that have been excreted and, most probably, consumed within the area served by a given sewer network. Detecting such residues in wastewater samples allows for non-invasive, near-real-time analysis of drug use. Wastewater analysis has some clear advantages over other approaches, as it is not subject to the biases associated with self-reported data and can better identify the true spectrum of drugs being consumed, which is particularly important as users are often unaware of the actual mix of substances they take. This tool also has the potential to provide timely information in short timeframes on geographical and temporal trends.

## Estimating community drug use through wastewater analysis

Wastewater-based epidemiology 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. First, representative composite samples of raw wastewater are collected and analysed for selected substances. Second, the back-calculation of drug consumption is performed by calculating the daily sewer loads of target residues; this is done by multiplying the concentrations of the measured target residues by the daily flow rates of sewage. From this value, the total consumption of a drug is estimated by applying a specific correction factor, which considers the average excretion rate of a given drug residue and the molecular mass ratio of the parent drug to its metabolite. In a third step, daily values are divided by the number of people served by the treatment plant in order to facilitate comparison among cities. This value can be expressed in daily amounts (or daily doses) per thousand population.

However, the findings of such an analysis are subject to uncertainties, mainly associated with sampling, biomarker analysis and stability, back-calculation of drug use, and the estimation of population size. Efforts to minimise the possible errors and standardise all procedures have achieved some success and continue to be made. The adoption of a standardised procedure will also improve the credibility and scalability of studies by ensuring that data from different sources are more reliable and comparable. Notably, the first Europe-wide study, performed in 2011 by the Sewage Analysis CORe group Europe (SCORE) network, provided a comprehensive insight into the uncertainties associated with all of these procedures. As a result, the group established a best-practice protocol with regard to sampling, sample handling, chemical analysis, back-calculation and data reporting. This protocol has been revised and updated during subsequent analytical campaigns in Europe, which were conducted annually.

## Application of wastewater-based epidemiology worldwide

Wastewater-based epidemiology has been applied in many countries to monitor the use of most of the commonly used illicit drugs. This worldwide application of wastewater-based epidemiology has demonstrated its potential for monitoring the use of cocaine, cannabis, amphetamine, methamphetamine and MDMA (3,4-methylenedioxymethamphetamine).

These studies detected geographical differences in drug use patterns, which were mostly consistent with data obtained by other approaches. Moreover, wastewater analysis has proven able to detect local and temporal patterns of drug use, demonstrating its potential to provide information that is complementary to that provided by standard techniques.

The detection of new psychoactive substances and the estimation of their use are challenges for drug epidemiology, including wastewater analysis. Pharmacokinetic data for most new psychoactive substances are essentially non-existent, as such compounds appear on the market at a rapid rate and the use levels of any particular substance are relatively low. Three conceptual approaches for dealing with new psychoactive substances using biomarkers in wastewater are presented in this report.

To date, few attempts have been made to compare drug use estimates obtained through wastewater analysis with conventional epidemiological data, obtained from population surveys. Although complicated and fraught with difficulties and limitations, comparing different approaches offers the possibility to cross-check data quality and accuracy, since each method tackles the task with different tools, and, therefore, combining different approaches should provide a more comprehensive assessment of drug use in a specific community. Some of the first attempts to compare results for cocaine use obtained using these two approaches are presented and discussed in this report. The first study was performed in Oslo, Norway, and compares results from three different datasets: two from survey methods and one from wastewater-based epidemiology. The second study analysed the temporal and spatial trends of cocaine use in Italy through wastewater-based epidemiology and compares the results with those obtained from local and national epidemiological surveys undertaken during the same period.

## Conclusions

For wastewater-based epidemiology to produce reliable estimates of illicit drug use and to inform the development of novel applications, the most urgent future research needs are as follows: (1) to improve the methodology by checking and reducing uncertainty factors for each single step; (2) to improve the comparability of results produced by different researchers or studies by adopting a common protocol of action, which will include ethical standards; and (3) to develop methods to integrate wastewater analysis with established methods of drug epidemiology.

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# Introduction

Sara Castiglioni

## Background to wastewater-based epidemiology

In modern society, humans are directly or indirectly exposed to a great variety of chemicals. Most of the chemicals that enter our body through the food or drink we consume or by other means such as smoking are excreted unchanged or as a mixture of metabolites in our urine and faeces, and ultimately end up in the sewer network. The concept of 'wastewater analysis' originates from research on monitoring the environmental pollution caused by pharmaceutical products and, in particular, from studies on the contamination of surface and sewage water caused by the excretion of therapeutic drugs by humans (Daughton, 2001).

The chemical analysis of wastewater was suggested for the first time as a 'new non-intrusive tool' to evaluate the use of illicit drugs and misused therapeutic drugs within a community in 2001 (Daughton, 2001). It involves sampling a source of wastewater, such as the sewage influent to a wastewater treatment plant. This allows scientists to estimate the quantity of drugs consumed by a community by measuring the levels of illicit drugs and their metabolites excreted in urine (Zuccato et al., 2008).

Some studies in the early 2000s showed that the concentrations of misused pharmaceuticals in the environment were higher than expected (e.g. Calamari et al., 2003), indicating that the monitoring of these substances in the environment could reflect their pattern of use in the population. The occurrence of amphetamines in treated wastewater was studied for the first time in 2004 in the United States (Jones-Lepp et al., 2004). In 2005, Zuccato and co-workers measured cocaine and its metabolites in raw wastewater samples, and, for the first time, used these data to back-calculate the consumption of cocaine by the population (Zuccato et al., 2005). This approach was called 'sewage epidemiology' or 'wastewater-based epidemiology' and was soon extended to the other main illicit drugs.

In recent years, wastewater-based epidemiology has been applied worldwide by several research groups at local and national scales, demonstrating the potential of the approach for quantifying illicit drug use at community level (Zuccato et al., 2008; Castiglioni et al., 2011; van Nuijs et al., 2011). In 2010, a Europe-wide network (the Sewage Analysis CORe group Europe – SCORE group) was set up with the aim of standardising the approaches used for wastewater analysis and coordinating international studies through the establishment of a common protocol of action (see Chapter 1). The work of the SCORE collaboration continues through the Earth System Science and Environmental Management (ESSEM) European Cooperation in Science and Technology (COST) Action ES1307 'Sewage biomarker analysis for community health assessment' ([1]).

From the outset, the European Monitoring Agency for Drugs and Drug Addiction (EMCDDA) showed a strong interest in exploring the potential of wastewater analysis to complement and extend the existing epidemiological tools. Wastewater analysis has some clear advantages over other approaches, as it is not subject to response and non-response bias and, as users are often unaware of the actual substances in the mix of drugs that they take, can better identify the true spectrum of drugs being consumed. The wastewater method is a flexible tool, as experiments can be designed to study drug use in a specific area or to compare the use between different areas during defined periods of the year or

([1]) Information on COST action ES1307 is available at www.cost.eu/domains_actions/essem/Actions/ES1307.

over successive years. As a result, the tool has the potential to provide timely information in short timeframes on geographical and temporal trends, including changing trends in particular locations, or during special events or public holidays.

Wastewater analysis offers an interesting and complementary data source for monitoring the quantities of illicit drugs used at population level, but it also has several limitations which should be carefully evaluated. Wastewater analysis cannot provide information on prevalence and frequency of use, route of administration, the main classes of users or the purity of the drugs. Additional challenges may arise as a result of uncertainties associated with the sampling of wastewater, the behaviour of the selected biomarkers in sewage, the reliability of interlaboratory analytical measurements, the different back-calculation methods used and the different approaches used to estimate the size of the population being tested (Thomas et al., 2012; Castiglioni et al., 2013). Furthermore, translating the total consumed amounts into the corresponding number of average doses is complicated as drugs can be taken by different routes and in amounts that vary widely, and purity levels fluctuate (Zuccato et al., 2008). Wastewater analysis is therefore proposed as a complement to, rather than as a replacement for, established monitoring tools.

This novel method of investigation has a strong multidisciplinary character, involving both environmental and social sciences. Until now, the main limitation was bringing together, and stimulating discussion and collaborations between, people working in different disciplines, namely chemists, water system engineers, pharmacologists and epidemiologists. In this framework, the first steps were taken by the EMCDDA, which organised, in 2013, in collaboration with the SCORE network and the EU-funded 7th framework programme Marie Curie Initial Training Network SEWPROF project, the first multidisciplinary conference, 'Testing the waters', on the detection of illicit drugs in wastewater. By uniting diverse disciplines, the conference created for the first time a forum for the discussion of future opportunities for combining wastewater analysis and drug epidemiology.

A second 'Testing the waters' conference took place on 11–15 October 2015. The conference brought together scientists and stakeholders from all involved disciplines to integrate results and contribute to the solution of a complex, societal problem such as drug use. The main aims of the conference were (1) to present monitoring studies integrating results from wastewater analysis and other epidemiological data; (2) to discuss scientific advances in individual disciplines in order to refine components of wastewater-based drug epidemiology; (3) to present improved methodologies for back-calculation of drug use and advances in analytical chemistry; (4) to discuss legal and ethical aspects of the approach; (5) to contribute to filling current gaps and provide guidance on future applications.

Research in this field is progressing very fast, with an increasing number of environmental chemists and engineers working together in a European network, in close collaboration with other groups in the United States, Canada and Australia, and with drug use epidemiologists, pharmacologists and addiction and prevention institutions in Europe, the United States and Australia. As a consequence, the number of publications available in this field is increasing and new knowledge and research advances are rapidly being added to the current knowledge. The rapid developments since the release of the EMCDDA's seminal work on wastewater epidemiology, the 2008 Insights 'Assessing illicit drugs in wastewater', have left that publication increasingly obsolete and in need of replacement by a publication that presents the latest findings in this research field.

This report presents the state of the art regarding wastewater-based epidemiology, including most of the findings from the 'Testing the Waters' conferences, and the results obtained from the initial years of activity of the SCORE network in Europe and from other

studies performed worldwide. Since 2008, when the approach was still in its infancy and when the EMCDDA published the first Insights report on this topic, great advances have been made in this research field. This is mainly as a result of the increasing number of groups that implement wastewater analysis worldwide, providing drug use estimates in a number of different countries, and because of the numerous studies focused on addressing critical issues and, therefore, improving the reliability of the approach.

## Overview of this publication

Chapter 1 provides a detailed description of the wastewater-based epidemiology approach, including a description of the best-practice protocol that was recently established to produce comparable data. The chapter also describes the most up-to-date procedures available for estimating illicit drug use within a community and summarises contributions regarding the optimisation of sampling and monitoring, chemical analyses and quality control, enantiomeric profiling of illicit drugs, stability of drug residues in urban wastewater and population size estimation.

Chapter 2 focuses on the main requirements for wastewater drug biomarkers, namely the specific substances selected as target drug residues in wastewater and used for back-calculating drug consumption values. A number of criteria that are essential for choosing proper target drug residues are presented. Back-calculation relies on specific correction factors which take into account mainly the urinary metabolism of a substance; unfortunately, human pharmacokinetic data are limited for most of the main illicit drugs, and, in general, the available studies were not performed recently and were based on a small number of participants. The further research needs are discussed and a critical overview of the current procedures used for back-calculating drug consumption is provided, giving some guidelines with regard to choosing or developing novel correction factors.

Chapter 3 presents an overview of the application of wastewater-based epidemiology worldwide. The results from four successive Europe-wide monitoring campaigns, coordinated by the SCORE network and performed in 2011 and each year since then, are presented in the first part of the chapter. The second part of the chapter reports a comprehensive summary of results obtained in the United States, Canada, Australia and Asia, and provides a comparison of all the available data.

Chapter 4, by Malcolm Reid and Kevin Thomas of the Norwegian Institute for Water Research, introduces a novel application for wastewater-based epidemiology, namely to detect the use of new psychoactive substances. This could be particularly useful because these substances constitute a heterogeneous group of synthetic products that are largely interchangeable, and very little is known about their use and prevalence. Conceptual approaches for dealing with new psychoactive substances using biomarkers in wastewater are discussed and an updated overview of the available applications is presented.

Chapter 5 is a collaboration between the Mario Negri Institute, Italy, and the Norwegian Institute for Water Research; it reports the first two case studies designed to integrate wastewater-based epidemiology with conventional approaches for estimating drug use within a community. In Italy, a nationwide monitoring campaign was performed in 17 cities to estimate drug use through wastewater analysis, and the results were compared with those obtained from an epidemiological survey conducted in the general population in the same period. In Norway, the results obtained from using three different methods for estimating the level of cocaine use in the general population were compared. The comparison applies to a set of regional-scale sample survey questionnaires, a representative sample survey on drug use among drivers and an analysis of the quantity of

cocaine-related metabolites in wastewater. These studies emphasise the challenges and opportunities for future studies that aim to bring together wastewater analysis and drug epidemiology.

Chapter 6 summarises the main findings from Chapters 1 to 5, and formulates the future research directions and final remarks.

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CHAPTER 1

# Estimating community drug use through wastewater-based epidemiology

Sara Castiglioni, Lubertus Bijlsma, Adrian Covaci, Erik Emke, Christopher Harman, Félix Hernández, Barbara Kasprzyk-Hordern, Christoph Ort, Alexander L. N. van Nuijs, Pim de Voogt and Ettore Zuccato

## 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 ($m^3$/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

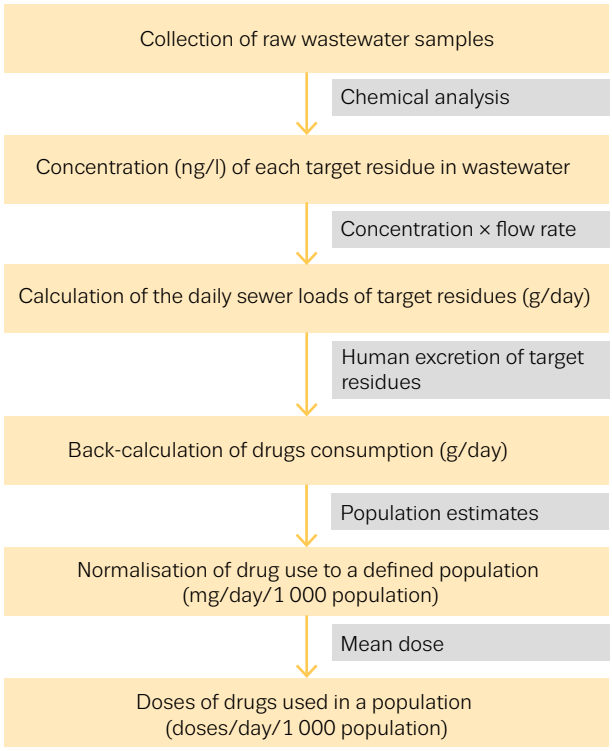
in wastewater and population size estimation is also discussed. In addition, new analytical techniques, such as the analysis of enantiomers of chiral compounds, which allows the amounts of drugs consumed to be distinguished from the amounts discharged in urban wastewater, are described.

## 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).

FIGURE 1.1

**The main consecutive steps of the wastewater analysis approach and the data required for each step**




NB: Modified from Castiglioni et al., 2014.

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).

### 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).

## 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.

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

TABLE 1.1

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

| Phase of the approach | Agreed procedures |
|---|---|
| **Sampling and sample handling** | *Sampling point*: wastewater treatment plant influent<br>*Sample type*: 24-hour flow-weighted composite<br>*Sampling container*: PET or glass container<br>*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<br>*After sampling — two possible options*:<br>1. Process the sample for analysis within 12 hours<br>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)<br>*Internal quality control*: use of labelled analytical standards for each compound<br>*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.

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

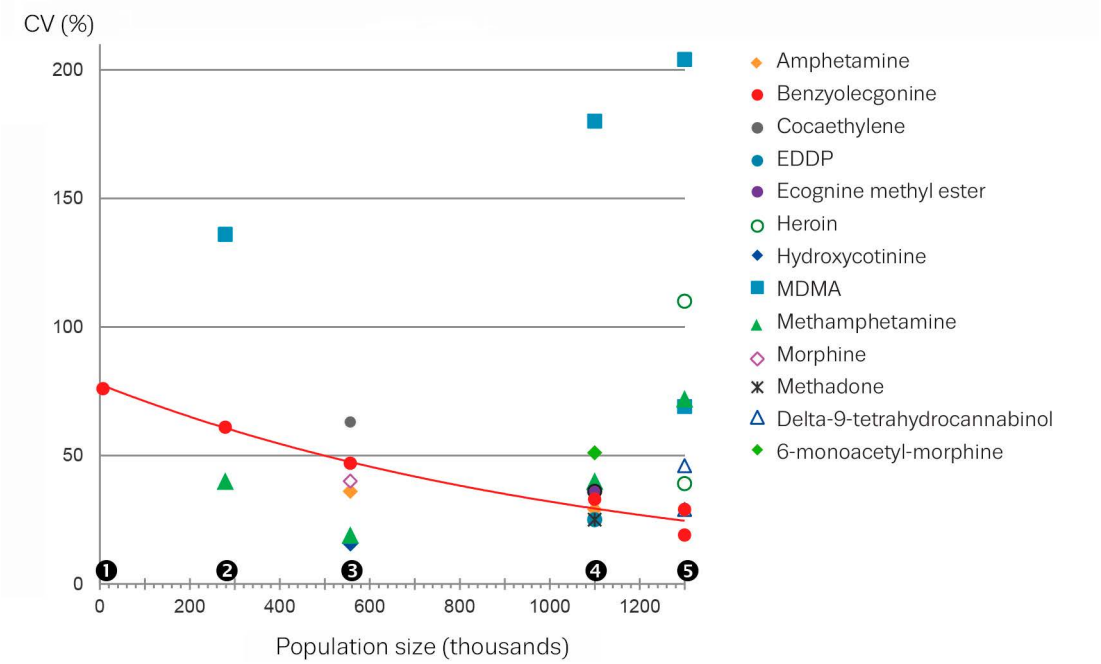
For various practical reasons, 24-hour composite samples of raw wastewater from the influent of wastewater treatment plants are normally collected (Ort, 2014). Thus, daily samples are the unit for analysis. Studies focusing on relatively large catchment areas and frequently used substances have concluded 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 benzoylecgonine, 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

FIGURE 1.2

**Variability of daily drug loads expressed as coefficients of variation (CV, standard deviation/mean) for five long-term studies**




NB: Population sizes (*P*) and the number of subsequent monitoring days (*d*) for the five studies were as follows: ❶ *P* = 7 160, *d* = 1 369; ❷ *P* = 278 000, *d* = 311; ❸ *P* = 557 000, *d* = 28; ❹ *P* = 1.1 million, *d* = 239; ❺ *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-methylenedioxymethamphetamine.

(*U*) can be calculated by $n = (CV/U)^2$. 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-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,

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).

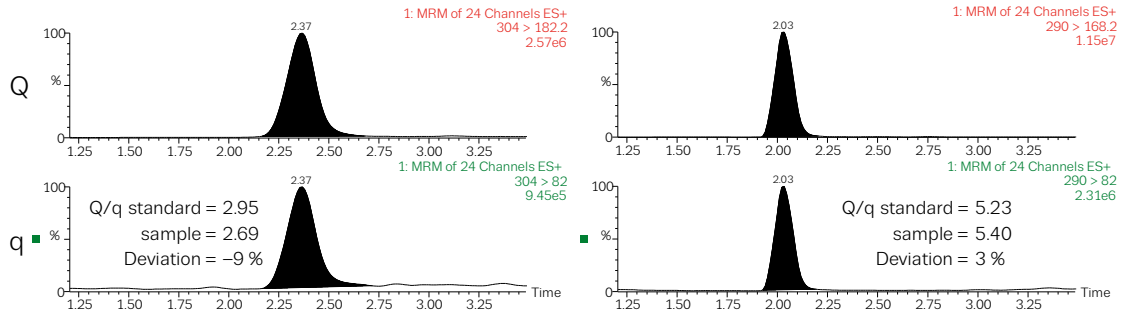
## Chemical analysis and quality control

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 main 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).

## Enantiomeric profiling of illicit drugs

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

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.

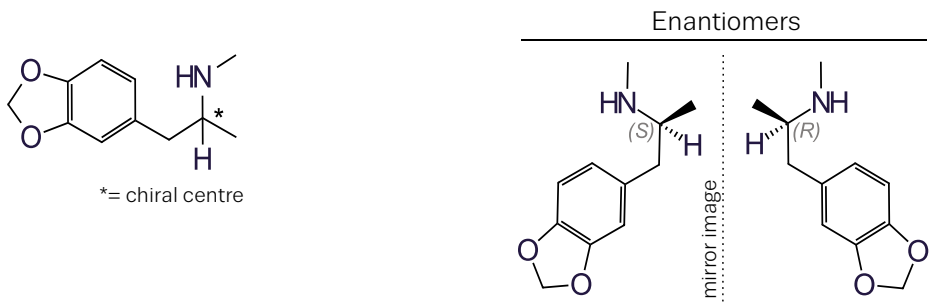
A chiral molecule usually has at least one chiral centre (e.g. an asymmetric carbon atom); as a result of this, it shows optical activity. Chiral molecules exist as two enantiomers (if only one chiral centre is present), which are non-superimposable mirror images of each other (Figure 1.4). Many of the 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 1*R*,2*S*(–)-ephedrine or 1*S*,2*S*(+)-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

FIGURE 1.4
**Enantiomers of 3,4-methylenedioxymethamphetamine (MDMA)**



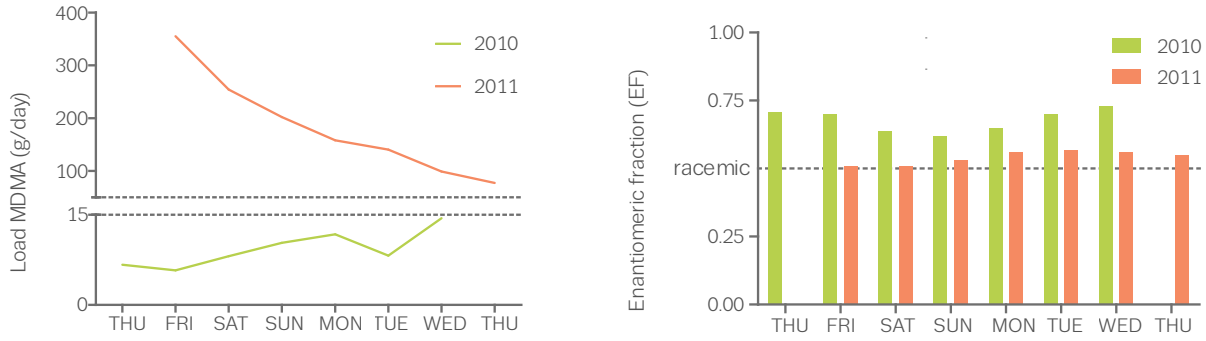
Leuckart route or various reductive amination reactions (Renton et al., 1993). All of these methods produce racemic MDMA. *S*(+)-MDMA is, however, metabolised in preference to *R*(–)-MDMA, which leads to the relative enrichment of the MDMA *R*(–)-enantiomer and the preferential formation of *S*(+)-3,4-methylenedioxy-amphetamine (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

**Summary of experiments used to assess the stability of the main illicit drugs and several metabolites (percentage change after incubation)**

| Reference | Time (hours) | Temperature (°C) | pH | Cocaine, % | Benzoylecgonine, % | Ecgonine, % | Amphetamine, % | Methamphetamine, % | MDMA, % | THC-COOH, % | 6-MAM, % |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Castiglioni et al., 2006 | 72 | 4 | 7.5 | –36 | 14 | NA | 5 | 0 | 1 | –8 | –14 |
| Gonzalez-Marino et al., 2010 | 24 | 4 | 7.5 | –7 | 7 | NA | 0 | NA | NA | 2 | NA |
| Bisceglia, 2010; Bisceglia and Lippa, 2014 | 12 | 23 | 7.4 | –50 | 10–14 | –40 | –15 | 0 | 0 | NA | –15 |
| Baker and Kasprzyk-Hordern, 2011 | 12 | 19 | 7.4 | –8 | 7 | NA | 47 | 8 | 1 | NA | –42 |
| Castiglioni et al., 2011 | 24 | 4 | 7.5 | –25 | 20 | –50 | NA | NA | NA | NA | NA |
| van Nuijs et al., 2012 | 12 | 20 | 7.5 | –40 | 6 | –20 | 3 | 2 | 3 | NA | –20 |
| Plosz et al., 2013 | 7 | 21 | 7.4 | –60 | 18 | –29 | NA | NA | NA | NA | NA |
| Thai et al., 2014 | 12 | 20 | 7.5 | –20 | 14 | NA | NA | 0 | 0 | NA | –25 |
| Chen et al., 2013 | 24 | 20 | 7.0 | –9 | NA | NA | NA | –5 | 1 | NA | –53 |
| Senta et al., 2014 | 24 | 20 | 7.5 | –35 | 15 | NA | –5 | –10 | –10 | 0 | –15 |

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, compising 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 advantegous 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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2

CHAPTER 2

# Target drug residues in wastewater

Sara Castiglioni and Emma Gracia Lor

## Introduction

Wastewater-based epidemiology relies on the chemical analysis of the metabolic residues of a certain substance in urban wastewater in order to determine the consumption of that substance in the contributing population. So far, this approach has been applied to estimate the consumption of the most widely used illicit drugs (Zuccato et al., 2008; van Nuijs et al., 2011a) and, more recently, alcohol (Reid et al., 2011) and nicotine (Castiglioni et al., 2015) by measuring specific 'target residues' in raw urban wastewater.

Humans come into contact with thousands of foreign chemicals, medicines and xenobiotics (substances foreign to the body) through intentional consumption and accidental exposure to environmental contaminants, as well as through food. These substances can be eliminated from the body after being chemically altered (metabolised), or they may be eliminated unchanged. The human body has several means available to eliminate xenobiotics rapidly, so that they do not accumulate in the tissues and cause harm. The ability of humans to metabolise drugs is a natural process that involves the same enzymatic pathways and transport systems as those used for the metabolism of 'normal' dietary constituents (Brunton et al., 2011). This metabolism consists of a number of biochemical processes, which may include oxidation, reduction, hydrolysis, hydration, conjugation, condensation and isomerisation, and makes a drug easier to excrete, normally as a result of its transformation into a polar form that is more readily excreted by the kidneys (in urine) and the liver (in bile). For many substances, metabolism occurs in two phases. Phase I reactions involve the formation of a new or modified functional group or cleavage (by oxidation, reduction or hydrolysis), while phase II reactions involve conjugation with an endogenous substance (e.g. glucuronic acid, sulphate ions or glycine).

Urinary excretion through the kidneys is the main route of excretion for a complex panel of metabolic products. Each substance can be excreted in a variety of different combinations: the main metabolite; a mixture of metabolites excreted in similar amounts; the main metabolite and several minor metabolites; a mixture of minor metabolites; an unchanged form; or conjugated metabolites. All of these excretion products enter urban wastewater through human urine. It is therefore feasible to select some substances ('target residues'), measure their levels using sophisticated and sensitive analytical techniques and back-calculate the amount of the corresponding parent substances ingested collectively by a community. This is the principle commonly applied to the estimation of illicit drug consumption by measuring selected 'target drug residues' in urban wastewater. Current research is focused on optimising the choice of these specific target residues in order to improve the reliability of the wastewater-based epidemiology approach (Khan and Nicell, 2011; Castiglioni et al., 2014). Moreover, the principles and specific guidelines developed for illicit drugs could be applied to a wide range of other substances.

This chapter collects and summarises the most recent guidelines for choosing target residues for wastewater-based epidemiology: it describes the main requirements of a target residue that allow it to be successfully employed as a biomarker of drug consumption, it provides an overview of the target drug residues currently employed for estimating illicit drug consumption, and it describes some novel suggestions for the refinement and standardisation of the choice of specific target residues.

## Target residue requirements for drug biomarkers

The selection of specific substances as target residues is not an easy task, since an ideal target drug residue should fulfil several specific requirements in order to ensure the reliability of back-calculated estimates. The

main requirements are that it should be excreted in consistent amounts in urine; it should be detectable in urban wastewater; it should be stable in wastewater; and its only source should be human excretion.

A target drug residue should be excreted in urine in amounts sufficient to ensure that it will be still detectable in urban wastewater after considerable dilution. The dilution factor of a target residue in urine or wastewater will vary according to the size of the population, the sewer system and the presence of satellite waters (i.e. rain water, river water) converging on the system. A 'typical' dilution factor for a medium-sized city would be in the range of 200- to 400-fold during dry weather conditions, but two to five times higher than this during rain events. Moreover, a stable daily per-capita excretion with low intra- and interindividual variability, and therefore a stable flow in wastewater, is also a desirable characteristic for a target drug residue. This can be evaluated from the consumption frequency of a substance and from its pharmacokinetic profile (if available), for example its excretion rate and plasma and urine half-lives; however, the interindividual excretion rate can vary and this largely depends on individuals' metabolism. In a sewer system collecting waste from thousands, or even millions, of people (i.e. cases in which wastewater-based epidemiology is typically applied), these differences are levelled out by the large number of people contributing to the waste; therefore, interindividual variations in excretion are likely to result in serious biases for only very small communities (< 10 000 population).

The analytical techniques used to measure target drug residues in wastewater should be specific and selective enough to guarantee the detection of a compound, even if it is present at only trace levels (in the low ng/l range). However, substances excreted at very low levels could be difficult to detect, and this should be checked carefully in advance. Unfortunately, urban wastewater is a complex matrix and analyses for specific substances can be affected by a high signal suppression, known as the 'matrix effect', because of a large number of components within a sample. Thus, the use of specific purification techniques and reference standards (as described in Chapter 1) should be evaluated when establishing an analytical method.

Another essential requirement for a target drug residue is that it should be stable in wastewater, during transport in the sewer system, during sampling and during analysis; stability was recently evaluated for several of the target drug residues currently used for wastewater-based epidemiology (see Chapter 1). The degradation of a substance in wastewater can easily occur as a result of the high microbial activity typically found in sewers. For instance, it was shown that metabolites excreted as glucuronide conjugates are completely transformed to the free forms by β-glucuronidase enzymes from faecal bacteria in raw wastewater (D'Ascenzo et al., 2003; Castiglioni et al., 2006). An example of this is morphine-3β-glucuronide, the excretion of which accounts for up to 38 % of a dose of heroin (Baselt, 2004), and which was found to completely revert to morphine within 24 hours of storage in wastewater (Castiglioni et al., 2006). The evaluation of the stability of substances in wastewater is crucial for choosing a useful target residue, as demonstrated for cocaine metabolites (Chapter 1). Several studies have demonstrated that of the two most abundant metabolites of cocaine, benzoylecgonine is more stable in wastewater than ecgonine methyl ester (Gonzalez-Marino et al., 2010; Baker and Kasprzyk-Hordern, 2011; Castiglioni et al., 2011; van Nuijs et al., 2012), with the latter showing losses of up to 40 % after 12 hours in wastewater (Bisceglia and Lippa, 2014). Some inconsistencies in the degradation rates of these two compounds were also observed in various studies, even within the same laboratory in which the same experimental conditions were used and only the wastewater used was different (Castiglioni et al., 2011). It was assumed that experimental set-ups and, particularly, the nature and composition of the wastewater substantially influence the biological degradation of these substances; thus, these parameters should be tested carefully in each specific case.

So far, stability experiments have been conducted only in laboratories in which 'real conditions' for temperature and sewage composition are simulated, because of the obvious difficulties with regard to performing such experiments under 'real' conditions. An alternative method is based on modelling studies, but only one such study has been performed (Plosz et al., 2013). Therefore, it is important that sewer experiments are designed and additional modelling studies are performed to investigate the in-pipe biotransformation of target residues and to confirm the current data.

Adsorption of compounds onto solid particulate matter is another phenomenon that can occur in wastewater and which can affect the stability of a substance. However, it was recently demonstrated that this is not an important factor in relation to illicit drugs because the percentage of such compounds adsorbed onto particulate matter is usually relatively low. For example, the adsorption of cocaine is less than 3.1 %, for benzoylecgonine adsorption is less than 0.5 %, for amphetamine it is less than 8.6 %, for MDMA it is less than 2.4 % and for methamphetamine it is less than

2.3 % (Baker and Kasprzyk-Hordern, 2011; Baker et al., 2012).

Finally, a target drug residue should be a product unique to human metabolism, and not a result of discharges from exogenous sources, which could lead to an overestimation of the final results. For instance, it was demonstrated that cocaine is not a suitable target drug residue for the estimation of cocaine consumption because its levels in wastewater are affected by factors other than consumption, such as trafficking of cocaine and handling (i.e. dumping) (van Nuijs et al., 2011a; Thomas et al., 2012). Recently, the profiles of nicotine and its main urinary metabolites, cotinine and *trans*-3'-hydroxycotinine, were evaluated in urban wastewater (Castiglioni et al., 2015). Nicotine mass loads were higher than expected for urinary excretion, and showed random variability during the sampling period. This may indicate that other sources of nicotine, such as the direct disposal of ash and washout from cigarette butts, may contribute to the amount of nicotine in wastewater. Therefore, nicotine itself cannot be used as a target residue for the estimation of nicotine consumption within a community.

## Target residues currently used for the back-calculation of drug consumption

After the selection of an appropriate target residue and its measurement in wastewater, specific correction factors are employed to back-calculate the consumption of the parent substance (see Chapter 1). These correction factors should be selected carefully in order to ensure the reliability of results. A brief overview of the target drug residues currently measured in urban wastewater and used to estimate illicit drug consumption is presented in Table 2.1. Usually, the correction factor for a given residue is based on the average proportion of the drug consumed that is

TABLE 2.1

**Overview of target drug residues measured in wastewater and the corresponding correction factors currently used for back-calculation of drug consumption**

| Drug | Target drug residue measured in wastewater | Percentage of excretion (mean selected) | Correction factor used for back-calculation | References |
|---|---|---|---|---|
| **Cocaine** | Benzoylecgonine | 45<br>35<br>32.5<br>29 | 2.3<br>3<br>3.2<br>3.59 | a,b,c,d,e,f,g,h<br>i,j,m<br>k<br>l |
| | Cocaine | 7.5 | 13 | i |
| **Amphetamine** | Amphetamine | 30 | 3.3 | a,c,e,f,g,h,j,k |
| **Methamphetamine** | Methamphetamine | 43<br>39<br>33 | 2.3<br>2.6<br>4.06 | a,e,f,h,j,k<br>i<br>m |
| **3,4-Methylenedioxy-methamphetamine (MDMA)** | MDMA | 65<br>26<br>20<br>15 | 1.5<br>3.9<br>5<br>6.7 | a,c,e,g,h<br>f<br>j,k<br>i |
| **Cannabis** | 11-*nor*-9-Carboxy-delta-9-tetrahydrocannabinol (THC-COOH) | 2.5<br>0.6 | 36.4<br>152 | f<br>a,c,e,g |
| **Codeine** | Codeine | 70 | 1.4 | c |
| **Heroin** | Morphine | 42 | 3.1 | a,c,e,h |
| | 6-Monoacetylmorphine | 1.3 | 86.9 | f,j |
| **Methadone** | Methadone | 27.5 | 3.6 | f |
| | 2-Ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP) | 30.9<br>13 | 3.4<br>6.3 | k<br>g |
| **Ephedrine** | Ephedrine | 75 | 1.3 | e,f,h |
| **Oxycodone** | Oxycodone | 14 | 221 | h |
| **Ketamine** | Ketamine<br>Norketamine | 30<br>1.6 | 3.3<br>65 | h<br>m |

(a) Zuccato et al., 2008; (b) van Nuijs et al., 2009; (c) Terzic et al., 2010; (d) Metcalfe et al., 2010; (e) Postigo et al., 2010; (f) Postigo et al., 2011; (g) Nefau et al., 2013; (h) Yargeau et al., 2014; (i) Lai et al., 2013a; (j) van Nuijs et al., 2011b; (k) Baker et al., 2012; (l) Castiglioni et al., 2013; (m) Lai et al., 2013b.

excreted in the form of that residue. It also takes into account the molecular mass ratio of the parent drug to the metabolite (Zuccato et al., 2008). As shown in Table 2.1, the target drug residues currently used are either the illicit drugs themselves (i.e. amphetamine, methamphetamine and MDMA) or metabolites of the drugs (i.e. benzoylecgonine for cocaine, 11-*nor*-9-carboxy-delta-9-tetrahydrocannabinol (THC-COOH) for cannabis, and morphine or 6-acetylmorphine for heroin).

### Cocaine

Most of the wastewater-based epidemiology studies available concern the estimation of cocaine consumption; benzoylecgonine is the most frequently used target residue in these studies because of its suitability as a biomarker, as discussed previously. However, different correction factors for the back-calculation of cocaine consumption have been employed in previous years, ranging from 2.3 to 3.2. More recently, a refined correction factor was proposed in order to standardise the back-calculation of cocaine use (Castiglioni et al., 2013). This was made possible by a thorough review of all the pharmacokinetic studies available in the literature and the development of a novel method of obtaining a refined correction factor (see Improved method to calculate correction factors, page 39, for detailed information).

### Amphetamine-type stimulants

The parent drug is used as the target residue for estimations of amphetamine-type stimulant use. For amphetamine, only one correction factor has been developed, while for methamphetamine and MDMA, correction factors range from 2.3 to 4.1 and from 1.5 to 6.7, respectively. In the case of MDMA, some new studies have been published in recent years which have allowed the revision of the previously used excretion percentages. Another promising tool for the differentiation of consumption and direct disposal of illicit substances is enantiomeric profile analysis (see 'Enantiomeric profiling of illicit drugs' in Chapter 1). These drugs are metabolised in an enantioselective manner in the human body; therefore, the enantiomeric profile in urine and wastewater after human consumption and metabolism is different from the profile that results from the direct disposal of the parent substance. In the case of substances for which the parent drug is directly measured in wastewater as the target residue, the presence of additional sources should be carefully assessed to avoid overestimation of drug use levels (van Nuijs et al., 2011a). For instance, amphetamine can also result from the metabolism of methamphetamine (up to 7 % of methamphetamine is excreted as amphetamine) and other illicit substances, such as the stimulant product fenethylline (27 % is excreted as amphetamine), the appetite suppressant fenproporex (38 % is excreted as amphetamine) (Baselt, 2004) and the anti-Parkinson drug selegiline (Maurer and Kraemer, 1992). Amphetamine can also be prescribed to treat specific disorders, such as attention deficit–hyperactivity disorder (Burgard et al., 2013). Similarly, methamphetamine is also a metabolic by-product of selegiline and of the analgesic and antipyretic agent famprofazone, and of the anorectic pharmaceutical benzphetamine (Maurer and Kraemer, 1992; Baselt, 2004).

### Cannabis

The main metabolite of cannabis, THC-COOH, is used as a target residue and, usually, a single correction factor (of 152) is used. Because of the relatively low percentage of cannabis that is excreted as THC-COOH — which results in a high correction factor and, thus, less precise estimates of consumption levels — specific studies should be performed to address the possibility of also using other metabolites, such as 11-hydroxy-delta-9-tetrahydrocannabinol (THC-OH), to estimate cannabis use levels. Nevertheless, there are some analytical challenges related to the chemical analysis of these metabolites, and the potential for these substances to partition to particulate matter, which could reduce their availability in wastewater, should also be investigated.

### Heroin

Morphine is the most abundant metabolite of heroin and can be used as a target residue to estimate heroin consumption; but morphine found in wastewater is also an indicator of the therapeutic use of morphine and codeine. Hence, when back-calculating heroin consumption based on wastewater morphine levels, correction factors that compensate for the contributions from therapeutic morphine and codeine must be applied (Zuccato et al., 2008). Alternatively, 6-acetylmorphine, a minor but exclusive metabolite of heroin (excretion rate 1.3 %; Baselt, 2004), could be used to estimate the consumption of this illicit drug; however, in general, only low concentrations of 6-acetylmorphine are found in wastewater and some degradation has been reported (van Nuijs et al., 2012).

### Other substances

Other substances have been included as target residues in a few studies (mostly only in one), as listed in Table 2.1, but, in general, only one correction factor is used to estimate their consumption.

This overview highlights that for a number of substances (cocaine, MDMA and methamphetamine) different correction factors have been used in the various studies carried out to date and these differences can limit the comparability of results between studies. Further research is therefore required to refine correction factors in order to harmonise and standardise the methods employed for the estimation of drug use. Several attempts to do this have been made in Europe by the SCORE group (Chapter 1) and some additional proposals are reported below.

## Improved method to calculate correction factors

It has been known for some time that most of the bias associated with correction factors is due to the limited number of pharmacokinetic studies available for illicit drugs and the small number of subjects involved in most of these studies (Zuccato et al., 2008). More recently, the need to conduct a comprehensive meta-analysis of metabolic disposition studies in order to construct excretion profiles for illicit drugs was suggested, and this has been implemented for some substances (Khan and Nicell, 2011, 2012).

This suggested approach was further improved in a subsequent study in which correction factors were refined by taking into account not only the excretion profiles resulting from different routes of administration, but also the number of subjects involved in each study and the frequency of use of a substance for each route of administration (Castiglioni et al., 2013). Cocaine was chosen for this preliminary study because its excretion profiles are relatively well described for the main routes of administration (i.e. intravenous, intranasal and oral administration, and through smoking). Since benzoylecgonine is the most reliable target drug residue for the back-calculation of cocaine consumption, all of the pharmacokinetic studies available in the literature that report data on the excretion profile of benzoylecgonine were reviewed.

Table 2.2 shows the mean percentage of benzoylecgonine, calculated for each route of administration by weighting the mean excretion of each study by the number of subjects included. The mean excretion profile of benzoylecgonine ranged from 14 % (when administered by smoking) to 55 % (oral administration) of an administered dose of cocaine depending on several factors, such as the route of administration, the habits of consumption, the amount of a dose and an individual's metabolism. Notable differences were observed among the different routes of administration, but also among the subjects treated through the same route of administration. All available information, with the exception of data from the very small study on oral cocaine use, was therefore used to revise the estimated excretion rate (taking into account the number of subjects in each study), which resulted in a mean excretion rate of 27 %, which was finally weighted by the frequency of cocaine use for the different routes of administration to obtain a mean of 29 %. Considering this excretion value, a new refined correction factor of 3.59, calculated considering the mean excretion rate and the molar mass ratio of the parent drug to its metabolite, as described elsewhere (Zuccato et al., 2008), was proposed.

TABLE 2.2

**Benzoylecgonine excretion (mean ± standard deviation) after different routes of administration of cocaine: summary of the data available in the literature**

| Route of administration | Number of studies | Number of subjects (range per study) | Mean excretion (%) [1] weighted by subjects (%) | Mean excretion (%) [2] | Mean excretion (%) weighted by route of administration [3] |
|---|---|---|---|---|---|
| **Intranasal** | 9 | 56 (2–7) | 29.4 ± 7.4 | 27.1 ± 11.4 | 29.2 ± 7.8 |
| **Intravenous** | 7 | 28 (1–7) | 37.3 ± 9.6 | | |
| **Smoking** | 3 | 20 (5–9) | 14.8 ± 5.8 | | |
| **Oral** | 1 | 2 | 55 ± 7.1 | | |

(1) Excretion of benzoylecgonine as a percentage of cocaine consumed.
(2) Oral administration was not considered in the analysis because it was used by only two subjects and was, therefore, a minor route of administration.
(3) Calculated by assuming the following pattern of consumption of cocaine: 95 % used intranasally, 2 % used intravenously and 4 % consumed by smoking (Prinzleve et al., 2004).

TABLE 2.3
**List of the main pharmacokinetic studies reporting the excretion profile of MDMA and the calculated mean percentages of excretion for each study**

| Dose | Subjects treated | Duration of the study (hours) | Mean excretion (%) | References |
|---|---|---|---|---|
| **50 mg** | 1 | 0–72 | 65 | Verebey et al., 1988 |
| **125 mg** | 1 | 0–24 | 30 | Ortuño et al., 1999 |
| **100 mg** | 4 | 0–24 | 15.0 | Segura et al., 2001 |
| **100 mg** | 6 | 0–24 | 23.9 | Pizarro et al., 2002 |
| **100 mg** | 7 | 0–72 | 22 | Pizarro et al., 2004 |
| **40 mg** | 8 | 0–24 | 33.1 | Fallon et al.,1999 |
| **1 mg/kg** | 5 | 0–120 | 13.7 | Abraham et al., 2009 |

TABLE 2.4
**MDMA metabolites excreted in human urine**

| Target residue | Mean excretion (%) |
|---|---|
| **3,4-Methylenedioxy-methamphetamine (MDMA)** | 13.4 |
| **3,4-Methylenedioxy-amphetamine (MDA)** | 1.1 |
| **4-Hydroxy-3-methoxymethamphetamine (HMMA)** | 9.6 |
| **4-Hydroxy-3-methoxyamphetamine (HMA)** | 0.9 |
| **3,4-Dihydroxy-methamphetamine (HHMA)** | 17.7 |

Since most of the correction factors currently used (Table 2.1) were developed several years ago, they should be revised to include the most recent information on pharmacokinetics, if available in the literature, and should adopt the new comprehensive methodologies of processing and analysing data. The methods proposed for cocaine could be applied to other substances, not only to update the currently used correction factors, but also to increase the panel of potential target drug residues.

New information can also be used to revise the correction factors used for the wastewater-based estimation of MDMA consumption, for which the drug itself is the target residue. MDMA has one main route of administration (oral) and the current correction factor (1.5) is based on a mean percentage excretion of 65 %; however, this excretion rate value can now be revised to take into account the most recent information on the drug's excretion profile and by applying the method described above. Considering this additional information, presented in Table 2.3, the revised mean percentage of excretion for MDMA is now estimated to be approximately 20 %, which is markedly different from the previous value of 65 %.

High loads of MDMA, which could not be explained solely by the consumption of this illicit drug by the specific community, were recently observed in a sewer system; these high loads may have resulted from the direct disposal of unused drugs or production waste from, for example, illegal manufacturing facilities (Emke et al., 2013). This makes the epidemiological estimation of community-wide drug use via wastewater analysis difficult and potentially unreliable; therefore, new approaches are required to distinguish between drugs in wastewater that result from consumption and those that result from the direct disposal of unused drugs. In this regard, one option that has been explored is the enantiomeric profiling of drugs in wastewater by chiral chromatography (see Chapter 1). Another potentially valid option is to search for target drug residues among urinary metabolites that can originate only from human consumption of a substance. A review of all of the MDMA metabolites excreted in human urine and reported in pharmacokinetic studies (Table 2.4) allowed us to identify a panel of metabolites (e.g. 4-hydroxy-3-methoxymethamphetamine (HMMA) and 3,4-dihydroxy-methamphetamine (HHMA)) that are excreted in percentages (> 10 %) sufficient for detecting target drug residues.

## Research needs and conclusions

The application of back-calculation approaches to the estimation of drug use relies on specific correction

factors, which mainly take into account the urinary metabolism of a substance. Unfortunately, human pharmacokinetic data are very scant for most of the main illicit drugs, and the available studies, in general, were not performed recently and are based on a small number of subjects. Some of these studies also considered doses that are lower than those commonly used by drug users and administered through atypical routes (Verstraete, 2013), while other studies included only one sex (e.g. males) or only one race (e.g Caucasian) (Bruno et al., 2014). Thus, the results from such studies are unlikey to reflect a typical, real-world situation in which both males and females are likely to consume a given drug, sometimes in multiple doses, via different routes or in conjunction with various other substances.

All of these factors could affect the metabolic profile of a drug and hence the accuracy of the excretion profile used to back-calculate consumption. The main limitation of this type of approach is related to the complexity of performing pharmacokinetic studies, as these require specific authorisations and adherence to strict ethical rules and can, therefore, be performed only in specific research centres. Despite this, there is an urgent need for new pharmacokinetic studies of illicit drugs. Such studies should include an adequate number of subjects and test realistic drug dosages using all of the main routes of administration. It would also be desirable to have data from studies on new psychoactive drugs for which human metabolism is mostly unknown.

Because of the difficulties associated with performing pharmacokinetic studies, an alternative technique has recently been suggested for the identification of the main metabolic products of illicit drugs; this technique involves using sub-cellular human liver models, such as pooled liver microsomes or heterologically expressed human enzymes (Meyer and Maurer, 2011). A few studies using these liver models have recently been conducted on some new psychoactive substances, such as the N-ethyl homologue of mephedrone, 4-methyl-*N*-ethyl-cathinone (4-MEC), which belongs to the beta-keto amphetamine (cathinone) group (Helfer et al., 2015), and the hallucinogenic designer drug 2,5-dimethoxy-4-propylphenethylamine (2C-P) of the phenethylamine class (Wink et al., 2014). The aim of these studies was to investigate the phase I and phase II metabolism of the selected substances in human urine, as well as in pooled human liver microsome incubations. The metabolites were identified by gas chromatography–mass spectrometry and by liquid chromatography–high-resolution tandem mass spectrometry. Based on the metabolites identified in urine or pooled human liver microsomes, several metabolic pathways were proposed for each substance.

It is vital that more information on the pharmacokinetics of illicit drugs is obtained in order to improve the reliability of wastewater-based epidemiology; thus, the use of sub-cellular models is likely to be a valuable alternative tool to 'classical' pharmacokinetic studies. Nonetheless, it is also very important that new pharmacokinetic studies, able to reflect 'real situations' of drug consumption within a population, are performed. Finally, until further pharmacokinetic information is available, it is highly recommended that reliable correction factors, based on meta-analyses of all available data and determined in accordance with the methodologies recently proposed, are used.

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3

CHAPTER 3

# A global overview of wastewater-based epidemiology

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## Introduction

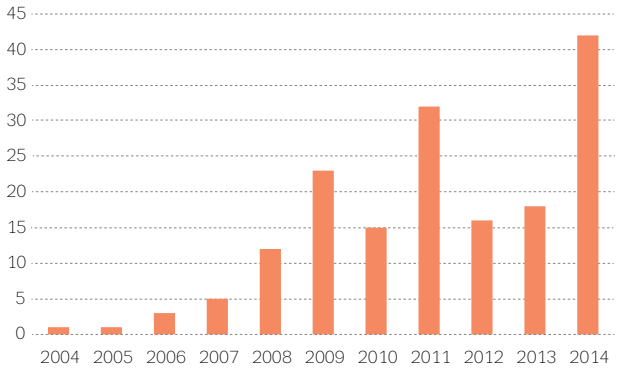
Wastewater-based epidemiology is considered to be a powerful approach for monitoring patterns and trends of illicit drug use within a community (van Nuijs et al., 2011a). Since 2005, this approach has been applied in several countries worldwide (Castiglioni et al., 2014). The number of research groups working in this field is growing continuously and the available knowledge has enormously improved. Figure 3.1 shows how the number of publications related to wastewater analysis has increased progressively since 2004.

Spatial and temporal patterns of use of the main illicit drugs (cocaine, cannabis, amphetamine, methamphetamine and MDMA) have been evaluated in urban areas, including several main cities and megalopolises, as well as in rural areas and during special events. These assessments have highlighted some common profiles of use, such as the weekly patterns of consumption, but also notable geographical differences in the consumption of specific substances. To date, wastewater analysis has mostly been used to estimate local consumption in major cities (see the Appendix), but it has also been applied on larger scales to allow the identification of differences in cocaine use in large and small cities in Belgium (van Nuijs et al., 2009); in cocaine, methamphetamine and MDMA use in urban and rural areas in Oregon, United States (Banta-Green et al., 2009); and in cocaine, MDMA, amphetamine and cannabis use in 25 cities in France (Nefau et al., 2013). Moreover, a European study was performed in 2011 and showed, for the first time, the distinct spatial patterns of drug use across 19 European cities; the results of this study were, in general, in good agreement with officially reported prevalence data (Thomas et al., 2012) (see below for a more detailed description). An increase in methamphetamine consumption at weekends was found by 1-month monitoring campaigns in Oslo, Norway (Reid et al., 2011a), and in Adelaide, Australia (Irvine et al., 2011).

The monitoring of drug use though wastewater analysis also allowed changes in drug use over time to be tracked, and new drug use patterns to be identified. For example, a wastewater analysis study conducted in the north of Italy between 2008 and 2009 (Zuccato et al., 2011) demonstrated a marked decrease in cocaine and heroin use in two cities during this time; this decrease was subsequently confirmed in 2012 by national epidemiological surveys (DPA, 2012). Other changing patterns of use have been observed in Australia as a result of wastewater analysis. For example, a decrease in cocaine consumption was observed in Queensland between 2009 and 2010 (Prichard et al., 2012), and a simultaneous decrease in MDMA use was also identified in Adelaide (Chen et al., 2011, 2013). In addition, some differences in the use of cocaine (increase) and methamphetamine (decrease) were recently found in the United States compared with the use levels estimated by previous studies on these drugs (Subedi and Kannan, 2014). The temporal patterns of consumption evaluated by a 4-year monitoring campaign in about 20 European cities were reported recently (EMCDDA, 2015); the results of this campaign are described in detail below (see next paragraph).

FIGURE 3.1

**The number of publications (combined PubMed search) related to wastewater-based epidemiology per year since 2004**



Wastewater analysis has also been applied successfully on a small scale to assess drug use in specific populations, such as in prisons (Postigo et al., 2011; Brewer et al., 2014), in schools (Panawennage et al., 2011; Burgard et al., 2013), in an airport (Bijlsma et al., 2012) and in different districts within a city (Reid et al., 2011b). It was also used to study fluctuations in drug consumption, mostly increases in cocaine and MDMA use, during special holidays or in vacation areas or holiday resorts (Reid et al., 2011b; van Nuijs et al., 2011b; Lai et al., 2013a) and during special music or sporting events (Bijlsma et al., 2009; Gerrity et al., 2011; Lai et al., 2013c).

The numerous studies available in the literature on wastewater analysis confirm the potential of this approach for monitoring the temporal and spatial trends of drug use on different scales (i.e. local, national and international). The particular pattern of drug use may also be studied at specific sites, such as rural or vacation areas, at different times of the year and during special events, in order to provide information to complement epidemiological surveys that normally collect information about the previous month or year, or the lifetime prevalence of use. Several studies have also shown that wastewater analysis is able to provide repeated estimates of drug use and that the approach can be used to quickly identify changing patterns of use.

This chapter provides an overview and comparison of the results obtained by applying wastewater-based epidemiology in Europe, the United States, Canada, Australia and Asia.

## A European collaboration: results of a 4-year long monitoring campaign

In 2010, a Europe-wide network (Sewage Analysis CORe group – SCORE) was set up with the aims of standardising the methodologies used for wastewater analysis and coordinating international studies. The first coordinated monitoring study was performed in March 2011, whereby wastewater analysis was simultaneously applied in 19 European cities over a 1-week period (Thomas et al., 2012). Within this study, a common protocol of action (best-practice protocol), as described in Chapter 1, was applied for the first time. Urinary target residues of cocaine, amphetamine, MDMA, methamphetamine and cannabis were measured by different laboratories using in-house optimised and validated analytical methods. This international study provided the first ever comparative information on the regional differences in illicit drug use in Europe based upon wastewater analysis. Additional studies were performed in 2012, 2013 and 2014 on an increasing number of cities (23, 42 and 50, respectively), each year with an intercalibration exercise (Ort et al., 2014; EMCDDA, 2015).

This project revealed distinct geographical and temporal patterns of drug use across European cities. A general description of the study findings is provided in this chapter, and detailed figures are presented in EMCDDA (2015).

Although substantial fluctuations in individual cities were observed, the general geographical patterns of drug use were relatively stable over the four years (2011–2014). Cocaine use, estimated by measuring benzoylecgonine loads, as described in Chapter 2, was highest in cities in the west and south of Europe and lowest in cities in the north and east (Figure 3.2). Methamphetamine consumption was highest in the Czech Republic, Slovakia and northern Europe, while in all other countries, methamphetamine use seemed to be very low or even negligible. For amphetamine, the highest loads were detected in northern and north-western European cities.

Relatively low levels of urinary biomarker loads related to MDMA were found in most of the European countries studied. For 2011–2013, the highest loads by far were detected in Belgian and Dutch cities. In 2014, London and Oslo also reported high loads of MDMA in wastewater.

With regard to cannabis, the quantification of THC-COOH (11-*nor*-9-carboxy-delta-9-tetrahydrocannabinol) loads in wastewater poses some analytical challenges, and, as a result, not all samples were analysed for this metabolite of THC. Therefore, in contrast to the other illicit drugs under investigation, it was not possible to establish regional patterns for cannabis use.

The study also highlighted differences among cities within the same country, which could be explained in part by the different social and demographic characteristics of different cities (e.g. whether or not they have universities or nightlife areas, and the age distribution of the population). In the majority of countries with multiple study locations, cocaine and MDMA loads were generally higher in large cities than in towns. No such differences were detected for amphetamine or methamphetamine loads.

In addition to geographical patterns, wastewater analysis can detect fluctuations in weekly patterns of

FIGURE 3.2

**Loads of benzoylecgonine and methamphetamine (mg/day/1 000 population) in the European cities included in a 4-year investigation (2011–2014)**

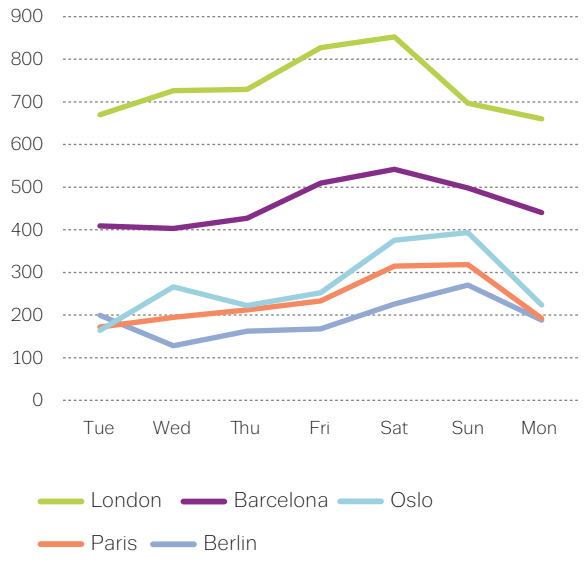

NB: This figure includes cities for which the benzoylecgonine and methamphetamine loads were measured at least three times out of four.
Sources: Thomas et al., 2012; Ort et al., 2014; EMCDDA, 2015.

illicit drug use. In the majority of cities, higher loads of benzoylecgonine and MDMA were detected on Saturdays and Sundays than on weekdays (Figure 3.3). In contrast, cannabis and methamphetamine use were found to be distributed more evenly over the whole week.

The results delivered by the SCORE project were consistent with standard monitoring data, demonstrating that wastewater-based epidemiology can be successfully applied to the assessment and comparison of the use of illicit substances at local and international levels, and to the detection of changes in the use of a substance. The results were generally in good agreement with officially reported national prevalence data for Europe (EMCDDA, 2010). Nevertheless, some limitations should be noted in order both to improve future studies at the international level and to acknowledge the caution that must be applied when comparing results from different sources. Firstly, the ranking of the city-based estimates reported in this study would not agree with

FIGURE 3.3

**Loads of benzoylecgonine (mg/day/1 000 population) in a selection of European cities (in 2014)**




Source: EMCDDA, 2015.

national-based estimates, because of the differences in demographics. The wastewater studies mainly included one or two cities per country, often chosen from the main cities, while most available standard drug epidemiological data reflect national levels of drug use. According to the epidemiological literature, urban areas tend to have a higher prevalence of drug use than rural areas. For that reason, it is difficult to extrapolate city-based estimates to the national level and, therefore, specific criteria should be taken into account (Ort et al., 2014).

The 2013 SCORE study covered a population of 25 million people (about 5 % of the EU population); however, in addition to the problems discussed with extrapolating estimates to national levels, it is also difficult to extrapolate these results to the whole of Europe. Finally, this study used 1-week sampling periods for each year, which were assumed to indicate the pattern of use for the entire year; however, whether or not this sampling strategy is sufficient to obtain reliable annual estimates, considering potential variation due to time of year and special events, remains to be verified. In fact, some drugs may display seasonal or event-related variation which might only be captured by longer and specifically designed sampling campaigns. Future monitoring campaigns should therefore include more cities with varying demographics within a country, and evaluate monitoring design strategies to find an optimum with feasible logistics, economic effort, sufficient quality control and representativeness for an entire year and an entire country. An additional confounding factor which should be considered in future studies is the presence of dumping or discharge from production laboratories. This was particularly apparent for amphetamine and MDMA in the Netherlands (and to some extent in Belgium) (Thomas et al., 2012; Emke et al., 2014; EMCDDA, 2015), and some methods to overcome this limitation have been suggested (see 'Enantiomeric profiling of illicit drugs' in Chapter 1).

Some studies have been conducted to evaluate changes in drug use during special events or holiday periods. The first of these studies was published in 2009 and reported increases in benzoylecgonine and MDMA during a major music event in Spain (Bijlsma et al., 2009). In Norway, increases in the cocaine flow in sewage were observed during a weekend that included a national day of celebration in Oslo and also during the closing party weekend at a Norwegian ski resort, compared with regular weekends (Reid et al., 2011b). A 1-year study conducted in Brussels, Belgium, on the main illicit drugs highlighted interesting patterns of use: cocaine, amphetamine and MDMA use levels were significantly higher during the New Year holiday than during other periods throughout the year (van Nuijs et al., 2011b).

The first study to evaluate the use and trends of use of drugs in a prison through wastewater analysis was conducted in Spain (Postigo et al., 2011). Daily use of cannabis and cocaine was detected, while heroin, amphetamine, methamphetamine and ecstasy use was detected only sporadically. Some other substances, such as methadone, used to treat heroin dependence, the benzodiazepine alprazolam and ephedrine, were also found in all samples tested. This study demonstrated for the first time that wastewater-based epidemiology can provide 'near-real-time' information on collective drug use in an anonymous way in small communities.

## Wastewater-based epidemiology in the United States and Canada

### Estimation of illicit drug use in the United States

The first ever measurements of illicit drugs (methamphetamine and MDMA) in urban wastewater were performed on the effluents of three wastewater treatment plants in Nevada, Utah and South Carolina (Jones-Lepp et al., 2004), where these substances were found at very low concentrations (about 1 ng/l). Later, in 2009, methamphetamine was detected at higher concentrations in the effluent from one wastewater treatment plant in Nevada (350 ng/l) and in several rivers upstream and downstream of this wastewater treatment plant at concentrations ranging from 1 to 60 ng/l (Bartelt-Hunt et al., 2009). The first study to use wastewater-based epidemiology was performed during the same period in seven different wastewater treatment plants in the United States, and several classes of illicit drugs (cocaine, amphetamines, opioids, ketamine and LSD (lysergic acid diethylamide)) were measured in influent wastewater (Chiaia et al., 2008). Methamphetamine loads were the highest yet reported, while cocaine loads were similar to those observed in western European locations. Ketamine was detected at very low levels, indicating sporadic use. One of the most comprehensive studies performed to date to evaluate the spatial epidemiology of illicit drugs was conducted in the state of Oregon, where 96 municipalities, representing 65 % of the population, were investigated with regard to cocaine, methamphetamine and MDMA use (Banta-Green et al., 2009). Benzoylecgonine loads, which indicate cocaine use, were significantly higher in urban than in rural areas and were, in fact, below the level of detection in many rural areas. Conversely, methamphetamine was present in all municipalities,

whether rural or urban, and MDMA was found in less than half of the communities, with a trend towards higher loads in more urban areas. The distribution of the wastewater-derived drug loads corresponded with expected epidemiological drug patterns; thus, this study provides evidence for the utility of the wastewater approach for spatial analyses and shows its potential to improve the existing estimates of the level and geographical distribution of drug use (Banta-Green et al., 2009).

A recent study conducted in the Albany area, New York, found evidence of comparatively high levels of use of cocaine, amphetamine and MDMA (Subedi and Kannan, 2014). Cocaine use estimates (mean benzoylecgonine loads of 2 315 mg/day/1 000 population) were four times higher than those reported previously for other states (Chiaia et al., 2008) and two times higher than the mean values reported for Europe (Ort et al., 2014). The amphetamine and MDMA loads reported by this study were, in general, higher than those reported previously for other parts of the United States and in Europe. In particular, amphetamine loads (mean of 244 mg/day/1 000 population) were two and eight times higher than the mean loads found in the United States and Europe, respectively, and MDMA loads (mean of 52 mg/day/1 000 population) were four and two times higher than the mean loads found in the United States and Europe, respectively. In contrast, methamphetamine loads were much lower than those found in other parts of the United States in 2009 (8.6 versus 427 mg/day/1 000 population) and those reported recently as European mean values (25 mg/day/1 000 population).

Some interesting studies of wastewater-based epidemiology have been conducted in the United States, for example to identify a correlation between major sporting events and illicit drug use (Gerrity et al., 2011) and to assess the pattern of drug use in small communities, such as schools (Panawennage et al., 2011; Burgard et al., 2013) and prisons (Brewer et al., 2014).

Gerrity et al. (2011) measured the content of illicit drugs in wastewater during the weekend of the National Football League's Super Bowl and found that the only changes relative to a baseline weekend were in the concentrations of methamphetamine and MDMA, which showed a slight increase.

In another study, the use of the main illicit drugs (cocaine, amphetamines and cannabis) by a student population was monitored by wastewater analysis during a normal class session, the final exam period and the summer break (Panawennage et al., 2011). The concentrations of these substances detected confirmed the expected trend: substance levels were generally highest during the final exam period, medium during a normal class session and mostly below the limits of detection during the summer. These results indicate a possible increase in the use of psychoactive substances during periods of high stress. This was confirmed in a second study conducted on a college campus in which amphetamine and ritalinic acid, contained in medications used to treat attention deficit–hyperactivity disorder, were monitored during low-stress and high-stress periods. Amphetamine use increased during periods of high stress, such as during the mid-term exam period, during the final week of classes and during the final exam period, when the highest peak of amphetamine use over baseline (760 %) occurred (Burgard et al., 2013).

Wastewater analysis was also used to monitor cocaine and methamphetamine use in a prison in the United States (Brewer et al., 2014) and the results indicate that this approach can provide information regarding the frequency of use of illicit drugs that cannot be obtained by conventional approaches, such as random urine analysis. In fact, methamphetamine was detected in all investigated wastewater samples, whereas the drug was detected only in 6 out of 243 tested urine samples. However, the wastewater study may be biased, as it was unable to differentiate between methamphetamine originating from inmates and that from employees and visitors; therefore, this should be evaluated in future studies. Cocaine was not found in any of the wastewater samples from this prison. This study also showed a different pattern of drug use from a previous Spanish prison study in which cocaine and benzoylecgonine were quantified in all daily samples; in this study, methamphetamine was infrequently detected (Postigo et al., 2011).

### Estimation of illicit drug use in Canada

Wastewater-based epidemiology was applied in Canada in two successive studies (Metcalfe et al., 2010; Yargeau et al., 2014), which were conducted in three and two Canadian cities, respectively. The size of the cities ranged from 1.6 million inhabitants (a large city in Canada) to 75 000 inhabitants (a small community). The results of the two studies were consistent, and cocaine was found to be the most used illicit substance tested, with the highest levels of consumption in the largest city. The highest levels of methamphetamine use were also detected in the largest city, while the use of amphetamine and MDMA was similar in large and small cities. Generally, the community drug consumption

values obtained in this study were more similar to values reported in Europe than to those obtained in the nearby United States. MDMA use peaked at weekends, as observed in almost all European studies. Ketamine was also detected in wastewater from the largest city (Yargeau et al., 2014) at levels similar to those previously detected in the United States (Chiaia et al., 2008); these findings indicate a potential problem related to the abuse of this veterinary anaesthetic in North America.

## Wastewater-based epidemiology in Australia

Wastewater-based epidemiology was first used in Australia between April and October 2009 in the state of South Australia to analyse wastewater from a number of metropolitan (population served of 150 000–800 000) and regional (population served of 400–23 000) treatment plants (Irvine et al., 2011). This study suggested a different pattern of drug use from that estimated in Europe, the United States and Canada. The use of cocaine was found to be much lower, and benzoylecgonine loads ranged from 5 to 10 mg/day/1 000 population. In contrast, MDMA and methamphetamine use were similar to the use levels observed in other countries, with loads ranging from 15 to 30 mg/day/1 000 population and from 40 to 65 mg/day/1 000 population, respectively. In Australia, MDMA was more popular in rural areas, whereas methamphetamine and cocaine were mainly consumed in metropolitan areas; this is slightly different from the profiles observed in the United States, where methamphetamine use was widespread in rural and urban areas (Banta-Green et al., 2009). As observed in other countries, the use of these substances increased at weekends, with the highest increases found for MDMA (a five-fold increase at the weekend compared with weekdays).

This finding agrees with data from the 2009 *World Drug Report* (UNODC, 2009) showing that use of MDMA and methamphetamine was higher in Australia than in all the other countries during the period investigated. However, the consumption of MDMA and methamphetamine described by the Australian study was 10 to 30 times higher than in some European cities (i.e. Milan and London; Zuccato et al., 2008), and was more similar to the profiles found in northern and eastern Europe (Ort et al., 2014). In addition, survey data (UNODC, 2009) suggest a similar use of cocaine in Europe and Oceania, while the Irvine et al. (2011) study showed a different picture.

Another survey was conducted in South-East Queensland and reported similar results for MDMA and methamphetamine, but benzoylecgonine loads were five to six times higher (Lai et al., 2011). This may suggest the presence of different geographical patterns of cocaine use within Australia, as indicated by the differences between South Australia and South-East Queensland. Cannabis consumption was investigated for the first time in this study and it was found to be the most-used substance. In general, the rank order of illicit drug consumption in Australia was cannabis followed by methamphetamine, cocaine, then MDMA. Shortly after this initial study, another study was conducted in the same area to assess the temporal trends of drug consumption, and two sampling campaigns were performed in November 2009 and 2010 in the same wastewater treatment plant (Prichard et al., 2012). The results indicated notable changes in the levels of drugs identified in the two sampling periods. The average load of cocaine (estimated from benzoylecgonine loads) in 2009 (221 mg/day/1 000 population) was more than four times higher than the average load measured in 2010 (52 mg/day/1 000 population); thus, a significant decline in benzoylecgonine loads was observed. Conversely, methamphetamine loads increased in the same period from 158 to 228 mg/day/1 000 population. No changes were observed for MDMA loads (about 135 mg/day/1 000 population).

One of the first studies to detect the use of new synthetic stimulants, cathinones and piperazines, through wastewater analysis was also performed in Australia (Chen et al., 2013). Wastewater samples were collected from multiple wastewater treatment plants in Adelaide in a 3-year monitoring campaign (2009–2011). MDMA was also included in this study as it was one of the most 'popular' synthetic drugs used in the years prior to the campaign. Firstly, a large decrease in MDMA levels was observed from 2009 to 2010; the levels then remained stable and relatively low in 2011. Methcathinone was found in all wastewater treatment plants and in all the years investigated, indicating a widespread and constant use of this substance. The other investigated drugs (mephedrone, methylone, methylenedioxypyrovalerone (MDPV), benzylpiperazine (BZP) and 3-trifluoromethylphenylpiperazine (TFMPP)) showed local increases, mainly in 2011, although mephedrone use levels had already increased in 2010. These results suggest that the decline in MDMA use may have been associated with an increase in the use of a number of other synthetic stimulants. However, the highly regionalised use of all of these substances and the delay between the decrease in MDMA use and the increase in the use of other substances indicates that

there was not a direct population-wide substitution of MDMA (Chen et al., 2013).

Wastewater analysis was also used in Australia to study changes in drug use during specific periods of the year (i.e. important annual holidays) and events (i.e. a music festival). In the first case, an urban area, a semi-rural area and an island popular for vacations were investigated (Lai et al., 2013a). In the semi-rural area, drug consumption was generally low and a decrease in cannabis use was found during holidays. In the urban area, the consumption of all drugs, especially cannabis and cocaine, increased during holidays. In the vacation area (the island), the consumption of cocaine, methamphetamine and MDMA markedly increased during holiday periods, but cannabis use declined. In the second case, the same music festival was monitored for two consecutive years (2010 and 2011) to assess the use of conventional illicit drugs (cannabis, cocaine, methamphetamine and MDMA) and emerging illicit psychostimulants (benzylpiperazine, mephedrone and methylone) (Lai et al., 2013c). The first group of substances was found in all samples taken and their use was found to be generally stable over the two festival years, apart from a decrease in methamphetamine use. The second group of substances was found only on specific days and no defined trends of use could be identified. The use of conventional drugs in a nearby urban community was also monitored and compared with the use at the music festival. MDMA was the only substance for which use was higher at the festival than in the nearby community (Lai et al., 2013c).

## Wastewater-based epidemiology in Asia

The levels of illicit substances were assessed, for the first time, in wastewater treatment plant effluents and surface water in Asia in Taipei, Taiwan, by Lin et al. (2010). Morphine, codeine, methamphetamines and ketamine were observed in significant quantities (up to hundreds of ng/l) in hospital effluents, wastewater treatment plant effluents and in river waters.

Since this initial study, few studies have been conducted to assess drug use in Asia by using wastewater-based epidemiology. The first of these was a pilot study in which Hong Kong's largest wastewater treatment plant, serving a community of approximately 3.5 million people, was analysed for cocaine, ketamine, methamphetamine and MDMA (Lai et al., 2013b). The overall drug use pattern detected was as follows: ketamine was the most used substance with loads ranging from 270 to 300 mg/day/1 000 population as the parent compound, and 20 mg/day/1 000 population as norketamine (the urinary metabolite of ketamine); the second most used drug was methamphetamine (60–70 mg/day/1 000 population), followed closely by cocaine (50–60 mg of benzoylecgonine/day/1 000 population). In contrast to what has been observed in all other countries investigated, MDMA was not detected in this study.

Wastewater-based epidemiology was also applied to assess the levels of ten illicit drugs in several wastewater treatment plants in four Chinese megacities (Beijing, Guangzhou, Shenzhen and Shangai) (Khan et al., 2014). The results obtained demonstrate, in a quantitative way, that the drug use patterns of Chinese people are different from those of Europeans, North Americans and Australians. In fact, the use of cocaine, which in some cases was not detected (i.e. Beijing and parts of Shangai), and MDMA appeared to be much lower in China than in Europe, the United States and Australia, with median loads of 5.6 and 1.5 mg/day/1 000 population, respectively. In Chinese megacities, much higher loads were found for methamphetamine (median 109 mg/day/1 000 population), ketamine (median 230 mg/day/1 000 population) and amphetamine (median 42 mg/day/1 000 population). The other substances investigated, cannabis, heroin and new psychoactive substances (mephedrone and methylenedioxypyrovalerone), were not found, which indicates their very low or non-existent use. The use of most of the drugs detected showed a geographical trend, with a much higher use observed in the southern (Shenzhen and Guangzhou) than in the northern cities (Beijing and Shanghai). The results observed for the southern cities were also very similar to those previously reported for Hong Kong (Lai et al., 2013b). The overall results were largely consistent with trends reported by the United Nations Office on Drugs and Crime (UNODC, 2013).

Different areas of Beijing were further investigated with regard to amphetamine and methamphetamine use (Li et al., 2014). Methamphetamine loads ranged from 4 to 230 mg/day/1 000 population and were similar to those previously reported by Khan et al. (2014). Amphetamine loads were low (0.4–13 mg/day/1 000 population) and this confirmed the hypothesis that, in Beijing, amphetamine comes mainly from methamphetamine metabolism, while the use levels of amphetamine itself are very low. Different patterns of use were observed for different areas of the city: methamphetamine loads were higher in the centre of the urban area, indicating a correlation with economic factors and the availability of

entertainment activities. This study also evaluated seasonal differences and showed that methamphetamine loads were higher in summer than in winter. A difference between use levels on weekdays and at weekends was also observed in winter. Methamphetamine consumption in five South Korean cities, in which the estimated average consumption during a Christmas and New Year period was 22 mg/day/1 000 population (Kim et al., 2015), was lower than the consumption levels observed in Australia, Hong Kong and China.

## Concluding remarks

This chapter presents an overview of the available results of studies carried out worldwide using wastewater-based epidemiology. The approach highlights interesting differences in drug use on different geographical scales. For instance, in Europe, cocaine was found to be mostly used in southern and western cities, whereas methamphetamine and amphetamine were mostly used in northern and central Europe. On the international scale, the use of cocaine in the United States was found to be similar to that in parts of Europe, while the use of amphetamine and methamphetamine was generally higher in the United States than in Europe. In Australia and Asia, the patterns of use were different from those in Europe and the United States: a lower use of cocaine was found in all studies, while the use of methamphetamine and amphetamine was generally similar to use in the United States, but lower than the use in Europe. The use of the recreational drug MDMA was steady over time in Europe and the United States, but decreased between 2009 and 2010 in Australia and was non-existent in Asia. Conversely, another recreational drug, ketamine, was found to be more highly consumed in Asia than in Europe and the United States. Finally, the use of cannabis was monitored in few countries, using its main metabolite (THC-COOH), but it was recorded in almost all of those countries as being among the most highly used substances, except in Asia, where THC-COOH was not found.

The patterns of drug use determined using wastewater-based epidemiology in the different countries were generally in agreement with data from epidemiological monitoring, as reported by EMCDDA for Europe and the UNODC for other continents. The wastewater-based studies were also able to identify local and temporal patterns of drug use, thus highlighting the high potential of the approach to provide additional complementary information to that obtained from conventional tools.

However, trying to compare results from different wastewater-based studies can be particularly challenging if non-homogeneous data are reported and this overview of results presented some examples. For instance, in most of the studies, the results were reported as loads per day of a target substance (e.g. benzoylecgonine for the assessment of cocaine use) and they were then normalised to 1 000 population; but, in some cases, the loads were transformed into the consumed amount of the parent substance (e.g. cocaine calculated from benzoylecgonine loads using specific correction factors) or they were converted into doses/day/1 000 population. Moreover, it was observed that, in some cases, different conversion factors had been used to back-calculate the consumption of a substance, as discussed in Chapter 2. Because of the enormous potential of this approach to provide rapid, objective and up-to-date information on the use of illicit drugs at local, national and international scales, it is imperative that a best-practice protocol is adopted in order to improve the reliability and comparability of results.

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4

CHAPTER 4

# New psychoactive substances: analysis and site-specific testing

Malcolm Reid and Kevin Thomas

## Introduction

The term 'new psychoactive substances' refers to chemical entities that produce effects similar to those produced by illicit substances, but that are not directly controlled by international conventions (specifically, the 1961 United Nations Single Convention on Narcotic Drugs and the 1971 United Nations Convention on Psychotropic Substances). These substances are often not newly developed from a scientific perspective, but are newly available as products on the (illicit) drugs market. To date, more than 450 such compounds have been reported to the EMCDDA, Europol and the European Medicines Agency (EMA) since the establishment of the 'Action on new drugs' via the Council of the European Union (Decision 2005/387/JHA, May 2005) on the information exchange, risk assessment and control of new psychoactive substances (EMCDDA, 2015). However, the potential number of compounds that could fit into this category is limited by only the imagination of synthetic chemists and their ability to side-step legislation. The new psychoactive substance market is, in this respect, very dynamic and adaptation to changes in the legislation plays a role in these dynamics. During 2014, 101 new psychoactive substances were reported to the EU Early Warning System (EWS) for the first time.

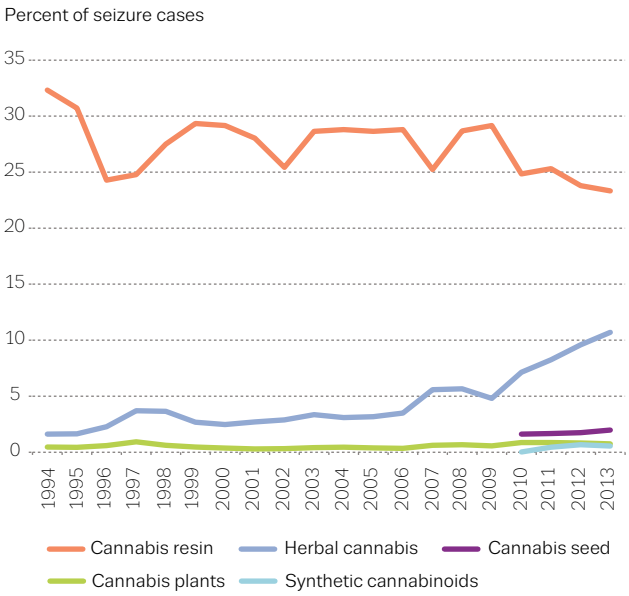
The development, management and amendment of effective drug policies relies heavily on the availability of accurate and timely information on the drug situation. That is to say, information is required on exactly what drugs are being produced, transported and used (and in what quantities). In Europe, this information is acquired via key epidemiological indicators, such as general population surveys and demand for treatment, among others, and a range of drug market information sources. However, the difficulty with these indicators, in light of the new psychoactive substance situation, is that many users are unaware of exactly which substances they are using. For example, a survey respondent may admit to the use of 'ecstasy', which formerly would imply the use of MDMA, but now could, in fact, imply that they have used one (or several) of any number of synthetic phenethylamines, or indeed another class of psychotropic substances.

Analysis of wastewater has been proposed as a tool for providing useful information on temporal and regional trends in the use of new psychoactive substances, because this technique can potentially provide accurate information on the identity and quantity of the drugs being used at any given time (Reid et al., 2014). While this technique has proved successful with regard to assessing the use of established illicit drugs, such as cocaine and amphetamines (Thomas et al., 2012; Ort et al., 2014), the new psychoactive substance market presents a number of challenges and, therefore, alternative solutions, using altered sampling and analysis methodology (as opposed to standard methods of wastewater analysis, as described in Chapters 1 and 2), may be necessary. The challenges include the following: the large number of individual substances on the market; the dynamics of the market and the rapidity at which 'old' drugs are substituted with 'new' alternatives; the relatively small size of the new psychoactive substance market with respect to the illicit drug market (e.g. the size of the synthetic cannabinoid market in Norway is relatively small when compared with the market for cannabis products; see Figure 4.1); and the lack of (clinical) data from rigorous pharmacokinetic profiling studies that provide the information necessary to identify and determine the rates of excretion of these drugs and their metabolites.

The last two challenges in this list relate to the selection of suitable, specific chemical substances that can be measured in wastewater and used as 'biomarkers' of drug use. The analysis of wastewater for drug epidemiology is essentially an extension of the toxicological analysis of urine, so the chosen biomarkers in wastewater analysis are generally the

FIGURE 4.1

**The size of the synthetic cannabinoid market in Norway relative to cannabis products, as measured by the occurrence of National Criminal Investigation Service (NCIS) seizures**




Source: NCIS Norway.

same as the well-established and well-understood urinary excretion products of target drugs used in toxicology. It follows that the requirements for biomarker suitability for wastewater analysis are the same as those for toxicology, that is that the analyte must be a specific marker of the target drug and not formed exogenously, it must be stable (chemically and enzymatically) within the sewer system, and it must be present at concentrations high enough to allow its detection and quantification. While information on these criteria is readily available for established illicit drugs, it is lacking (or completely absent) for many new psychoactive substances; therefore, alternative data sources are required.

The approaches proposed as potential solutions to the above challenges can be broadly divided into three groups:

1) the use of computer-based modelling tools to predict the identity and fate of new psychoactive substance residues in urine and wastewater which will serve as a proxy for *in vivo* or *in vitro* studies in the laboratory;
2) a shift from the analysis of wastewater from large, non-specific general populations to wastewater from more targeted populations in which the use of new psychoactive substances is expected (e.g. wastewater from nightclub toilets or festivals or similar);
3) a shift from the targeted analysis of selected known drug residues (as is common for the analysis of 'classical' drugs) to suspect screening (Chiaia-Hernandez et al., 2014) and retrospective analysis of data acquired from non-targeted (or unbiased) analytical methodologies where little or no knowledge is available on which exact chemical species (drugs or metabolites) may be present when the initial wastewater sample is collected, processed and analysed.

These necessary adjustments in approach will affect the way in which the obtained results are interpreted and used in drug monitoring.

## Identification and selection of appropriate new psychoactive substance biomarkers by computer modelling

New psychoactive substances encountered by healthcare services, law enforcement agencies (including customs authorities) and national medicines agencies are reported to the EMCDDA and Europol under the framework of the EU Early Warning System (EMCDDA, 2007). One of the critical outputs of this framework is the European database on new drugs (EDND), which presents up-to-date information on the occurrence of new psychoactive substances in the European Union (and individual Member States). This database is an excellent 'spring-board' for wastewater analysts, as it provides an up-to-date list of the compounds known to have been encountered on the drugs market in Europe. Substances on this list can then be passed through pharmacokinetic and physicochemical modelling to identify appropriate biomarkers that could subsequently be added to analytical databases.

Pharmacokinetic profiling involves reviewing the rates of absorption, distribution, metabolism and excretion of new psychoactive substances after administration (or use). Of particular importance to wastewater analysts and toxicologists alike are the metabolism and excretion partitions, because it is these two factors that play the most significant role in determining the identity and expected amount of a particular biomarker in urine (and thereby wastewater).

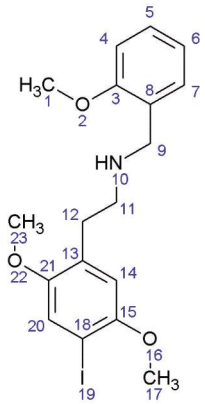
It is expected, however, that clinical and laboratory data on the pharmacokinetics of recently identified new psychoactive substance will be limited or non-existent;

therefore, the identification and selection of appropriate biomarkers will require alternative data sources, such as those generated by computer models (so-called *in silico* modelling). The SMARTCyp model was the first web-based application to be developed to predict the metabolism of drugs via the cytochrome P450 family of enzymes (Rydberg et al., 2010a, b). SMARTCyp models drug reactivity and predicts the likelihood of metabolism of particular sites of a drug molecule by applying scores to each atom: a lower score implies a greater chance of metabolism. The tool has been used to successfully identify the urinary metabolites of MDMA, mephedrone, JWH-018 and MAM-2201 (Reid et al., 2014). In the current report, the results of an exercise carried out on 25I-NBOMe, AH-7921 and methoxetamine, as examples of a substituted phenethylamine, a synthetic opioid and an arylcyclohexylamine, respectively, are presented (Table 4.1). In these examples, the *in silico* predictions are in good agreement with the (albeit limited) literature.

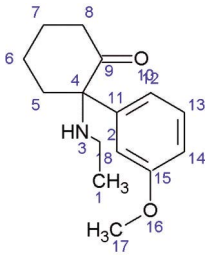
The next step in the process is to assess the stability and fate of these predicted biomarkers in wastewater. Drug residues are subject to a number of different processes as they are transported through wastewater networks to the point of sampling and eventual analysis (Baker and Kasprzyk-Hordern, 2011; Castiglioni et al., 2013; Plósz et al., 2013). The two main processes that can affect new psychoactive substance biomarkers during transit and sample handling and storage are transformation and sorption. The term 'transformation' refers to the

TABLE 4.1

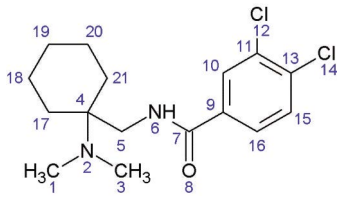
***In silico* prediction of metabolism, biodegradation and adsorption potentials for 25I-NBOMe, methoxetamine and AH-7921**



25I-NBOMe



Methoxetamine



AH-7921

| | SMARTCyp scores (1) | | | | | Metabolite | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Atom number | Standard | 2C9 | 2D6 | Average | Putative metabolism | Identified in literature | Log *P* | Biodegradation potential (%) (2) | Adsorption potential (%) (3) |
| **25I-NBOMe** | 17 | 52 | 60 | 66 | 59 | *O*-demethylation | Stellpflug et al., 2014 | 3.18 | 0 | 41 |
| | 1 | 52 | 60 | 80 | 64 | *O*-demethylation | Stellpflug et al., 2014 | 2.69 | 0 | 41 |
| | 23 | 52 | 66 | 80 | 66 | *O*-demethylation | Stellpflug et al., 2014 | 3.53 | 0 | 41 |
| **Methoxetamine** | 2 | 33 | 46 | 94 | 58 | *N*-dealkylation | Meyer et al., 2013 | 2.15 | 48 | 1 |
| | 17 | 52 | 60 | 73 | 61 | *O*-demethylation | Meyer et al., 2013 | 2.61 | 29 | 3 |
| | 8 | 52 | 65 | 99 | 72 | Acetylation | Meyer et al., 2013 | 1.99 | 6 | 24 |
| **AH-7921** | 1, 3 | 32 | 45 | 93 | 56 | *N*-dealkylation | Vorce et al., 2014 | 3.61 | 2 | 35 |
| | 19 | 66 | 74 | 101 | 81 | Hydroxylation | | 2.61 | 1 | 5 |
| | 18 | 67 | 81 | 115 | 88 | Hydroxylation | | 2.54 | 1 | 5 |

(1) Calculated via www.farma.ku.dk/smartcyp. Only the best (lowest) three scores for each new psychoactive substance are displayed.
(2) Non-linear MITI (Ministry of International Trade and Industry) Biodegradation Model (BIOWIN 6, U.S. Environmental Protection Agency, USA).
(3) EPISuite (U.S. Environmental Protection Agency, USA): WWTPWIN sludge adsorption potential based on BIOWIN 6-derived half-lives.

degradation or metabolism of a biomarker that results in a reduction in the concentration of that marker in wastewater. This loss or reduction would, in turn, lead to artificially low estimates of drug consumption or, indeed, false negatives if it is not appropriately accounted for. Transformation can also refer to the formation of the biomarker by chemical or enzymatic processes in the sewer system, which would lead to artificially high estimates of drug consumption. Transformation in wastewater is determined by the same phases (phases I and II) as drug metabolism in the human body; the enzymatically mediated processes associated with the cytochrome P450 family of enzymes, peroxidases and esterases are not exclusive to the human body and these enzymes are also involved in the transformation of new psychoactive substance biomarkers in wastewater (Plósz et al., 2013).

Adsorption is the process whereby new psychoactive substance biomarkers bind to solid particulate matter or surfaces within the wastewater system. Such processes are governed by a number of physicochemical properties of the given chemical (biomarker), but, in general, the most important features are surface energy and lipophilicity (as measured by log *P* or log *D*).

As with pharmacokinetic prediction, there are a number of *in silico* tools that can be used to predict and identify whether or not transformation and adsorption are likely to be a concern for analysts working on new psychoactive substances in wastewater (Reid et al., 2014). Table 4.1 presents the results of applying these models to the predicted metabolites of new psychoactive substances. The predicted metabolites of 25I-NBOMe and AH-7921 are likely to be stable in wastewater (biodegradation potential of < 5 %), but adsorption to particulate matter is a potential concern for 25I-NBOMe (adsorption of 41 %). The predicted dealkylation metabolites of methoxetamine are expected to be unstable in wastewater, as the biodegradation potential for both of these metabolites is in the 25–50 % range, which indicates that they are less stable than cocaine (a biodegradation potential of 21 % was determined using the same model).

The major limitation associated with the use of *in silico* models is that the results are (by definition) just predictions of what may occur, and this by no means guarantees that the results are accurate. The SMARTCyp model, for example, is reported to have a 76 % success rate (Rydberg et al., 2010b). Likewise, the BIOWIN-6 biodegradation model used in Table 4.1 is derived from a dataset of 884 chemicals (Tunkel et al., 2000), and these substances may not always have physicochemical properties that are adequate proxies for new psychoactive substances. In general, the best that can be expected from these models is that they produce a concise list of 'possible' sewage biomarkers for new psychoactive substance use, and provide a warning if one or more of these biomarkers is expected to be unstable in wastewater. Confirmation of these predictions would be reliant on the use of laboratory-based experiments such as human-liver microsome incubations (for *in-vitro* metabolism) and biodegradation studies.

## Utility of targeted or site-specific sampling

While the use of new psychoactive substances appears to be an expanding problem and there is a very large number of drugs (or substances) that fall under this definition, it is difficult to describe the exact size of the new psychoactive substance market in relation to that of the established illicit drug market. The large number of these substances does, however, imply that the market is diverse and that the total use of any one particular new psychoactive substance is likely to be low compared with that of an established illicit drug (see Figure 4.1 for an example of synthetic cannabinoid use in relation to cannabis product use in Norway). This characteristic gives rise to a very significant challenge for wastewater analysis in that the measurable concentration (or amount) of a particular new psychoactive substance biomarker is expected to be low. A possible solution to this problem is to move away from sampling large-scale municipal wastewater towards the sampling of wastewater from targeted populations that are expected to have higher use levels of new psychoactive substances than the general population and, therefore, will produce higher measureable concentrations of biomarkers in wastewater. The analysis of pooled urine wastewater from portable public urinals (pissoirs) achieves this objective in two ways: firstly, pissoirs can be placed in areas in which target populations are expected to congregate or in which the use of new psychoactive substances is expected (e.g. at music festivals) and, secondly, urine from portable toilets is less dilute than urine from municipal wastewater systems because dilution occurs in municipal wastewater as a result of the domestic use of water (showers, washing machines, etc.) and the infiltration of rain water. The concentrations of new psychoactive substance biomarkers in pissoir-derived wastewater are, therefore, expected to be orders of magnitude higher than those in municipal wastewater, which will increase the likelihood of detection.

TABLE 4.2
**Summary of new psychoactive substances identified in pooled urine samples**

| New pychocative substance | Sample type | Reference |
|---|---|---|
| Mephedrone | Nightclub urinal, London (2011) | Archer et al., 2013a |
| 3-Trifluoromethylphenylpiperazine | | |
| 2-AI (2-aminoindane) | | |
| Cathine | City centre urinals, London (2012) | Archer et al., 2013b |
| Methylhexaneamine | | |
| 4-Methyl methcathinone | | |
| Methiopropamine | | |
| Methoxetamine | | |
| 4-Methyl methcathinone | City centre urinals, London (2012) | Archer et al., 2014 |
| Methylhexaneamine | | |
| Methcathinone | | |
| 4-Ethylmethcathinone | | |
| Methiopropamine | | |
| Pipradrol | | |
| Cathinone | | |
| 5-(2-Aminopropyl)benzofuran | | |
| 1,4-Trifluoromethylphenylpiperazine | | |
| 4-Methylbuphedrone | | |
| 4-Methylethcathinone | | |
| 1,4-Methoxyphenylpiperazine | | |
| 4-Fluoroephedrine | | |
| 1-(2-methoxyphenyl)piperazine | Pooled portable toilet, Oslo (2013) | Reid et al., 2014 |

A number of studies have shown that analyses of pooled urine samples from city centre, nightclub and music festival toilets allows the identification of new psychoactive substances, thereby providing timely data on exactly which drugs are currently in use at a particular location or within a particular population (Archer et al., 2013a, b, 2014; Table 4.2).

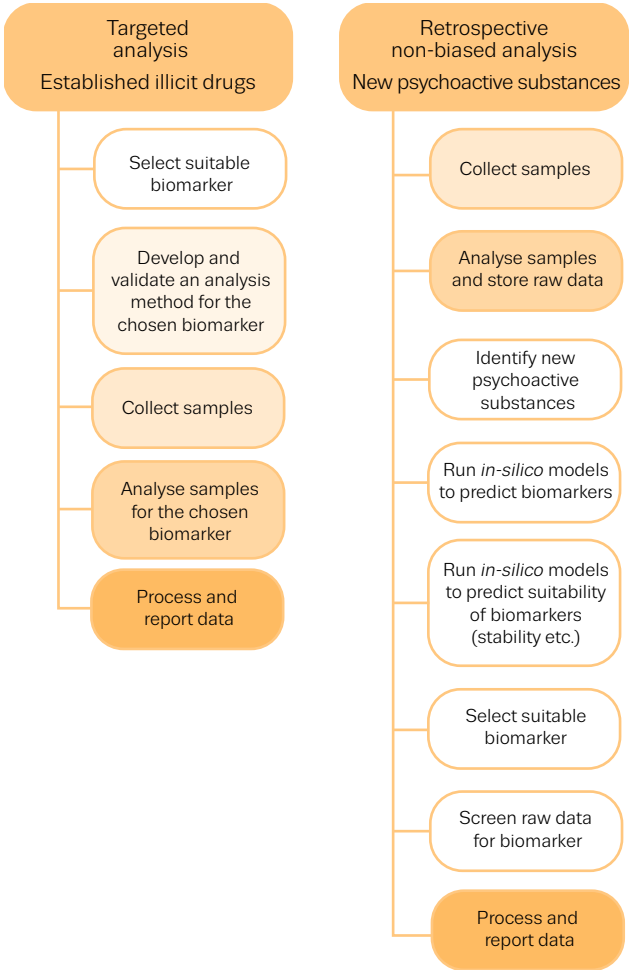
## Utility of unbiased data acquisition and (retrospective) analysis

The analysis of established illicit drugs in blood, urine and wastewater is based on the detection and quantification of a parent drug or well-documented metabolites of this drug, and the methods for sample preparation, extraction and detection can be developed and validated in advance (Figure 4.2). With new psychoactive substances, however, the identity of the biomarkers is not always known and, therefore, methods must be developed to allow the detection of all compounds in a sample without prior knowledge of what the eventual target compound is likely to be. Within this paradigm (Figure 4.2), an analyst will perform unbiased data acquisition and, at a later stage, will investigate the collected data by searching for specific drugs or biomarkers within an electronic record (Ibáñez et al., 2014; Reid et al., 2014). A suitable analogy would be a photographer taking a high-definition digital photograph then later analysing that picture for specific colours; for the purpose of detecting a new psychoactive substance in wastewater (or urine), the 'camera' is a high-resolution mass spectrometer.

In the example shown in Figure 4.3, 2 786 individual chemical species (represented by coloured dots) were identified by high-resolution mass spectrometry of a single sample of pissoir-derived wastewater. The high-resolution mass spectrometry data that were recorded and stored for each of the 2 786 compounds identified include data on chromatographic retention times, exact mass and isotopic abundance; from these data, a nominal chemical formula can be derived. This record can be filed and then later screened for the presence of any given new psychoactive substance (or associated biomarker). Filtering this extensive high-resolution mass spectrometry data record for target new psychoactive substance biomarkers is aided by the fact that many new psychoactive substances share structural elements or moieties. This allows for common-fragment and mass-defect filtering to be performed on the high-resolution mass spectrometry data, thereby

FIGURE 4.2

**Flow charts for the targeted analysis normally carried out for established illicit drugs, and for the retrospective analysis paradigm required for new psychoactive substance screening**




focusing the search on little-known chemical species and making it more manageable. This filtering approach is illustrated in Figure 4.3: only 475 species (of the total 2 786) remain after mass-defect filtering around AH-7921 and, of the 475 retained species, only one has a chlorine isotope pattern that implies that it could be related to AH-7921, and none has a dichlorobenzaldehyde moiety (associated with a mass spectral fragment with mass to charge ratio of 172.955), as expected for AH-7921 and related metabolites (Vorce et al., 2014).

In this example, neither AH-7921 nor any probable related metabolites were found to be present, but it is possible that other new psychoactive substance residues are present in the sample. This sample and the associated high-resolution mass spectrometry data record could be investigated again if another new psychoactive substance is identified by the Early Warning System or predicted by *in-silico* modelling in the future. This technique therefore allows the rapid and comprehensive screening of complex samples for biomarkers of specific new psychoactive substances without the need for prior development and validation of specific analytical methods.

High-resolution mass spectrometry also allows the later processing, detection and tentative confirmation of the presence of a compound without the need for a standard reference compound. The screening of samples with the aid of a reference library of new psychoactive substance spectra and retention indices in a database is a possibility, with the potential to add new compounds as they are identified. There is also the potential for retrospective analysis, that is re-investigation of previously analysed samples if additions are made to the database.

## Utility of new psychoactive substance screening results

The analysis of the residues of illicit drugs (such as cocaine, cannabis and amphetamines) in municipal wastewater provides quantitative data which can be used to back-calculate the total amount of a drug used by the general population. These data are particularly useful for regional comparisons on drug consumption and the eventual generation of time-series, which allow the study of changes in drug use over time (Table 4.3).

The screening of new psychoactive substances in wastewater will not, unfortunately, be able to generate the same level of data as can be generated for established illicit drugs, particularly in the early stages of use of a new psychoactive substance. This is because the methods used for the back-calculation of

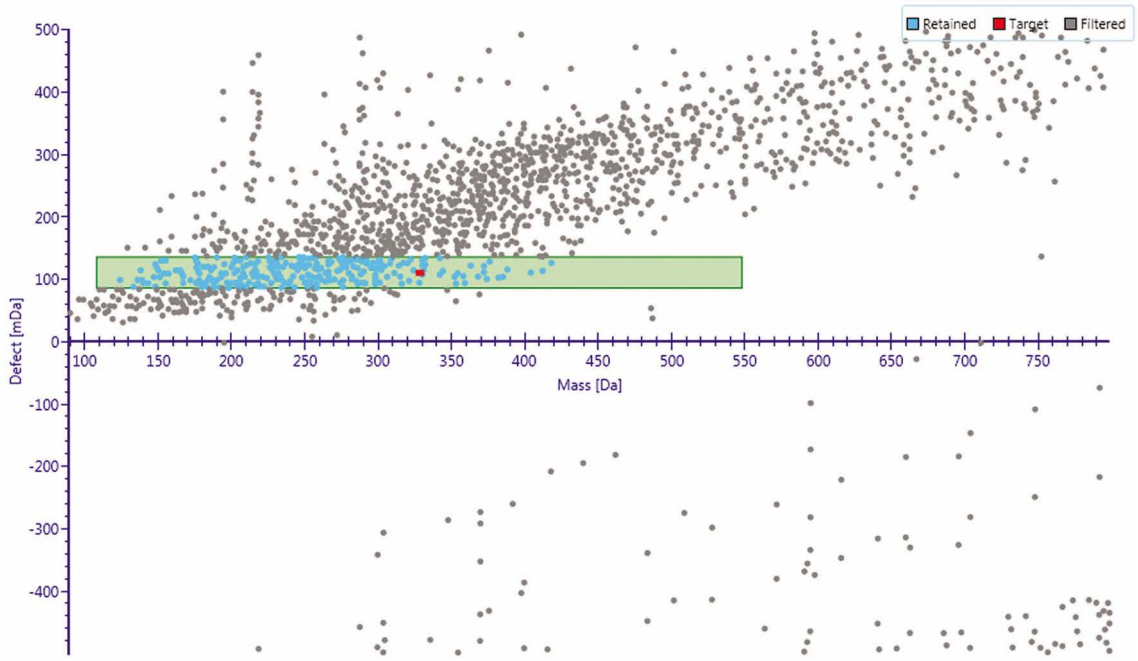
TABLE 4.3

**The utility of the analysis of drug biomarkers in wastewater**

| Established illicit drugs in municipal wastewater | Early new psychoactive substance screening in pissoir-sourced wastewater |
|---|---|
| Targeted analysis on pre-selected and validated biomarkers<br>Quantitative results<br>Back-calculated use data (quantity/volume)<br>Generation of time-series and regional comparisons | Retrospective analysis on *in silico* predicted biomarkers<br>Non-quantitative results<br>Time-series and regional comparison by frequency of detection |

FIGURE 4.3

**Mass defect plot for a sample of pissoir-derived wastewater**




NB: The plot shows a total of 2 786 individual chemical species (coloured dots) with molecular weights in the range of 90–800 Da. The target new psychoactive substance (in this case AH-7921) is highlighted in red. A total of 475 species (blue) pass the filter (green area) on the mass defect associated with AH-7921 (50 mDa tolerance around a defect of 118 mDa).

drug use are reliant on the availability of well-documented excretion ratios, which describe the percentage of the initial drug dose that is excreted in urine as a particular biomarker. Few or no clinical trial data are available on the excretion ratios of the most recently identified new psychoactive substances so it is not possible to quantitatively relate measured biomarker concentrations in wastewater to absolute estimates of new psychoactive substance consumption. Back-calculation methods are also reliant on accurate measurements of sewage volume as these provide the key link between measured drug biomarker concentrations and calculated drug loads. The screening of new psychoactive substances in pissoir-sourced wastewater does not provide the same opportunity because volume measurements are often not available, and do not necessarily have any correlation with the total daily urine volume of a target population.

It is important to note that the representability of the population visiting an event may be limited because a percentage of the attendees will not make use of a pissoir, and indeed the availability of urine from pissoirs at an event cannot always be guaranteed because of privacy regulations, or because festival organisers or club managers do not wish to be stigmatised.

Data derived from screening new psychoactive substances in pissoir-sourced wastewater are mostly qualitative, but there are certainly significant opportunities for building semi-quantitative datasets (Table 4.3). The generation of time-series and the eventual comparison of data from differing populations could be based on frequency. In this way, the measured value is not a drug consumption estimate, but instead the frequency at which a particular new psychoactive substance is detected. In addition, if levels of use of a new psychoactive substance are sufficiently high among the general population that appropriate biomarkers are detectable in municipal wastewater, it will be possible to generate time-series data and run population-based comparisons on the biomarker itself. Although analysts may not be able to give precise estimates of the amount of new psychoactive substances used, they will be able to provide details on how much the use has changed over time and how this relates to use in other populations.

## Conclusions

The new psychoactive substance market presents a unique set of challenges to all drug epidemiologists, including those working with wastewater, because this

market is extremely dynamic and new compounds are being introduced at a rapid rate. The lack of experimental data on pharmacokinetics and unanswered questions related to biotransformation pathways severely impede the identification, detection and quantification of these new psychoactive compounds in samples of wastewater. There are, however, a number of *in-silico*-based tools that can be used to predict these unknown parameters and provide a concise list of potential biomarker targets. It should be noted, however, that by no means do these models guarantee the formation of a given metabolite or biotransformation product; therefore, extensive non-targeted screening by retrospective analysis of high-resolution mass spectrometry data will be required.

Screening for potential biomarkers of new psychoactive substances by high-resolution mass spectrometry provides the ability to detect and (tentatively) confirm the presence of a compound without the need for standard reference material, and the fact that many new psychoactive substances share structural elements also allows common-fragment searches and mass-defect filtering to be performed. These techniques focus the search on unknown metabolites and reduce spectral noise, leaving only the information that is most likely to be related to a series of new psychoactive substance biomarkers.

The concentrations of new psychoactive substance residues in wastewater are often below the lower limit of detection; therefore, the collection and analysis of wastewater from pissoirs has, thus far, been used as a relatively successful alternative to municipal wastewater. This technique may be the primary alternative for new psychoactive substance detection, but it is important to remember that pissoir-derived screening exercises provide data that are more qualitative than quantitative in nature and comparisons between regions or over time will most likely be based on frequency of new psychoactive substance detection rather than on the magnitude of consumption.

In summary, the combination of these tools and alternative data sources provides an excellent framework in which to maximise the likelihood of successfully identifying and detecting biomarkers for new psychoactive substances in wastewater, albeit with differing interpretation outcomes.

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5

CHAPTER 5

# Integrating wastewater analysis with conventional approaches for measuring illicit drug use

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## Introduction

Wastewater-based epidemiology can provide near-real-time estimates of drug use within a defined population and can track changes in drug use over time. This information is complementary to that available from established drug-use monitoring methods. Realising the full potential of wastewater-based information will depend on integrating it into the existing set of epidemiological indicators. In Europe, our knowledge of drug use is built on the findings of a range of epidemiological indicators. Surveys of the general and school populations provide the 'big picture' on the use of drugs at national and European level. Data collected on users entering treatment are a vital source of information on the more risky forms of drug use, and this is supplemented by data gathered through special studies, using methods designed to sample hard-to-reach populations and stigmatised behaviours. Further indicators report on the harms associated with drug use, such as drug-related diseases and deaths. In addition, monitoring of drug market (price, purity, seizures) and drug-related crime indicators allows a comprehensive overview of the drug situation.

How wastewater analysis can fit into the monitoring of drug use has been addressed by Castiglioni et al. (2014), who looked at how epidemiological approaches based on wastewater analysis and surveys compare and can complement each other (Table 5.1).

To date, few attempts have been made to compare the drug use estimates obtained through wastewater analysis and epidemiological surveys. Although complicated and fraught with difficulties and limitations, the comparison of these different approaches provides

TABLE 5.1

**Summary of the main features of established epidemiological approaches and wastewater-based epidemiology**

| Information provided | Drug epidemiology (established approaches) | Wastewater-based epidemiology (wastewater analysis) |
|---|---|---|
| **Methods employed to estimate drug use** | | |
| High costs of studies | Yes [a] | No |
| Real-time estimates | No | Yes |
| Retrospective analysis | No | Yes |
| **Drug use estimates** | | |
| Frequency and patterns of drug use | Yes | No |
| Changes in population levels of drug use in a short time period (daily, weekly, annual) | No | Yes |
| Mode of drug use | Yes | Yes/no [b] |
| Main groups of users | Yes | No |
| Purity of drugs | Yes | No |
| Emerging trends in drug use (e.g. appearance of new drugs) | No/not systematic [c] | Yes |

([a]) Except for cases in which routinely available data were analysed.
([b]) Subject to the availability of characteristic biomarkers.
([c]) Only in specifically targeted surveys or studies.

the possibility to indirectly check quality and accuracy, since they approach the challenge from different viewpoints and, together, can provide a better assessment of drug use in a specific community than either could alone. In the current chapter, some of the first attempts to compare results obtained from these two approaches, with regard to cocaine use, are presented and discussed, and the limitations and requirements for further research in this field are highlighted. The first study of this type, performed in Oslo, Norway, compared the results from three different datasets, two obtained by population surveys and one by wastewater-based epidemiology. The second study analysed the temporal and spatial trends of cocaine use in Italy as estimated by wastewater-based epidemiology, and compared these results with those obtained from local and national epidemiological surveys undertaken during the same period.

## Norwegian case study

### Introduction

In an attempt to understand how data from wastewater analysis can be used in an epidemiological context and to determine how these data can be integrated with more established epidemiological methods, Reid et al. (2012) carried out an analysis on data describing the use of cocaine in Oslo, Norway. The analysis was designed around the seemingly simple task of comparing the results of three distinct datasets with the aim of identifying how they align and, most importantly, where additional information is needed.

Three distinct datasets from three different sources formed the basis of this analysis:

1) A set of regional general population surveys were carried out between 2000 and 2010 and included a total of 14 438 respondents. In this dataset, the annual prevalence of cocaine use by the population, aged 15–64 years, was 2.9 % (range 2.6–3.2 %). More than 50 % of users in the general population reported using the drug on between one and four occasions in the 6 months preceding the survey, while less than 5 % reported daily or almost daily use. Self-reported per-user cocaine consumption rates were, on average, 9.8 g (of pure cocaine) per user per year.
2) A sample survey of drug use among drivers (roadside survey) was conducted between 2008 and 2009 and included a total of 2 341 respondents. The analysis of oral fluid for cocaine and metabolites showed that 0.7 % (range 0.36–1.03 %) of drivers tested positive for cocaine use.
3) Cocaine metabolites in wastewater from a sewage network that processed waste from 570 000 inhabitants were analysed. The results from samples collected throughout September 2009 provided an estimated combined cocaine consumption rate of 1 458 g (of pure cocaine) per week (range of 1 158–1 758 g/week).

The study methods, results and conclusions are covered in detail in the original published text (Reid et al., 2012). Therefore, in the present report, only a synopsis of a case study is provided in which one attempt at integrating wastewater analysis with established drug use measures is described.

### Methods of comparison

#### Comparing consumption estimates

Total population cocaine use was estimated from the general population survey dataset via a 'bottom-up' method, in which the number of users is multiplied by the reported frequency of use and the reported amount (mass) of cocaine used. This is then directly comparable with the wastewater-based estimate. No consumption estimate was derived from the survey of drivers.

#### Comparing prevalence estimates

Wastewater analysis provides only a direct measure of the total amount of cocaine used within a population; therefore, any attempt to estimate the prevalence of use from these data must be derived from per-user consumption figures. More specifically, the number of users in a population is calculated by dividing the total consumption of the population (in g per year) by the estimated per-user consumption amount (9.8 g/year per user) from the general population survey dataset.

The general population surveys and the roadside survey deliver prevalence estimates, but with differing timeframes. General population surveys generally involve questions related to use within the last 6 to 12 months, while the analysis of oral fluid (as carried out in the roadside survey) has a cocaine-detection window of less than 24 hours. The comparison of these datasets, therefore, requires a timeframe adjustment. The general population survey data include self-reported frequency-of-use statistics, so it is possible to derive a statistical probability of use for the last 24 hours which is directly comparable with the driver survey. For instance, on this

basis, a person reporting having used the drug on 18 use days in the last 6 months (i.e. about 180 days) would be assumed to have a 10 % probability of using cocaine on any given day.

## Results of the original analysis

### Comparing consumption estimates

Applying a bottom-up approach to the general population survey dataset provided a combined annual consumption estimate for the total population of 117 kg per year (range of 70–165 kg/year). This is comparable to the wastewater estimate of 76 kg per year (range of 60–91 kg/year).

### Comparing prevalence estimates

The data from the combined general population survey revealed a last 12-month prevalence of 2.9 % (2.6–3.2 %), whereas a prevalence rate of 1.9 % (1.0–4.0 %) was derived from a combination of wastewater-sourced total population consumption data and self-reported per-user consumption data.

By adjusting the timescale of the last 12 month prevalence data, a 24-hour prevalence figure, that can be directly compared with data from the roadside survey, was derived. The roadside survey showed that 0.7 % (0.36–1.03 %) of the drivers tested positive (weighted for undersampling/oversampling relative to the general population; Reid et al., 2012) for cocaine use, whereas the prevalence rate derived from the general population survey was 0.22 % per day (0.13–0.30 %/day).

## Discussion and conclusions

Reid et al. (2012) carried out the study described above in order to better understand how data from wastewater analysis can be used in an epidemiological context, and to determine how these data can be integrated with data from epidemiological surveys. Comparing the three datasets was not a trivial exercise because there is little published data on per-user cocaine consumption, and because the extent of overlap of these three study populations is unknown.

Per-user consumption estimates are the only means by which the wastewater-derived total population consumption can be directly compared with prevalence estimates. Reid et al. (2012) made some general assumptions with regard to per-user consumption, based on limited published data and self-reported use in a small sample of the Norwegian user population, but there were no direct measurements made of the weight or purity of the drug used, which could have been used to validate the results. Further work has since been carried out in an attempt to arrive at a more reliable estimate of per-user cocaine use (Amundsen and Reid, 2014) and, while the results of this later study agree (for the most part) with the initial estimate, there were still no measurements of weight or purity which could be used to support the findings. A positive outcome of this study is that, because per-user consumption is a common denominator for both general population survey prevalence and wastewater-derived mass measurements, these two datasets could be combined to triangulate a more accurate per-user consumption figure in the future.

The validity of the sample populations was also highlighted as a confounding factor within this comparison. Inconsistencies between the sample populations, from which each of the three datasets were derived, arise from both temporal and spatial incompatibilities. Temporal inconsistencies arise because the general population survey dataset combines results from numerous surveys conducted over more than a decade, while the wastewater measurements that form the basis of the comparison were carried out over the course of only a single month in 2009. This study would best be conducted on data from a single year (2009), but the volume of data available from a single year is low, so additional data from multiple years were used with the knowledge that cocaine consumption in Norway has been relatively stable over the last decade.

Inconsistencies in the spatial domain are apparent because the population is mobile. This affects wastewater data, as it is possible for people to move in and out of the sewage catchment throughout the course of a day, but it is, of course, the driver population that is most susceptible to such error because this population is highly mobile. However, perhaps the largest unanswered question with regard to the roadside survey is how the driver population and the cocaine user population are aligned. It was somewhat surprising to see that the survey of the driver population showed the highest prevalence rates of cocaine use and, although it is entirely possible that cocaine use is indeed higher in the driver population, no formal conclusions can be made in this regard without first repeating the general population survey with specific questions related to the use of motor vehicles.

The numerous difficulties and shortfalls reported by Reid et al. (2012) highlight the fact that wastewater methods in

drug epidemiology cannot (in their current form) be used alone as a replacement for more survey-based indicators. Wastewater analyses generate only total community-wide consumption figures (amount of a drug used), which are difficult to compare with prevalence estimates without a wealth of supporting information. Wastewater analysis is best used as a source of supporting information to validate or confirm trends identified by the other indicators. For example, indications of a rise in drug use prevalence or demand for treatment services may be validated with supporting information from wastewater analysis. Data from wastewater analysis and data from other indicators are not expected to be in exact agreement, and a degree of overlap should be sufficient. Although a 'snapshot' in time and space might be difficult to obtain because of the lack of detailed information and inaccuracies in the precise estimation of consumption patterns and levels, a promising way of resolving these difficulties is to determine the congruence of wastewater and conventional indicators with regard to temporal and spatial patterns of use. Such validation of the use of wastewater-derived data as supporting information will, of course, require the more formalised collection of wastewater data at both the national and international level.

## Italian case study

### Introduction

The results from population surveys and wastewater analysis are difficult to compare, as the former is designed to estimate the prevalence of use and the latter to estimate the collective consumption of pure substances within a community. However, prevalence and consumption can be considered as two different measures of the same phenomenon and it is, therefore, important to verify whether or not these approaches produce convergent outcomes. To study this issue, we investigated the profiles and monitored the temporal and spatial trends of drug use by these two methods. In this case study, we compared the results obtained by the following consumption and prevalence studies:

1) Temporal trends of cocaine use in Milan: cocaine use was monitored through wastewater analysis in Milan between 2005 and 2010. The consumption trends identified were compared with the outcomes of population surveys carried out in the same period in Milan and in Italy as a whole.
2) Spatial distribution of cocaine use in Italy: cocaine consumption was studied in northern, central and southern Italy in 2011 by wastewater analysis. The results obtained were compared with profiles obtained by population surveys.

### Methods

#### Wastewater analysis

In the first study, 24-hour composite samples of wastewater were collected from the inlet to the principal wastewater treatment plant in Milan (Milano-Nosedo) by sampling wastewater every 20 minutes for 24 hours. Samples were taken daily, on consecutive days in November 2005 (7 days), March 2006 (16 days), March to April 2008 (35 days), and again in March (30 days) and September 2009 (7 days). Samples were analysed by liquid chromatography–mass spectrometry for cocaine metabolites (Castiglioni et al., 2011) and cocaine consumption was estimated by back-calculation from benzoylecgonine loads, as described elsewhere (Zuccato et al., 2005).

In the second study, wastewater samples were collected on 7 consecutive days from the wastewater treatment plants of 17 cities in Italy (six in northern, four in central and seven in southern Italy) in October 2011. Samples were collected and processed, as described above, to estimate cocaine consumption in the cities under study.

#### Population surveys

Survey data were extracted from published reports. The last month and last year prevalences of cocaine use in Milan in 2007 and in 2010 were obtained from a 2011 report on the consumption of psychotropic substances in Milan (ASL Milano, 2011). The last year prevalence data for cocaine use in Italy in 2008 and 2010 were obtained from the National Drug Policy Department's 2010 national report to parliament (DPA, 2010), while the 2012 last year prevalence data for northern, central and southern Italy were extracted from the 2012 national report (DPA, 2012).

#### Comparison

In the first study, the estimates of cocaine consumption in Milan for 2008 and 2009, as assessed by wastewater analysis, were compared with the prevalence of use in Milan (last month prevalence for 2007 and 2010) and in Italy as a whole (last year prevalence for 2008 and 2010). In the second study, the consumption of cocaine, as estimated by wastewater analysis in 17 cities in Italy in October 2011 and pooled according to their geographical

location in northern, central or southern Italy, were compared with 2012 prevalence data extracted from the national report (Dipartimento Politiche Antidroga, 2012).

## Results

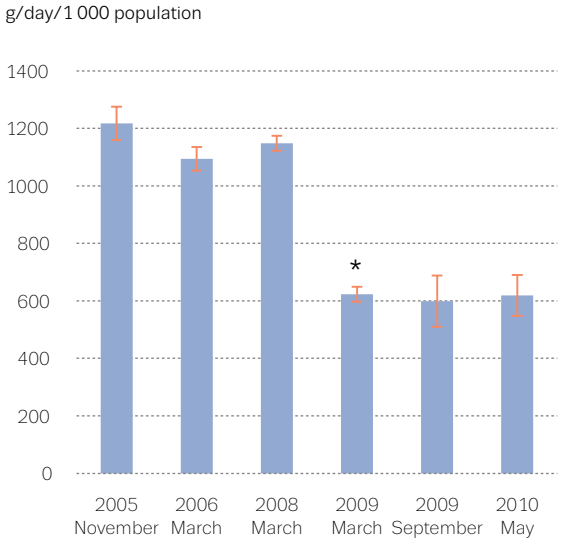
### Time trends of cocaine use in Milan

Daily cocaine consumption levels, as estimated by wastewater analysis, in Milan were the same in November 2005, in March 2006 and March/April 2008, but fell by 45 % in March 2009 and remained at the same level in September 2009 (as determined by analysis of variance [ANOVA], $p < 0.001$; followed by Tukey–Kramer HSD [honest significant difference] test, $p < 0.05$; Zuccato et al., 2011). Further analysis performed in May 2010 showed that the use of cocaine in Milan remained stable (Figure 5.1).

Population surveys also showed a parallel drop in last month (from 2.6 % in 2007 to 1.2 % in 2010, i.e. a decrease of 54 %) and last year (from 5.0 % in 2007 to 2.1 % in 2010, i.e. a decrease of 58 %) prevalence rates for cocaine use in Milan from 2007 to 2010 (ASL Milano, 2011), and in the last year prevalence rates (from 2.1 % in 2008 to 0.9 % in 2010, i.e. a decrease of 57 %) in the general population in Italy (DPA, 2010).

FIGURE 5.1

**Daily cocaine consumption, as estimated by wastewater analysis, in Milan**




NB: Study covers the catchment area of the Milan-Nosedo treatment plant. The results given are for the mean ± standard deviation, *$p < 0.001$ (ANOVA), followed by Tukey–Kramer HSD test (2009 vs. 2005–2008).

### Spatial distribution of cocaine use in Italy

Cocaine estimates for October 2011, pooled according to the geographical location of the 17 cities investigated, showed that there was a significantly higher use of cocaine in central than in northern or southern Italy ($p < 0.01$). Population studies carried out at the beginning of 2012, which investigated cocaine use prevalence in the previous year, showed a similar distribution, with a greater prevalence of cocaine use in central Italy than in northern or southern Italy (Table 5.2).

## Discussion and conclusions

Because of the different types of information provided by wastewater analysis (collective consumption of pure substances within a community) and population surveys (prevalence in the last month or year), a direct comparison of the data is difficult. It was only possible to compare the distribution of cocaine use, that is, the temporal trends of cocaine use at a local level (Milan, northern Italy) and the spatial pattern of use in Italy as a whole.

The spatial distribution of cocaine consumption in Italy (northern, central and southern Italy) revealed by wastewater analysis was in agreement with the prevalence findings from population surveys. Similar results were also obtained in a recent study aimed at quantifying spatial differences and temporal changes in the consumption of illicit drugs across European regions (Ort et al., 2014). A clear spatial difference in illicit drug use across Europe was demonstrated, with cocaine use being highest in western Europe and methamphetamine use being highest in northern Europe, the Czech Republic and Slovakia; these results were in agreement with available survey data.

The decrease in cocaine consumption observed in Milan between 2008 and 2009 (45 % decrease), as determined

TABLE 5.2

**Measures of cocaine use in Italy (northern, central and southern), as estimated by wastewater analysis and by population survey**

| Geographical location | Wastewater analysis | Population survey |
|---|---|---|
| | Consumption (g/day/1 000 population) | Last year prevalence (%) |
| Northern Italy | 0.44 ± 0.08 | 0.27 |
| Central Italy | 0.71 ± 0.11* | 0.34 |
| Southern Italy | 0.45 ± 0.07 | 0.22 |

NB: Prevalence data refer to the population aged 15–64.
*$p < 0.01$.

by wastewater analysis, was similar to the decrease reported by local and national epidemiological surveys (an approximately 55 % decrease). These results confirm the potential of wastewater analysis as a new method for rapidly detecting changes in the levels of use of a substance within a population. Nevertheless, the information obtained by wastewater analyses was not sufficient to fully understand the reasons for the observed decrease in cocaine use, which could have been caused by a decrease in the number of users, a decrease in the amounts used by the existing users or a change in the purity of the substance. It was, therefore, necessary to consider data on cocaine purity (which was stable during the period considered: 47 % in 2008 and 48 % in 2009) to conclude that the observed drop in cocaine consumption could be ascribed to a 'real decrease' in the number of consumers or the amounts being used by existing consumers, possibly related to the economic crisis (Zuccato et al., 2011). Furthermore, the general population survey data suggest that a decrease in the number of users was more probable than a decrease in the amounts used by existing users. These studies exemplify the complementary character of the two approaches that, when used together, can be a powerful tool that opens up a promising field of future investigation.

## Limitations and requirements for future research

The numerous limitations associated with the comparability and interpretation of the results reported in the previous examples indicate that wastewater-based methods for drug epidemiology cannot, alone, replace established indicators, but they can be used as a complementary tool that provides useful, novel information. These limitations primarily result from the fact that wastewater analysis generates only total community-wide use figures (amount of drug used), which are difficult to compare with prevalence estimates (usually confined to certain age ranges, generally 15–64 years) without a wealth of supporting information. Therefore, wastewater analysis is best used as a source of supporting information for population studies, for example to assess the extent and rank of use of different substances and to gain information on patterns of use, time trends and spatial differences of consumption; these types of data could be used to validate or confirm trends identified by the other epidemiological indicators. For example, epidemiological indications of a rise in prevalence or demand for treatment services could be validated with supporting information from wastewater analysis. Moreover, wastewater analysis has the advantage of providing data within a short time (days to weeks from sampling) and could, therefore, act as a first 'alert' tool in the identification of new trends in drug consumption or the use of new substances (see Chapter 4 for new psychoactive substances). Results from wastewater analysis can therefore anticipate results from population surveys, as shown in the study conducted in Italy (Zuccato et al., 2011) and described above. Moreover, wastewater analysis can easily provide information about large populations and can be applied on different scales to obtain information for almost the entire population of a country. In this case, it is necessary that specific sampling campaigns are designed and a representative set of wastewater treatment plants are selected in order to allow the extrapolation of consumption figures to the entire country.

Some additional limitations, related to the comparison of these different approaches, are ascribable to the potential lack of geographic correspondence between wastewater catchments and epidemiological surveyed areas; to resolve this, data from many wastewater catchment areas may have to be combined for comparison. In fact, the catchment areas of urban wastewater treatment plants can sometimes be larger than a single city, and, in these cases, specific adjustments will be required. Another important limitation is the different timeframes of the two approaches: drug use surveys typically gather data on use over a range of time windows (last month, last year, lifetime), whereas wastewater campaigns are typically on the scale of weeks or months.

Data from wastewater analysis and data from conventional epidemiological indicators are not expected to be in exact agreement, but a degree of overlap should exist and be sufficient to demonstrate the complementary character of these approaches, as demonstrated by the case studies presented above. These studies may be considered a reliable validation of the use of wastewater-based epidemiology as a novel indicator along with the existing, well-established multi-indicator system used to monitor drug use in Europe. If wastewater-based epidemiology is to be used to obtain supporting information, more standardised methods of wastewater data collection, at both national and international levels, will be required.

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# 6

# CHAPTER 6
# Conclusions and final remarks

Sara Castiglioni, Liesbeth Vandam and Paul Griffiths

Monitoring illicit drug use is difficult because of the hidden and complex nature of drug-using behaviours. The potential of wastewater analysis as an approach to complement established monitoring tools in the drug use area has been demonstrated. It has some clear advantages over other approaches, as it is not subject to response and non-response bias and can better identify the true spectrum of drugs being consumed; this latter advantage is important as users are often unaware of the actual mix of substances they take. This tool also has the potential to provide near-real-time information on geographical and temporal trends, and to provide data representative of relatively large population sizes.

Wastewater-based epidemiology involves 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 consumed by the population served by a wastewater treatment plant. It offers an interesting and complementary means of obtaining data that can be used to monitor the quantities of illicit drugs used at a population level, but it cannot provide information on prevalence and frequency of use, the route of administration, the main classes of users or the purity of the drugs. Additional challenges arise from uncertainties associated with the sampling of wastewater, the behaviour of the selected biomarkers in the sewer, the reliability of interlaboratory analytical measurement, the different back-calculation methods used and the different approaches used to estimate the size of a population being tested.

To improve the credibility and scalability of such studies, data from different sources need to be more reliable and comparable. Notably, the first Europe-wide study, performed in 2011 by the Sewage analysis CORe group Europe (SCORE) network, provided a comprehensive insight into the uncertainties associated with all of the wastewater-based epidemiology procedures. As a result, this group established a best-practice protocol for sampling, sample handling, chemical analysis, back-calculation procedures and data reporting. This protocol has been revised and updated during subsequent analytical campaigns in Europe (EMCDDA, 2015).

Wastewater-based epidemiology is considered to be a potent approach for monitoring patterns and trends of illicit drug use within a community. Since 2005, the approach has been applied in several countries worldwide. This worldwide application has demonstrated the potential of this approach with regard to monitoring the use of most of the main illicit drugs (cocaine, cannabis, amphetamine, methamphetamine and MDMA). These studies revealed geographical differences in drug use patterns, which were mostly consistent with data obtained by other approaches (as reported by EMCDDA and the United Nations Office on Drugs and Crime). Moreover, wastewater analysis has been proven to be able to detect local and temporal patterns of drug use, demonstrating its potential to provide information complementary to standard techniques.

In view of the main features of wastewater-based epidemiology, one direct and novel application of this approach is for the detection of the use of new psychoactive substances. While the technique has proved successful for established illicit drugs, the new psychoactive substance market presents a number of challenges which mean that alternative sampling and analysis methodology may be necessary. The challenges include the large number of individual substances on the market, the dynamics of the market, the relatively small size of the new psychoactive substance market and the lack of data from rigorous pharmacokinetic profiling. Thus, obtaining reliable estimates of new psychoactive substance use is not feasible at present. Three conceptual approaches for dealing with new psychoactive substances, using biomarkers in wastewater, are discussed in this report.

Some attempts have been made to compare drug use estimates obtained through wastewater analysis and epidemiological surveys. Comparing the different approaches provides the possibility for indirectly checking quality and accuracy, and of improving the assessment of drug use in a specific community. The

first attempts to compare results from the two approaches for cocaine use are discussed in this publication. The first study, performed in Oslo, Norway, compared the results from three different datasets, two of which were obtained by epidemiological surveys and one of which was obtained by wastewater-based epidemiology. The second study analysed the temporal and spatial trends of cocaine use in Italy using wastewater-based epidemiology and compared the results with those from local and national epidemiological surveys undertaken during the same period.

The numerous limitations associated with the comparability and interpretation of the results reported in these examples highlight the fact that wastewater methods for drug epidemiology cannot, at present, replace more established indicators, but they can be used as a complementary tool to provide useful and novel information. Wastewater analysis is best used as a source of supporting information for population studies, for example, to assess the extent and rank of use of different substances and to gain information on patterns of use, including time trends and spatial differences in consumption; these types of data could be used to validate or confirm trends identified by the other epidemiological indicators. Moreover, wastewater analysis has the important advantage of being able to provide data within a short time (days to weeks from sampling) and for a relatively large part of the population; therefore, wastewater-based epidemiology could be used as a first 'alert' tool in the identification of new trends in drug consumption or the use of new substances.

The potential for wastewater-based epidemiology to be used in the evaluation of the effectiveness of interventions that target drug supply (e.g. law enforcement) or drug demand (e.g. prevention programmes or public health campaigns) has not yet been explored. In order to start exploring these potential wastewater-based epidemiology applications, a close collaboration between the different stakeholders involved, including epidemiologists, legal authorities and the scientists applying wastewater analysis, is highly recommended.

Further developments can be expected with regard to widening the application of this interdisciplinary approach within more holistic epidemiological studies of societal health. In fact, wastewater-based epidemiology has the potential not only to provide estimates of a broad number of lifestyle factors that influence health (i.e. illicit drugs, alcohol, tobacco and the use of counterfeit medicines), but also to provide information about health and illness within a community. For instance, it could potentially give information about diet, diseases, health status and exposure to environmental and food contaminants. Some of these topics are now under investigation within a Marie Curie Initial Training Network project (SEWPROF) entitled 'A new paradigm in drug use and human health risk assessment: sewage profiling at the community level' (www.sewprof-itn.eu). The network links 16 leading European institutions from 12 countries and combines European expertise in wastewater-based epidemiology and related areas.

To conclude, future directions for wastewater research include studies aimed at advancing the identification of drugs and their metabolites, and minimising the uncertainties related to sampling, measurement and back-calculation methods. Better integration of this novel methodology with existing epidemiological indicators will allow for a more holistic understanding of societal health. The first multi-approach studies suggest that wastewater analysis can predict results from population surveys. Closer collaboration between epidemiologists and legal authorities will improve the perception of the 'true' drug situation, and allow for a better evaluation of interventions. Finally, the ethical rules for wastewater-based epidemiology are yet to be established. Since the approach is non-invasive, and does not allow for identification of drug-using individuals, it does not give rise to any obvious ethical issues. However, thorough study design and cautious management of relationships with research partners (e.g. prisons or forensic authorities) may be needed to protect the anonymity of sample providers in the case of studies of small communities in order to prevent stigmatisation. Special care is also suggested with regard to ensuring accurate communication of results to the media. The field awaits the establishment of best practices, taking into consideration the ethical aspects of wastewater research.

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# Appendix

A list of all the studies on wastewater-based epidemiology published by early 2015 is presented here. Studies in bold are those that report information on illicit drug use.

| Study | Country | Substances investigated | Application |
|---|---|---|---|
| Jones-Lepp et al., 2004 | United States | Methamphetamine, MDMA | Presence in effluents from three wastewater treatment plants in Nevada, Utah and South Carolina |
| **Zuccato et al., 2005** | **Italy** | **Cocaine, benzoylecgonine** | **Estimation of cocaine use in four Italian cities** |
| Castiglioni et al., 2006 | Italy, Switzerland | Cocaine and metabolites, amphetamines, opioids, cannabinoids | Presence in influents and effluents from two wastewater treatment plants in Milan and Lugano |
| Hummel et al., 2006 | Germany | Benzoylecgonine, opioids | Presence in influents and effluents from 12 wastewater treatment plants and 11 rivers |
| Huerta-Fontela et al., 2007 | Spain | Cocaine, amphetamines, ketamine, LSD, PCP, fentanyl | Presence in influents and effluents from 16 wastewater treatment plants and six rivers in Catalonia |
| **Bones et al., 2007** | **Ireland** | **Cocaine, benzoylecgonine** | **Estimation of cocaine use in Dublin and surroundings** |
| Boleda et al., 2007 | Spain | Opioids, tetrahydrocannabinol, fentanyl | Presence in influents and effluents from five wastewater treatment plants in Catalonia |
| **Zuccato et al., 2008** | **Italy, Switzerland, United Kingdom** | **Cocaine, heroin, amphetamines, cannabis** | **Back-calculation of consumption in three cities (Milan, Lugano and London)—comparison with prevalence data** |
| Gheorghe et al., 2008 | Belgium | Cocaine, benzoylecgonine, ecgonine methyl ester | Presence in five wastewater treatment plants and three rivers |
| Kasprzyk-Hordern et al., 2008 | England | Cocaine, benzoylecgonine, amphetamine | Presence in influent and effluent from one wastewater treatment plant and one river |
| Postigo et al., 2008 | Spain | Cocaine, benzoylecgonine, amphetamines, opioids, cannabinoids, LSD | Presence in influents and effluents from four wastewater treatment plants on the east coast |
| **Huerta-Fontela et al., 2008** | **Spain** | **Cocaine, amphetamines, ketamine** | **Presence in 42 wastewater treatment plants (influents and effluents) and loads per capita in north-east Spain** |
| **Kasprzyk-Hordern et al., 2008** | **United States** | **Cocaine and metabolites, amphetamines, ketamine, PCP** | **Calculation of loads (mg/person/day) in seven wastewater treatment plants** |
| **van Nuijs et al., 2009** | **Belgium** | **Cocaine, benzoylecgonine** | **Back-calculation of cocaine consumption from 41 wastewater treatment plants** |
| Bijlsma et al., 2009 | Spain | Cocaine and metabolites, amphetamines, THC-COOH | Presence in influent and effluent from one wastewater treatment plant (weekdays and weekends) in Castellon |
| Bartelt-Hunt et al., 2009 | United States | Amphetamine, methamphetamine | Presence in effluent from one wastewater treatment plant and four rivers in Nebraska |
| Cecinato et al., 2009 | Italy, Portugal, Serbia, Algeria, Chile, Brazil | Cocaine | Presence in airborne particles |
| **Kasprzyk-Hordern et al., 2009** | **United Kingdom** | **Cocaine, benzoylecgonine, amphetamine** | **Back-calculation of cocaine use in two wastewater treatment plants in South Wales** |
| **Mari et al., 2009** | **Italy** | **Cocaine, heroin** | **Calculation of loads in one wastewater treatment plant in Florence** |
| **Banta-Green et al., 2009** | **United States** | **Cocaine, methamphetamine, MDMA** | **Calculation of index loads (mg/person/day) in 96 municipalities in the state of Oregon** |
| Gonzalez-Marino et al., 2009 | Spain | Amphetamines | Presence in influents and effluents from four wastewater treatment plants in north-west Spain |
| **Postigo et al., 2010** | **Spain** | **Cocaine, amphetamines, heroin, cannabis** | **Estimation of consumption based on seven wastewater treatment plants in north-east Spain** |
| **Terzic et al., 2010** | **Croatia** | **Cocaine, amphetamines, heroin, cannabis** | **Estimation of consumption based on one wastewater treatment plant in Zagreb** |

| Study | Country | Substances investigated | Application |
|---|---|---|---|
| **Karolak et al., 2010** | **France** | **Cocaine, MDMA** | **Estimation of consumption based on four wastewater treatment plants in Paris** |
| **Metcalfe et al., 2010** | **Canada** | **Cocaine, amphetamines** | **Estimation of consumption based on three wastewater treatment plants** |
| Berset et al., 2010 | Switzerland | Cocaine, amphetamines, opioids | Presence in influents and effluents from five wastewater treatment plants |
| Gonzalez-Marino et al., 2010 | Spain | Cocaine, amphetamines, opioids, cannabis | Presence in influents and effluents from five wastewater treatment plants and four rivers in north-west Spain |
| **Zuccato et al., 2011** | **Italy** | **Cocaine, heroin, amphetamines and cannabis** | **A 4-year monitoring campaign for estimation of use in the north of Italy** |
| **Reid et al., 2011a** | **Norway** | **Benzoylecgonine, methamphetamine** | **Estimation of consumption based on one wastewater treatment plant in Oslo** |
| **Irvine et al., 2011** | **Australia** | **Benzoylecgonine, methamphetamine, MDMA** | **Estimation of consumption based on four metropolitan and three regional plants in South Australia** |
| **Postigo et al., 2011** | **Spain** | **Cocaine, cannabis, opioids, amphetamines, LSD** | **Estimation of consumption in a prison in Catalonia** |
| **van Nuijs et al., 2011** | **Belgium** | **Cocaine, amphetamines, heroin** | **A 1-year estimation campaign in Brussels** |
| **Gerrity et al., 2011** | **United States** | **Cocaine, amphetamines, morphine** | **Estimation of use during a sporting event (the National Football League Super Bowl)** |
| **Lai et al., 2011** | **Australia** | **Cocaine, amphetamines, THC-COOH** | **Estimation of use based on one wastewater treatment plant in South-East Queensland** |
| **Reid et al., 2011b** | **Norway** | **Cocaine, amphetamines** | **Estimation of use in different city locations and during special events in Oslo** |
| Kasprzyk-Hordern and Baker, 2012 | United Kingdom | Amphetamine, methamphetamine, MDMA, MDA | Profile of chiral drugs in wastewater |
| **Prichard et al., 2012** | **Australia** | **Cocaine, methamphetamine, MDMA,** | **Estimation of use in two consecutive years (2009 and 2010)** |
| **Baker et al., 2012** | **Czech Republic** | **Cocaine and metabolites, MDMA** | **Estimation of use based on one wastewater treatment plants (1-week monitoring)** |
| Bijlsma et al., 2012 | The Netherlands | Cocaine, amphetamines, ketamine, opioids, THC-COOH | Presence in influents and effluents from five wastewater treatment plants |
| **Brewer et al., 2012** | **United States** | **Cocaine, benzoylecgonine** | **Estimation of use in one municipality** |
| **Thomas et al., 2012** | **Europe (19 cities)** | **Cocaine, amphetamines, cannabis** | **Estimation of use in 19 European cities** |
| **Lai et al., 2013a** | **Australia** | **Cocaine, methamphetamine, MDMA, cannabis** | **Estimation of use during holidays in urban, rural and vacation areas** |
| van Nuijs et al., 2014 | Belgium | Mephedrone, ketamine, MDPV, cannabis | Monitoring in three wastewater treatment plants |
| Reid et al., 2013 | Norway | Cathinone, mephedrone, PMA, PMMA, synthetic cannabinoids | Monitoring in three Norwegian cities |
| Burgard et al., 2013 | United States | Amphetamine | Presence in wastewater from a college campus |
| Repice et al., 2013 | Italy | 12 licit and illicit drugs | Presence in a wastewater treatment plant in Verona, Italy |
| **Chen et al., 2013** | **Australia** | **MDMA, cathinones** | **Estimation of use in a 3-year campaign in Adelaide** |
| **Lai et al., 2013b** | **Asia** | **Cocaine, MDMA, methamphetamine, ketamine** | **Estimation of use in the largest wastewater treatment plant in Hong Kong** |
| **Lai et al., 2013 c** | **Australia** | **Cocaine, methamphetamine, MDMA, cannabis, benzylpiperazine, mephedrone, methylone** | **Estimation of use at an annual music festival** |
| **Nefau et al., 2013** | **France** | **Cocaine, MDMA, amphetamine, cannabis, methadone** | **Estimation in 25 wastewater treatment plants** |
| Mwenesongole et al., 2013 | United Kingdom | New synthetic drugs, amphetamine, methamphetamine, cocaine | Screening of 25 drugs in wastewater from Cambridgeshire |
| **Yargeau et al., 2014** | **Canada** | **Amphetamine, methamphetamine, MDMA, cocaine, heroin, ketamine** | **Estimation of use in two Canadian cities** |
| **Brewer et al., 2014** | **United States** | **Cocaine, methamphetamine** | **Estimation of use in a prison** |
| **Subedi and Kannan, 2014** | **United States** | **Cocaine, amphetamine, methamphetamine, MDMA, methadone, morphine** | **Estimation of mass loads in two wastewater treatment plants in the Albany area, New York** |

| Study | Country | Substances investigated | Application |
|---|---|---|---|
| Khan et al., 2014 | Asia | Benzoylecgonine, amphetamine, MDMA, methamphetamine, ketamine, methadone, heroin, THC-COOH | Estimation of use in four Chinese megacities |
| Li et al., 2014 | Asia | Amphetamine and methamphetamine | Estimation of use in Beijing |
| Mackulak et al., 2014 | Slovakia | Cocaine, amphetamine, methamphetamine, MDMA, cannabis | Estimation of use in eight cities |
| Östman et al., 2014 | Sweden | Benzoylecgonine, amphetamine, MDMA, methamphetamine, codeine, morphine, methadone, EDDP | Estimation of use in 33 municipalities |
| Kankaanpaa et al., 2014 | Finland | Benzoylecgonine, amphetamine, MDMA, methamphetamine, methadone, MDPV | Estimation of use in 10 municipalities |
| Vuori et al., 2014 | Finland | Benzoylecgonine, amphetamine, MDMA, methamphetamine, methadone, MDPV, THC-COOH | Estimation of use in nine municipalities |
| Damien et al., 2014 | Martinique, Caribbean | Cocaine | Estimation of quantity used based on four wastewater treatment plants |
| Kim et al., 2015 | South Korea | Amphetamine, methamphetamine, codeine | Estimation of use in five South Korean cities |

EDDP, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine; LSD, lysergic acid diethylamide; MDA, 3,4-methylenedioxyamphetamine; MDPV, methylenedioxypyrovalerone; PCP, phencyclidine; PMA, *para*-methoxyamphetamine; PMMA, *para*-methoxy-*N*-methylamphetamine; THC-COOH, 11-*nor*-9-carboxy-delta-9-tetrahydrocannabinol.

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