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DOI
10.1007/164_2018_111

Publication date
2018

Document Version
Final published version

Published in
New Psychoactive Substances

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H. H. Maurer, S. D. Brandt (eds.), *New Psychoactive Substances*,
Handbook of Experimental Pharmacology 252, https://doi.org/10.1007/164_2018_111

Contents

Abstract

Wastewater-based epidemiology (WBE) complements existing epidemiology-based estimation techniques and provides objective, evidence-based estimates of illicit drug use. After consumption, biomarkers – drugs and their metabolites – excreted to toilets and flushed into urban sewer networks can be measured in raw wastewater samples. The quantified loads can serve as an estimate for the collective consumption of all people contributing to the wastewater sample. This transdisciplinary approach, further explained in this chapter, has developed, matured and is now established for monitoring substances such as cocaine and amphetamine-type stimulants. Research currently underway is refining WBE to new applications including new psychoactive substances (NPS).

Keywords

Illicit drugs · New psychoactive substances · Public health · Wastewater-based epidemiology

1 From Sewage to Drug Consumption: The Concept of Wastewater-Based Epidemiology

Over the last decade, wastewater-based epidemiology (WBE) has been developed as a complementary approach to obtain an objective estimate for the collective drug consumption of an entire population, typically at a city level (EMCDDA 2016; Ort et al. 2014a). Along with other data such as information on drug-related criminality, hospital records, and population surveys, WBE allows a better understanding of drug use and gives policymakers a wealth of information useful to take evidence-based decisions and assess the impact of prevention strategies and enforcement actions (Been et al. 2016b, c; Thomaidis et al. 2016; Zuccato et al. 2016). WBE has demonstrated its value and is well established for monitoring conventional illicit drugs such as cocaine, MDMA, amphetamine, and methamphetamine (EMCDDA 2016). It also has

potential to monitor trends in new psychoactive substance (NPS) use following the same workflow used for "classical" drugs. In this chapter, the principles of WBE, strengths and challenges for conventional drugs are explained first. Furthermore, the focus of Sect. 5 is on the current status and potential of WBE for monitoring NPS.

WBE relies on the fact that human metabolic excretion products resulting from substance use are collected and pooled by central sewage systems. Therefore, the measurement of target metabolic residues in untreated urban wastewater allows for identifying the use of specific substances, providing valuable data on the amount and types of substances consumed by the population contributing to the sampled wastewater (van Nuijs et al. 2011a). Hence, consumption can be estimated following the subsequent steps (Gracia-Lor et al. 2017a; Zuccato et al. 2008; see also scheme in Fig. 1):

1. Collection of a representative composite sample – typically over 24 h – of influent wastewater (see Sect. 3.1). Result: a small, pooled wastewater sample (100 mL–1 L) resembling the properties of the entire wastewater volume entering a wastewater treatment plant (WWTP), normally several thousand cubic meters of wastewater per day
2. Chemical analyses for selected biomarkers (see Sect. 3.2). Result: average concentration of biomarker (ng/L) over the sampled period of time in the entire wastewater volume
3. Multiplication of average concentrations (ng/L) with measured flow rates of wastewater (m^3/day). Result: Biomarker mass load in the wastewater (mg/day)
4. Normalization of biomarker mass load by the number of people, who contributed to the sample, to facilitate comparison among cities of different sizes. Result: population-normalized biomarker load in the wastewater (mg/day/1,000 people)
5. Application of a specific correction factor (CF), which considers the average excretion rate of a given drug residue and the molecular mass ratio of parent drug/metabolite. Result: estimation of total consumption of a substance (mg/day/1,000 people)

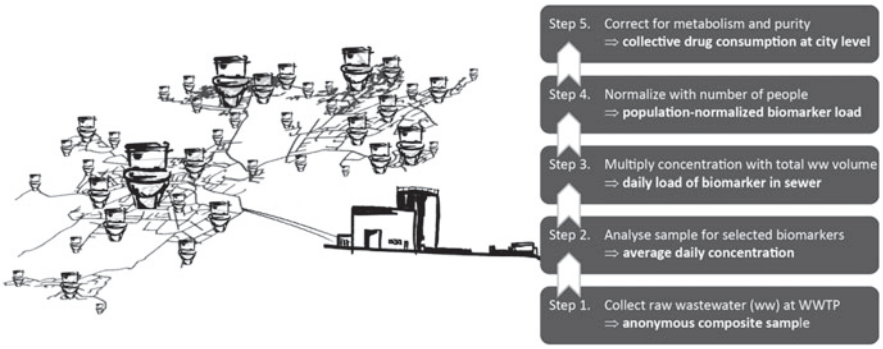

Fig. 1 Key steps in estimating illicit drug use at the community level based on urban wastewater collected at the influent of a wastewater treatment plant (WWTP) (modified from Thomas et al. 2012)

Although this concept is relatively simple, various factors influencing its reliability need to be fully understood before implementation on a large scale is possible (Castiglioni et al. 2013). Among the most important factors are human metabolic and excretion patterns of the investigated drugs, characteristics of sewer systems (the number of people contributing to a sample, wastewater volumes), and understanding the fate of biomarkers in the sewer system (e.g., stability, degradation, partitioning, or sorption in the sewer [McCall et al. 2016; Ramin et al. 2017]).

The WBE approach can be designed to study substance use in a specific area or to compare the use among different areas during defined periods of the year or over successive years (Ort et al. 2014a). As a result, it has the potential to provide almost real-time information on drug use patterns, both geographical and temporal, including during special events or holidays.

However, there are a few limitations of the approach, as WBE cannot provide information on prevalence and frequency of use, route of administration, main classes of users, and purity of substances. Furthermore, translating the total consumed amounts into the corresponding number of average doses is complicated as amounts of drugs taken vary widely, and purity levels fluctuate (EMCDDA 2016). WBE is therefore proposed to complement, rather than to replace established socio-epidemiological monitoring tools (Been et al. 2016a; EMCDDA 2016).

2 Choice of Biomarkers

Specific metabolic excretion residues (biomarkers) that enter urban wastewater may either be the parent substance or a metabolite. Metabolites are preferable since the presence of the parent compound may also originate from disposal/dumping of the unused drug into the sewer system. In cases where unique metabolic products are lacking, the presence of dumping can be addressed by chiral analysis and their characteristic stereoselective metabolism patterns if the drug of question contains asymmetric centers (see Sect. 3.2). The selection of a specific biomarker is not an easy task, because it should fulfill several requirements to ensure the reliability of back-calculated estimates. An ideal biomarker should be excreted in consistent amounts in urine, be detectable in urban wastewater, be stable in wastewater, and be released into sewers only as a result of human excretion (EMCDDA 2016). Therefore, all these characteristics should be verified carefully before a substance – parent or metabolite – can be used as a biomarker.

It is particularly relevant to assess the stability of a biomarker in wastewater, during transport in the sewer, sampling, and analysis. The degradation of a substance can easily occur in wastewater as a result of the high microbial activity typically found in sewers. For instance, it was shown that metabolites excreted as glucuronide conjugates (i.e., morphine 3β-glucuronide) are completely reverted into the free form in raw wastewater (Castiglioni et al. 2006; D'Ascenzo et al. 2003). An overview of the stability studies of the most common biomarkers was provided recently (EMCDDA 2016; McCall et al. 2016). It indicated that benzoylecgonine, the main metabolite of cocaine, and the parent substances, amphetamine, methamphetamine,

and MDMA are the most stable biomarkers for these illicit drugs. On the contrary, the specific metabolite of heroin, 6-monoacetylmorphine, was found to be highly unstable in wastewater, thus morphine appears to be so far the best option to estimate heroin consumption, although morphine therapeutic use should be taken into account. This requires a particular care to collect the most accurate figure of morphine use from prescriptions and sales reports, collected at local level if possible. Regarding cannabis, the main metabolite 11-nor-9-carboxy-delta-9-tetrahydrocannabinol (THC-COOH) was stable under relevant conditions (EMCDDA 2016). However, other important knowledge gaps as well as analytical challenges related to the determination of THC-COOH have been identified. A study focused on the initial aspects of the analytical procedure suggested a best practice protocol to handle samples in order to improve estimations of cannabis use (Causanilles et al. 2017). Recently, a new modeling framework to assess in-sewer stability was proposed taking into account realistic biotransformation conditions occurring in the sewer system (McCall et al. 2017). This approach, assessing the effect of instability in a specific catchment, is applicable to all substances and includes varying transformation potential of different biofilms in different pipes.

Once the specificity of a biomarker as indicator of human excretion is verified, it is possible to develop a correction factor (CF) to back-calculate the consumption of the parent substance. A CF takes into account the biomarker excretion profile and the molecular mass ratio of the parent drug to the metabolite (in the case the biomarker is a metabolite) (Zuccato et al. 2008). Due to the scant information on pharmacokinetic profiles of illicit drugs and the high variability of results, a comprehensive meta-analysis of the metabolic studies available was conducted in order to develop CFs as much reliable as possible. This was suggested first for cocaine (Castiglioni et al. 2013), and later implemented for the other main illicit drugs (Gracia-Lor et al. 2016). The procedure took into account not only the excretion profile of a drug but also the number of subjects involved in each study, and the frequency of use for each route of administration. This was necessary because different CFs were employed to estimate the use of the same substance. For instance, for cocaine the CFs ranged between 2.3 and 3.2 considering different excretion profiles from single studies. The meta-analysis study facilitated the integration of all available information and proposed a new corrected CF of 3.59 (Castiglioni et al. 2013). Nowadays, it is highly recommended to use reliable and homogeneous CFs to back-calculate illicit drugs use in different studies, ensuring a higher comparability of results from different research groups.

3 Best Practice Protocol

3.1 Sample Collection and Monitoring Schemes

WBE typically relies on routinely collected 24 h composite samples representing the wastewater from an entire day (daily average concentration). Typically, the sampling error for samples from large WWTPs, analyzed for high prevalence drugs, is usually

negligible compared to the overall uncertainty of WBE results (Castiglioni et al. 2013). However, in small WWTPs (e.g., Ort et al. 2014b) or effluents of individual premises – such as prisons or schools (e.g., Brewer et al. 2016; Burgard et al. 2013; Postigo et al. 2011) – and for low prevalence drugs analyzed in larger WWTPs, routine sampling may result in considerable sampling error (Ort et al. 2010c): When the toilet is flushed, the flush initially lasts a few seconds. In the sewer system, depending on layout, topography, and properties of pipes, the flush extends over time (effect of dispersion, Rieckermann et al. 2005) and may last several minutes at the influent of a WWTP (Ort and Gujer 2006; Ort et al. 2010b). If this flush was the only one containing the substance of interest, it would be challenging to properly capture this peak and obtain the true daily average concentration. With an increasing number of toilet flushes containing the same substance arriving at the WWTP, the temporal concentration pattern becomes less variable and, hence, it is less challenging to obtain a representative 24-h composite sample.

Besides the rather random, short-term variations described above, there can also be systematic diurnal variation (e.g., Brewer et al. 2012; Lai et al. 2013). To properly account for both types of variation, it is recommended to rely on a volume-proportional sampling mode with high temporal resolution. More examples and further details can be found in Ort (2014).

Most studies have to date investigated relatively short periods of consecutive days at different intervals, e.g., 1 week annually (e.g., Ort et al. 2014a) or selected months (e.g., Harman et al. 2011; Zuccato et al. 2011). There are, however, some data available from studies carried out at higher frequency over longer periods of time, i.e., daily over several months to years (e.g., Lai et al. 2015; Ort et al. 2014b; van Nuijs et al. 2011b). Such data are useful for studying long-term trends, or indeed seasonal variability of drug use. Ort et al. (2014b) have proposed a stratified random monitoring scheme as the most appropriate for this task. One implementation of "*stratified random*" is, for example, considering each weekday randomly once per quarter, resulting in 28 samples per year. The underlying day-to-day and seasonal variability is ideally quantified with a preliminary high-frequency monitoring campaign or, alternatively, with expert knowledge considering other evidence. Since wastewater samples are usually analyzed for multiple substances, the substance with the highest variability expected is decisive for the minimum number of monitoring days required to achieve a certain accuracy, e.g., to estimate an annual average. Monitoring over longer periods and with samples collected on nonconsecutive days does increase the time and effort required to coordinate the exercise, both from the perspective of the wastewater scientist and the wastewater treatment staff. Deciding on the most appropriate monitoring setup, therefore, depends on the research question and resources available and is best identified in a team consisting of epidemiologists being familiar with the region and experts performing the wastewater study.

3.2 Analytical Approaches and Recommendations

Analysis of drug residues in wastewater poses an analytical challenge due to the complexity of the wastewater matrix and the low concentration levels of drugs in question. This holds especially true for NPS because consumption of the latter has not reached levels comparable to those of more frequently used classic illicit drugs. While the most common way to pre-treat wastewater is by means of filtration to remove larger particulates, centrifugation is also being used (Bade et al. 2017; González-Mariño et al. 2016b; Hernández et al. 2018 [open access]).

The rather complex matrix requires specific treatment steps. Target compounds can interact with the particulates in the sample, the sample bottle material, the materials used for the sample handling (Causanilles et al. 2017), and matrix components can interfere during the ionization phase before entering the mass spectrometer. These parameters can lead to suppression of the detector signal up to 80% (Bijlsma et al. 2013) when extracting 100 mL of sewage water influent. Several options are available to tackle these effects: matrix matched calibration, standard addition, or the addition of isotope-labeled internal standards prior to sample handling.

Sample preparation methods (aimed to remove interferences and to concentrate analytes) usually employ solid phase extraction (SPE) to extract drug residues from the liquid phase of wastewater and accelerated solvent extraction or microwave assisted extraction to extract the residues from the solid phase of wastewater (i.e., suspended particulate matter) (Baker and Kasprzyk-Hordern 2011; Evans et al. 2015; Křesinová et al. 2016; Petrie et al. 2016b). Sorbents most often used in SPE for illicit drugs are reversed phase (RP) polymers or mixed mode RP-cationic exchange materials. The extracts are then analyzed by means of ultrahigh performance liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). For chromatographic separation, a C18 column is typically used for classic illicit drugs, but other options have been shown to be successful for NPS analysis, such as polar endcapped C18 (Senta et al. 2015), biphenyl (Kinyua et al. 2016), pentafluorophenyl (Borova et al. 2015), and HILIC (Kinyua et al. 2015). The development of analytical equipment also offers the possibility of directly injecting large injection volumes (Bagnati and Davoli 2011; Berset et al. 2010; Chiaia et al. 2008).

Usually, triple quadrupole mass spectrometers are used for sensitive and selective quantitative multi-residue measurements of target analytes (Hernández et al. 2018 [open access]). As an example, Fig. 2 represents UHPLC-MS/MS chromatograms corresponding to the detection of mephedrone and butylone in influent wastewater. However, high resolution mass spectrometry (e.g., QTOF and Orbitrap) is gaining popularity due to its high selectivity and mass accuracy and its ability to not only quantify target drugs but also search for unknown compounds and facilitate retrospective search for analytes (Bijlsma et al. 2013; González-Mariño et al. 2016a; Hernández et al. 2011, 2018 [open access]). The search for the presence of NPS is often carried out through screening using a list of suspected and known analytes that is based on existing inventories, such as the EMCDDA Implementation Reports published annually within the European Union (EMCDDA 2016).

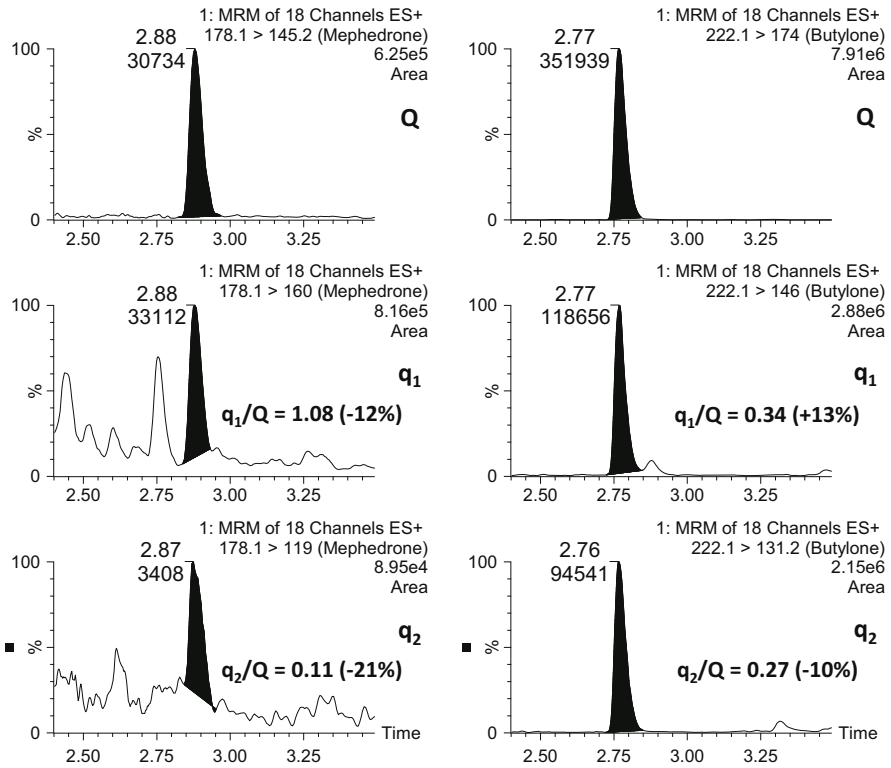

Fig. 2 UHPLC-MS/MS chromatograms corresponding to the positive finding of mephedrone (left) and butylone (right) in influent wastewater. **Q** quantification transition; $\mathbf{q_1}$ and $\mathbf{q_2}$ confirmation transitions. Retention times and ion ratios (q/Q) deviations were within tolerance limits (<0.1 min and <30%, respectively) in relation to reference standards, which allowed confirming the identity of the compounds in samples

Correct quantification of drug residues is of critical importance in wastewater analysis. Therefore, robust method validation, intra- and inter-day quality assurance, and comprehensive quantification protocols (van Nuijs et al. 2018) including labeled internal standards (preferably single isotope-labeled internal standards for each target compound) to correct for sample preparation and analysis derived errors, i.e., matrix effects, are now considered to be the norm. Several isotope-labeled NPS are commercially available (Borova et al. 2015).

The usage of chiral chromatography provides yet another dimension to the interpretation of wastewater-derived analysis since many NPS have one or more chiral centers. Chiral chromatography allows for verification of origins of biomarkers in wastewater (e.g., differentiation between consumption/direct exposure and other disposal routes, e.g., disposal of unused drugs) (Kasprzyk-Hordern and Baker 2012). This is due to stereoselective metabolism of chiral drugs taking place in humans. For example, MDMA is synthesized as a racemate. However,

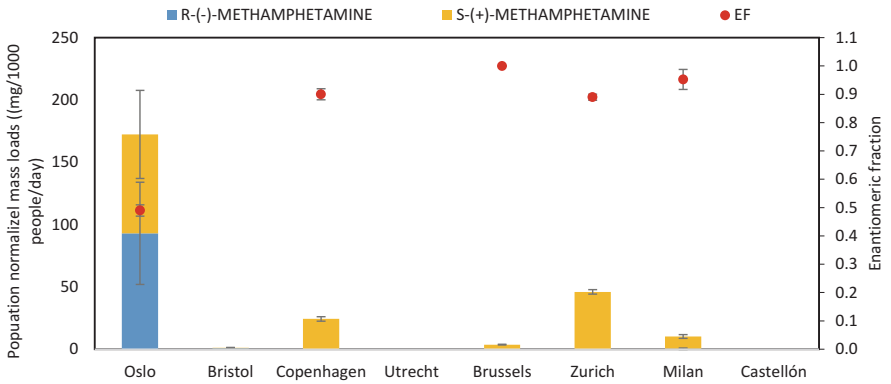

Fig. 3 Weekly average methamphetamine population-normalized mass loads and enantiomeric fraction values in a European monitoring campaign (modified from Castrignanò et al. 2018)

after its administration and stereoselective human metabolism, it is excreted with urine enriched with the (*R*)-(−)-enantiomer. Direct disposal of racemic MDMA as a result of police raid in the Netherlands (see Sect. 3.5) is one of the prime examples (Emke et al. 2014), where a chiral CBH column was used. Chiral chromatography can also help with verification of potency of drugs used in studied communities as seen in recent pan-European study investigating methamphetamine use across Europe. High loads of racemic methamphetamine were detected in Oslo. This is in contrast to other European cities where (*S*)-(+)-methamphetamine was the predominant enantiomer. This indicates different methods of methamphetamine synthesis and/or trafficking routes in Oslo, compared with the other cities tested (see Fig. 3) (Castrignanò et al. 2018).

3.3 Population Size Estimation

An accurate estimation of the size of the population that is served by a WWTP in order to normalize biomarker mass loads to the per capita level is currently one of the biggest bottlenecks of WBE. Accurate population size permits temporal and spatial comparisons to be made (Castiglioni et al. 2013; Daughton 2018). Until now, WBE studies used data that originated from public surveys (i.e., census data) and a wide array of demographic statistics to estimate the size of the population under investigation. However, this type of information is static and mostly captures only the de jure population (i.e., formal residents), whereas in WBE studies, it is more relevant to have knowledge on the de facto population, i.e., those being present in the WWTP catchment area, regardless of the location of their formal residence (Daughton 2012). Therefore, there is a need for alternative ways to estimating the de facto population of a WWTP catchment area.

Some research has been performed on the hydrochemical parameters that are routinely determined in the WWTPs (chemical oxygen demand (COD), biological oxygen demand (BOD), and total nitrogen and phosphorus) as markers of population size (Been et al. 2014; van Nuijs et al. 2011b). Been and co-workers showed promising results for normalization to NH_4^+–N load as dynamic measures used in WBE studies (Been et al. 2014). However, an important limitation of using hydrochemical parameters is that they often not only reflect human activities but that they can also be influenced by other external events, such as industrial or agricultural activities.

Further research into population estimation of a WWTP catchment area has been guided towards direct indicators of human activity and metabolism, i.e., naturally occurring and synthetic xenobiotics as well as products of endogenous metabolism in wastewater. However, to be a suitable candidate biomarker, several criteria have to be met (Daughton 2012; Gracia-Lor et al. 2017b):

1. Unique to human activity and excreted in substantial amounts
2. Low inter- and intra-individual variance in the daily excretion; excretion not affected by variables such as season, weather, and geographic location
3. Stable in wastewater during in-sewer transport and during storage until analysis

Several xenobiotics and endogenous compounds have been studied in this regard, such as pharmaceuticals, artificial sweeteners, nicotine, caffeine, creatinine, 5-hydroxyindoleacetic acid, cholesterol, cortisol, and androstenedione (Brewer et al. 2012; Chen et al. 2014; Lai et al. 2011, 2015; O'Brien et al. 2014, 2017; Senta et al. 2015). However, to date, none of the investigated population size markers have yet met all necessary criteria.

Recently, Thomas et al. proposed an alternative way based on mobile device-based activity to provide a dynamic way of the de facto population estimation which could be useful in WBE studies (Thomas et al. 2017). The authors concluded from their research that mobile device analytics might be promising since it addresses the uncertainties associated with both short- and long-term changes in population. In addition, it provides WBE scientists with population data that can be used to investigate and validate new (or improved) (hydro)chemical, hydrodynamic population markers of population, in cases where a mobile device monitoring option is not available.

3.4 Catchment and Sewer System Characterization

Besides the three most important variables to calculate population-normalized biomarker loads, that is: (1) biomarker concentration, (2) wastewater volume, and (3) population size, it is required to collect additional information about catchment characteristics. This is essential for data quality assurance and interpretation of data. This involves details on:

Technical	Nontechnical
• Type and operation of sewer systems • Sample collection (see Sect. 3.1) • Unusual operating conditions of sewer system	• Special events in the catchment (e.g., festivals and holidays)

Operators of WWTPs and sewer systems are the professionals who provide subsamples of their routinely collected samples, which they normally collect and analyze for standard parameters to confirm efficacy of the treatment process. These are the professionals who can also provide relevant details about the catchment and sewer system characteristics. Standardized questionnaires facilitate collection of these data. An example can be found in the supporting information of Castiglioni et al. (2013).

3.4.1 Type and Operation of Sewer Systems

In a separate sewer system, urban wastewater (from households and industry generated during irrespective of weather) and surface runoff (originating from precipitation) are collected in two different pipe systems. In contrast, in a combined sewer system, surface runoff during wet weather dilutes municipal wastewater. However, the increased wastewater flow results in the same illicit drug loads, assuming that illicit drugs are exclusively contained in the municipal wastewater as long as the WWTP can cope with the entire wastewater flow. If not, for example, during wet weather, a part of the wastewater can be discharged with no or only minor treatment into the environment and this part is not captured in the flow measurement in the influent of the WWTP. Potentially, during wet weather not all wastewater is arriving at the WWTP.

Pumps, detention tanks, and other special infrastructure can impact on the temporal pattern of pollutant loads and concentrations and influence the specification to obtain a representative sample (see Sect. 3.1 and cited literature therein). Abnormal operation in the sewer systems or the WWTP (e.g., maintenance) or normal operation during wet weather may imply that not all wastewater generated in the catchment arrives at the WWTP, or with a delay. Therefore, it is important to identify "normal" dry weather wastewater volumes and compare them with wastewater volumes measured during the monitoring period.

3.4.2 Special Events in the Catchment

Technical aspects related to sewer systems do not affect drug consumption, but can lead to undesirable consequences on sampling specification or observed biomarker loads (see above). If not specifically asked for from WWTP operators, this information is hidden and unknown to researchers applying WBE. In contrast, events such as holiday weeks, public holidays, festivals, sporting events, etc., can have two effects: (1) emergence of a different population size than normal (important for normalization of drug loads) and/or (2) different composition of the population. These nontechnical abnormalities are more obvious than the technical ones and therefore, not elaborated on in more detail. The quantification of the effect of the two aspects is difficult, but at least data can be flagged and compared to

baseline data for days where no such special events are known. Another special event that introduces further variables is represented by direct discharges of unconsumed drugs (see Sect. 3.5).

3.5 Direct Discharges of Unconsumed Drugs

One important issue that should not be overlooked in the evaluation of WBE data are the direct discharges into the sewer of biomarkers used for the community-wide drug use estimation. In the past 7 years, several types of evidence for direct discharges have become eminent.

The first signs of direct discharges of illicit drugs into sewer systems became apparent when monitoring the wastewater of Schiphol airport, a major international airport in the Netherlands (Bijlsma et al. 2012). The ratio of the levels of cocaine and its metabolite benzoylecgonine was not corresponding to commonly observed in-sewer ratios reflecting human metabolism, suggesting that drug traffickers were unloading their goods prematurely (Bijlsma et al. 2012). In another case reported in the UK, the direct disposal of an estimated 915 fluoxetine capsules was confirmed by chiral analysis (Petrie et al. 2016a).

The first multicity campaign in Europe executed in 2011 (Thomas et al. 2012) revealed another case of a direct discharge. The levels of MDMA observed in 2011 in the sewer of the city of Utrecht in the Netherlands were about 30 times higher than recorded by Bijlsma et al. (2012) in 2010 in the same city. Through the separation and quantification of the two MDMA enantiomers, it was concluded (Emke et al. 2014) that the MDMA observed in the wastewater had not passed the human body since the enantiomer ratio was racemic, whereas human metabolism changes the racemic composition to a higher contribution of the (*R*)-(−)-enantiomer (Moore et al. 1996). Information obtained from the police resulted in the conclusion that most likely under the pressure of a police raid, 30 kg of MDMA were dumped into the sewer close to the WWTP.

In the following years (2012–2017), during a European multicity monitoring exercise carried out by the Sewage Analysis CORe group Europe (SCORE), unexplained high levels of MDMA and amphetamine were encountered each year in the wastewater from the city of Eindhoven in the South of the Netherlands (Ort et al. 2014a; Thomas et al. 2012). The catchment area of the sewer system of Eindhoven is relatively large and includes many rural communities nearby. The southern part of the Netherlands is known for its production sites of MDMA and amphetamine (EMCDDA 2015). The illegal producers need to dispose of synthesis waste contaminated with final products as well as intermediate products and synthesis by-products. The synthesis waste is ranging from highly acidic (pH < 1) to highly basic (pH > 10) or it contains organic solvents which all pose a threat to relatively small WWTPs. A typical synthesis for the production of amphetamine results in 20–30 kg of chemical waste for 1 kg of amphetamine (EMCDDA 2015). Although the high levels of chemical waste, presumably from illegal syntheses of amphetamine or MDMA, disposed in the sewer system of

Eindhoven have not affected the functioning of its WWTP in the past years, it was impossible to calculate consumption figures for both illicit drugs.

In the Netherlands, a shift towards more professionalized laboratories has been reported. In 2014, a complete laboratory ordered in China was confiscated (Boerman et al. 2017). After mephedrone was banned in the EU at the end of 2010 following a risk assessment carried out under the auspices of the EMCDDA (2011), criminals could no longer import the drug directly and switched to local production of mephedrone for the international market. Also production sites for methamphetamine and captagon (fenethylline) were discovered in the Netherlands (Boerman et al. 2017). One drug that gained some popularity in specific subpopulations in the Netherlands was 4-fluoroamphetamine (4-FA) which is now a listed chemical in the Netherlands since 2017 (WHO 2017). Nowadays, 4-fluoromethamphetamine (4-FMA) appears to be gaining popularity. It is available through online shops and is marketed as an alternative for the now controlled 4-FA. Although the extent of illegal NPS production is not exactly known, it is expected that in the years to come direct discharges of the parent compounds and their synthesis waste products will be encountered.

In a case also reported in the South of the Netherlands described by Emke et al. (2018), it was shown that relatively small WWTPs are highly vulnerable to direct discharges of acidic waste into sewers that arise from drug synthesis. The active sludge of such WWTPs can become completely inactivated when exposed to the amounts of acidic waste dumped, resulting in malfunctioning or termination of the treatment process. Restoration of the process can only be done by renewed inoculation of active sludge. The study of Emke et al. (2018) also showed that synthesis markers (precursors, intermediates, and synthesis by-products such as benzylmethyl ketone, alpha-phenyl acetoacetamide, *N*-formylamphetamine, and 5-phenyl-4-methylpyrimidine), typically of the synthesis pathways employed, are usually detectable in the chemical waste discharged into the sewer system. The identification of specific synthesis markers in wastewater is not only useful to establish whether a direct discharge of waste has occurred but can also serve for forensics purposes to determine which (pre)precursors were used in the manufacturing of the drug.

3.6 Ethical Aspects and Communication (Media Attention)

WBE can be applied for different purposes. It was branded in the context of estimating illicit drug use, but it has also been the approach to quantify, e.g., the contribution of health care facilities to the load of pharmaceutically active ingredients in municipal wastewater (e.g., Langford and Thomas 2009; Ort et al. 2010a), alcohol or nicotine consumption (e.g., Baz-Lomba et al. 2016; Castiglioni et al. 2015; Gatidou et al. 2016; Reid et al. 2011; Senta et al. 2015), and other health parameters (e.g., obesity; Newton et al. 2015), in addition to NPS use

(e.g., Kinyua et al. 2015). Furthermore, numerous other potential applications can be thought of (Thomas and Reid 2011), such as sporting events (doping by athletes [Causanilles et al. 2018], but also changes in drug use in the general public, e.g., Gerrity et al. 2011).

As with many other research endeavors, it is highly recommended that researchers inform relevant partners about intentions of the study, particularly WWTP operators or owners of individual premises (e.g., hospitals, or prisons). If these stakeholders are not supportive, it can be difficult to obtain a wastewater sample. Before publication, they should also be proactively informed about the outcome of the study. From our experience, in most places, for most applications of WBE, no anonymization of data/location was required since no major ethical concerns arise when studies are carried out in the wastewater of large populations (Hall et al. 2012). It is impossible to identify individuals (respect for autonomy, i.e., consent and confidentiality) and there is no direct harm to participants (non-maleficence). In this setting, the approach also satisfies the principle of beneficence and distributive justice, since WBE can potentially inform on interventions aimed at improving public health and no social groups are singled out (Hall et al. 2012). However, this might be different, for example, if a socially disadvantaged suburb or district or individual premises (entertainment venues, prisons [e.g., Brewer et al. 2016; Postigo et al. 2011; Van Dyken et al. 2016], schools/colleges/universities [e.g., Burgard et al. 2013], and workplaces) are investigated, since indirect harms of WBE cannot be excluded. In such cases, potential policy responses (e.g., collective punishment in a prison) should be discussed with relevant stakeholders as part of the study, before carrying out the actual analyses. It should also be noted that the logistic and technical efforts to obtain representative samples from individual venues are substantially higher than collecting a sample from a WWTP (Ort 2014).

While factual reporting (method, accuracy, and potential benefit) by media to the general public is beneficial, negative media coverage can result in sensationalism and amplification of stigmatization (for more details, see Prichard et al. 2014). Typically, human research ethics committees have not required ethical review of WBE studies and, therefore, Prichard et al. (2014) recommended the development of ethical guidelines (see also SCORE 2017a). Special attention should be given to regions/groups where specific sensitivities may apply and how media communication of findings can be improved (e.g., asking journalists to send their article for comment before publication or avoiding quotations being taken out of context in a TV interview).

4 Applications of Wastewater-Based Epidemiology

Rapid progress in the field of WBE has been facilitated through successful international collaboration. The SCORE group, established in 2010, brought together experts working on illicit drugs analysis from several European countries (www.score-cost.eu) with the aim of undertaking international studies comparing illicit drug use between major European cities and evaluating different analytical

procedures being used in different labs. Supported by the EMCDDA, the group established a wider European network (now including >20 countries) that for the first time allowed an international study on the analysis of wastewater to be performed with the aim of estimating the use of illicit drugs on a European scale (Thomas et al. 2012).

Europe-wide monitoring campaigns are now undertaken for 1 week every year for amphetamine, cocaine, MDMA, and methamphetamine. This started with 19 cities in 11 countries in 2011 and covered over 80 WWTPs in 23 countries in 2017. Over the 7 years, more than 120 cities with a total population of approximately 61 million people were monitored at least once (including a few cities outside Europe investigated by associated partners) and 25 cities have participated in at least five of the campaigns (Fig. 4).

The most recent study in 2017 employed a 1-week monitoring period where samples from the wastewater of a total of over 36.7 million people were collected every day and analyzed by project partners. The results show distinct spatial differences across Europe with the data reported online by the EMCDDA (http://www.emcdda.europa.eu/activities/wastewater-analysis). For example, daily per capita loads of benzoylecgonine, the main excretion product of cocaine, ranged from "not quantifiable" (i.e., near zero) in, e.g., Cluj-Napoca (Romania) to over

Fig. 4 European monitoring campaigns: Up to 67 cities participated per year between 2011 and 2017 with a peak total population of approx. 38 million in 2015 (modified from SCORE 2017b)

400–800 mg/1,000 people/day in Amsterdam, Antwerp, London, and Zurich. Furthermore, differences within countries could be observed, with lower per capita loads in smaller towns compared to higher values in metropolitan areas. The analysis of methamphetamine revealed a completely different picture: Budweis and Prague, Piestany and Bratislava, Oslo, Turku, and Dresden, i.e., cities in Northern and Eastern Europe dominated the scene. Whereas spatial differences are obvious and findings from wastewater analyses were consistent with information held by epidemiologists in a quantitative manner, a comprehensive analysis of temporal changes will require a longer time series. Therefore, it is desirable that cities participate every year. To obtain more representative country-wide averages, it seems appropriate to expand the number of cities, also including small towns and villages (Ort et al. 2014a). In 2016, the European Commission contracted the WATCH consortium for a wastewater analysis report on the European stimulant illicit drug market.

In 2012, in order to develop transdisciplinary and cross-sectoral European research capability for the next generation of scientists working in the field of WBE, the group received funding from the European Commission to start a European Marie Curie Initial Training Network, SEWPROF ITN. SEWPROF ITN established interdisciplinary and cross-sectoral research capability and trained the next generation of scientists working in the new and exciting field of WBE. It developed and validated new, integrated tools towards public health monitoring at a community level based on innovative WBE techniques. SEWPROF ITN undertook the first Europe-wide profiling of community-wide health and lifestyle including oxidative stress (Ryu et al. 2016), exposure to pesticides (Rousis et al. 2017a), presence of synthetic cathinones and phenethylamines (Bade et al. 2017; González-Mariño et al. 2016b), and geographical differences in potency of methamphetamine use across Europe (Castrignanò et al. 2018). In order to facilitate international collaboration, the SCORE group received further EU funding in 2014 to establish a COST action (www.score-cost.eu). This was initiated in April 2014, developed and expanded the existing pan-European interdisciplinary network, bringing together experts from relevant disciplines interested in the application and development of the quantitative measurement of human biomarkers in sewage to evaluate lifestyle, health, and exposure at the community level.

Outside of Europe, Australia has led the way in establishing a National Wastewater Drug Monitoring Program (https://www.acic.gov.au/publications/intelligence-products/national-wastewater-drug-monitoring-program-report) that has measured the level of illicit drug consumption of around 60% of the Australian population every 3–4 months (dependent on location) since 2016. The Australian National Wastewater Drug Monitoring Program monitors 54 sites and 14 substances with the goal of providing concrete data on illicit drug consumption to inform policy in health, education, and law enforcement. The program has to date shown that methamphetamine is the most prevalent illicit drug tested in Australia, and that the opiates oxycodone and fentanyl exceed heroin consumption and the rise of 3,4-methylenedioxyamphetamine (MDA) as a tangible problem in some regional areas.

Outside of Europe and Australia, research projects have been performed in China, New Zealand, Costa Rica, Canada, and the USA although, to the best of the authors' knowledge, the establishment of formal monitoring programs has yet to be consolidated.

5 Wastewater-Based Epidemiology Beyond Traditional Illicit Drugs: New Psychoactive Substances and Public Health Monitoring

The NPS market is very dynamic as traditional drugs are quickly substituted with new alternatives. There have been in excess of 620 compounds reported to the EMCDDA early warning system up to 2016. A total of 423 individual substances were detected on the drug market in 2015 alone (EMCDDA-Europol 2016).

Data derived from the analysis of wastewater can provide quantitative information on NPS use, which can then be integrated with epidemiological information. Analysis of wastewater may in this way prove to be a useful tool in the development, management, and amendment of effective policies in the management of NPS and associated outcomes. The scale and dynamics of the NPS market do, however, present some significant challenges to wastewater scientists. The large number of individual substances and the relatively small size of the NPS market relative to traditional drugs make it difficult to identify and detect individual NPS in municipal wastewater (Reid and Thomas 2016). There is also a lack of (clinical) data from rigorous pharmacokinetic profiling which would provide necessary information on the identity and rates of excretion of the drugs and/or metabolites, which are critical for back-calculations from measured loads in sewers to the actual amounts of drugs used.

There are currently three key strategies for dealing with the challenge of NPS and wastewater. These include:

(a) Rigorous maintenance of target (NPS) databases to provide analytical chemists with the most up-to-date list of drugs that may be present in a given wastewater sample.
(b) Targeted analysis of wastewater from populations for whom the use of NPS is expected, such as from toilets at nightclubs and festivals. This is most easily achieved via analysis of pooled urine from pissoirs and portable toilets at and around the target venues.
(c) The use of nontargeted analytical methodologies, which allow for retrospective screening of new drugs after their inclusion in NPS databases.

These strategies have successfully been employed by NPS-Euronet which is an EU funded network (HOME/2014/JDRU/AG/DRUG/7086) tasked with developing an integrated chemical analytical epidemiological approach to improve the capacity to identify and assess new drugs (http://www.npseuronet.eu/). This network and other groups have detected numerous NPS in samples of pooled urine and wastewater (Bade et al. 2017; Borova et al. 2015; Chen et al. 2013;

González-Mariño et al. 2016b; Kinyua et al. 2016; Tscharke et al. 2016). The NPS-Euronet continues to work closely with authorities to provide information useful to the implementation and effective practice of prevention.

Wastewater analysis has proven invaluable in drug community-wide use estimation and is now evaluated in the context of identification of NPS. WBE has also a clear potential to revolutionize public health assessment at the community level (Reid et al. 2011; Ryu et al. 2015; Thomas and Reid 2011; Yang et al. 2015a, b). This is because wastewater reflects the health status of a population and surrounding environment as it pools endo- and exogenous biomarkers of that population. It provides opportunities to develop a wide range of innovative solutions to quickly and quantitatively assess patterns of factors related to health and disease within populations, while also providing a means of collecting complementary data for epidemiological and socioeconomic studies in order to undertake comprehensive evaluation of public health. As an example, Ryu et al. (2016) reported a Europe-wide monitoring of 8-iso-PGF2α (an oxidative stress biomarker). Increased levels of 8-iso-PGF2α were observed at the inner-city level correlating with the degree of urbanization and levels of nicotine use. Rousis et al. reported different levels of community-wide exposure to pyrethroid pesticides in eight Italian cities (Rousis et al. 2017b). Gracia-Lor et al. profiled caffeine use in ten European cities (Gracia-Lor et al. 2017a). Lopardo et al. identified new biomarkers of internal exposure to endocrine disruptors (Lopardo et al. 2017).

6 Conclusions

WBE has rapidly become a complementary tool, alongside existing epidemiology-based techniques, for providing objective, evidence-based estimates of illicit drug use. Adopted by EMCDDA, WBE now provides spatial and temporal drug use trends in Europe and is being rapidly implemented worldwide, taking Finland and Australia as prime examples. Ongoing developments in this field focus on: (1) comprehensive evaluation of uncertainties, (2) collation of long-term datasets on spatio-temporal drug usage trends in different geographical locations that will allow for comprehensive exposure assessment, and (3) new applications including an introduction of an early warning system for NPS. New applications of WBE, that are likely to transform public health monitoring, include an estimation of public exposure to food, environmental, and industrial chemicals as well as tracking the development and spread of infectious disease in vulnerable communities.

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