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Monitoring of regional drug abuse through wastewater-based epidemiology—A critical review

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Abstract Wastewater-based epidemiology is a new approach to monitor drug abuse. It involves collecting wastewater, analysis of residues of drugs or its metabolites in wastewater, and back-calculation of drug consumption by taking into account wastewater flow, stability of drug target residues in wastewater, and excretion rates of drugs/metabolites. Wastewater-based epidemiology has the advantages of being inexpensive and yielding more consistent and near real-time results. It has the great potential to supplement the existing drug monitoring methods. It can be used to build large-scale (regional, national, or even continental) monitoring networks that would yield spatial patterns and temporal trends in drug abuse. This paper described in detail the principle and procedures of this wastewater-based approach. Application of this approach across the globe was also reviewed. The uncertainties involved in the approach and knowledge gaps were identified. Finally, necessity, benefits, and feasibility to set up nation or province-wide monitoring networks based on wastewater analysis in China were discussed.

Keywords Drug abuse, Monitoring, Wastewater-based epidemiology, Review

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1. Introduction

Illicit drugs are a group of substances that cause strong stimulating, sedative, and hallucinogenic effects of the central nervous systems and that are scheduled under the Single Convention on Narcotic Drugs of 1961 or the Convention on Psychotropic Substances of 1971. Illicit drugs sometimes are also called controlled drugs, drugs of abuse, or simply drugs. Common illicit drugs include heroin, opium, methamphetamine (METH, commonly called ice), cocaine, ecstasy (3,4-methylenedioxymethamphetamine, MDMA), cannabis, etc. Abuse of illicit drugs is a worldwide problem that has severe societal consequences, such as loss of lives and health of abusers, increased treatment costs, and higher incidence of crimes (Degenhardt et al., 2009; Kilmer and Pacula, 2009; Nutt et al., 2007). United Nations Office of Drugs and Crime has recently estimated that a total of 247 million people, corresponding to 5% of the world population aged between 15 and 64, had used illicit drugs at least once in 2013 (UNODC, 2016).

In order to take targeted actions to control illicit drug abuse, law enforcement need detailed information such as number of drug users, consumption of various drugs, and spatial and temporal trends of drug abuse. However, drug abuse monitoring is not an easy undertaking. Traditionally drug abuse information is obtained by means of population surveys, consumer interviews, individual medical records, crime statistics, and seizure data. All of these approaches are indirect measures and therefore have several disadvantages. Population surveys and consumer studies yield subjective

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results and are time-consuming and expensive. In addition, consumer interviews are typically carried out within relatively small areas (e.g., in addiction treatment centers) and among specific populations (e.g., AIDs patients). As such, it is unlikely that the survey results reflect regional or country-level patterns. Drug seizures are largely subject to randomness, providing limited information that can reflect the overall drug use trend at best. As a result, the traditional approaches disallow spatial and temporal comparison of drug abuse.

About a decade ago, a novel approach called wastewater-based epidemiology (WBE) was developed to estimate drug prevalence and consumption by a particular population. The concept of the approach, tracking drug consumption through monitoring drug concentrations in wastewater, was first brought about in 2001 (Daughton, 2001). The first WBE study was implemented in Italy by Zuccato et al. (2005). Since then, the approach has been applied in many countries and regions across the globe. While a number of uncertainties are involved in WBE, this approach has demonstrated huge promise for drug abuse monitoring.

The objective of this paper is to review the procedures, advantages, and uncertainties of WBE, as well as its application across the globe. Knowledge gaps that are to be closed for accurate estimation of drug consumption are also elaborated. Finally, rationales to apply WBE in China are discussed and recommendations on how to implement are made.

2. Fundamentals of wastewater-based epidemiology

The basis of WBE is the fact that after drug abuse, drugs or their metabolites are excreted and released into domestic wastewater. Thus by collecting wastewater samples from wastewater treatment plants (WWTPs) and measuring the concentrations of drug residues or its metabolites in the samples, the drug loads and consumptions of the communities served by the sampled WWTPs can be back-calculated. The back-calculation needs to take into account the flow rates of WWTPs, populations of the communities, as well as correction factors that account for excretion rates and stabilities of the drugs (Zuccato et al., 2008).

WBE has a set of advantages over traditional drug abuse monitoring approaches. First, it is more objective, as it does not depend on answers or opinions of drug abusers, but rather relies on sample collection, analysis, and back-calculation that can follow standard protocols. Thus, more consistent results are obtained through WBE. Second, it is much quicker. Wastewater samples can be acquired from WWTPs who carry out routine collection. Concentrations of dozens of drugs and their metabolites can be determined within 24 h. In addition, it is cheaper. Estimating drug abuse in a community only needs to collect and analyze wastewater samples for several days from the WWTP that serve the community. For megacities such as Beijing and Shanghai, the urban populations are typically served by less than 15 WWTPs. As a result, abuse of drugs in the urban areas of the cities can be monitored by analyzing less than 105 wastewater samples (assume each WWTP is sampled from 7 consecutive days).

The near real-time estimation of drug consumption at low cost allows regular monitoring of drug abuse at regional, national, and even continental scales. For example, a monitoring network can be built that cover the urban population of all the provincial capitals and equivalent cities of China. Such a network would include less than 400 WWTPs that serve over 250 million people. Regular monitoring of drug abuse via this network is feasible technically and economically, as only less than 4200 samples (assuming samples are collected twice per year and seven consecutive days per time) need to be analyzed. Large-scale monitoring networks would yield spatial patterns and temporal trends in drug abuse. Based on spatial patterns and temporal trends, natural and social impacts on drug abuse can be revealed.

3. Procedures of wastewater-based epidemiology

WBE studies typically involve investigation of WWTPs (treatment capacities, service areas, served populations, etc.), sample collection, sample analysis, drug load and consumption estimation. Figure 1 is a schematic diagram of WBE procedures. If average dose and abuse frequency of a particular drug are known, the number of drug abusers can also be estimated.

3.1 Selection of drug target residues

The drug residues or metabolites that are selected and measured for back-calculation of drug consumption are referred to as drug target residues (DTR). An ideal DTR is a major and exclusive excretion product (metabolite or unchanged parent drug) of the drug of interest. It also needs to be stable in wastewater. For example, benzoylecgonine is the major and exclusive excretion product of cocaine. There is no other source for benzoylecgonine in wastewater. It is also quite stable in wastewater (more stable than parent drug cocaine). However, such a DTR does not exist for many other drugs of interest. For example, 6-acetylmorphine is an exclusive excretion product of heroin. However, it is not the major excretion product (excretion rate around 1%) (Khan and Nicell, 2011). More importantly, 6-acetylmorphine is easily degraded in wastewater. On the other hand, morphine (in free and conjugate forms) is the major excretion product of her-

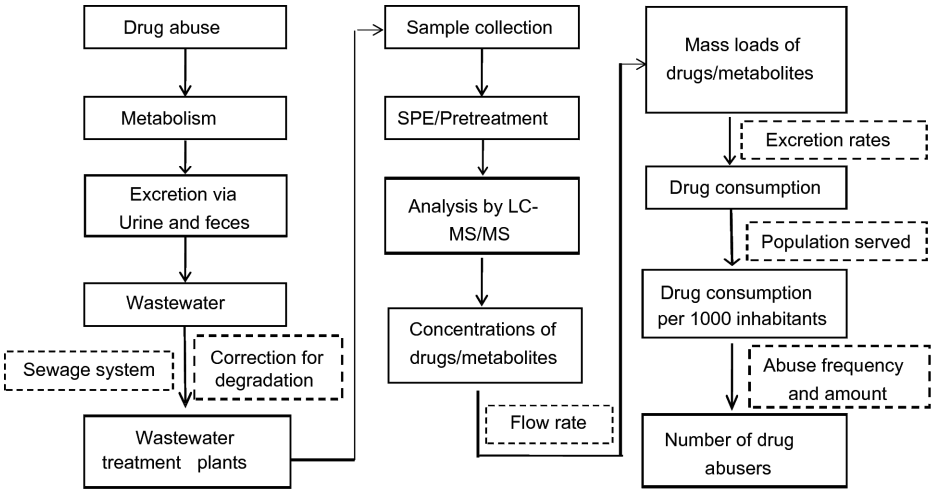

Figure 1 Schematic diagram of wastewater-based epidemiology.

oin. However, morphine in wastewater has other sources, such as therapeutic use of morphine and codeine (a drug used to treat coughing).

In cases where an ideal DTR does not exist, a DTR has to be selected by weighing the stability, excretion rate, and interferences from other sources. Usually, stability is the top priority in selection as unstable DTRs results in greatest uncertainties in back-calculation. Table 1 lists the DTRs of common illicit drugs of interest and the associated correction factors for drug consumption estimation.

3.2 Sample collection and preservation

In WBE studies, 24-h composite samples were usually collected to determine DTR concentrations. Flow at a particular WWTP may vary considerably during 24 h. Furthermore, each WWTP and sewer system is unique due to different topology and layout of the catchment. Thus, the fluctuation patterns of flows at different WWTPs are different. In addition, toilet flushes that contain the majority of the illicit drug load can lead to significant short-term variations in the range of minutes. As such, collecting composite samples is not straightforward. Improper sampling strategies may lead to much larger uncertainties in drug consumption estimation than chemical analysis (Ort et al., 2010a, 2010b).

There are three approaches to collect composite samples. Time-proportional sampling takes a constant sample volume at constant time intervals and mixes the subsamples to form a composite sample. Flow-proportional sampling takes a subsample volume proportional to the flow in the sewer at constant time intervals. Volume-proportional sampling takes a constant sample volume at variable time intervals, after a certain volume of wastewater has passed the sampling point. Time-proportional sampling can be accomplished using a standard auto-sampler or even manually (e.g., a sampling worker collects a subsample every one hour and mix them at the end of the day). Flow-proportional sampling needs a flow meter and an auto-sampler with adjustable sampling volume that is proportional to the wastewater flow. In volume-proportional sampling, a flow meter is also needed to send a flow signal to an auto-sampler. Once a predefined flow volume is reached, the auto-sampler is triggered to take a subsample.

Among the three sampling approaches, time-proportional sampling is easiest to implement. However, this approach will generally yield underestimation of drug consumption, as low flows, with a higher proportion of wastewater of lower drug loads, are over-represented in a composite sample. The volume-proportional sampling approach (sometimes mistakenly referred to as flow-proportional) takes samples more frequently during higher flow periods and less frequently during lower flow periods; but the sampling volume remains constant. Thus, it cannot provide a true average concentration because only the frequency changes, but the individual samples are not weighted properly according to the wastewater flow. Flow-proportional sampling is the only sampling mode that correctly weighs individual subsamples to form a composite samples. In the literature, however, flow proportional sampling was implemented in only one-fourth to one-third of WBE studies (Ort et al., 2010b).

During sampling, it is ideal to maintain the samples at around 4°C. The collected samples are commonly acidified using HCl or other acids to pH=2, put into polyethylene or polypropylene bottles, and carried to laboratory with ice. If the samples are not analyzed immediately, they should be stored in freezers at −20°C.

3.3 Analysis

The collected samples were pretreated before concentrations

Table 1 Drug target residues (DTRs) of common illicit drugs

Drugs	DTR	Mean excretion (%)	Correction factor	References
Methamphetamine (METH)	METH	22.7±6.4	4.4	Gracia-Lor et al., 2016
Amphetamine (AMP)	AMP	36.3±8.4	2.77	Gracia-Lor et al., 2016
MDMA	MDMA	22.5	4.4	Gracia-Lor et al., 2016
Ketamine (KET)	KET	30	3.3	Yargeau et al., 2014
Methadone (MET)	MET	27.5	3.6	Postigo et al., 2011
	EDDP	55	2.0	Thai et al., 2016a
Heroin (HER)	morphine (MOR)	42	3.1	Zuccato et al., 2008
Codeine (COD)	COD	30	3.3	Thai et al., 2016a
Cocaine (COC)	Benzoylecgonine (BE)	29	3.59	Castiglioni et al., 2013

of DTRs were determined using liquid chromatography-tandem mass spectroscopy. Sample pretreatment typically involves filtration, addition of internal standards, solid phase extraction, drying and re-dissolution, and final cleaning. Wastewater samples were filtered using filter papers to remove suspended particles. Following filtration, deuterated analogs (e.g., benzoylecgonine-d_8, morpine-d_6) were added as internal standards to account for recovery and matrix effects. Oasis MCX or HLB cartridges from Waters Corp. have been commonly used for solid phase extraction to remove or suppress matrix interferences and to concentrate analytes (i.e., to reduce limits of quantification). The cartridges can be conditioned using methanol and acidified ultrapure water, loaded with wastewater samples (50 mL or more), and then eluted using methanol and/or alkaline methanolic solution or with other solvents such as acetone or ethyl acetate (Bones et al., 2007; Hummel et al., 2006). The eluates can be evaporated to dryness under gentle nitrogen stream and redissolved typically in acetonitrile or methanol solutions.

The process of solid phase extraction, dying, and re-dissolution is both laborious and time-consuming (usually takes several hours). To save labor and time, large-volume injection (LVI) using a special injector kit was developed. In this mode, a large total volume of wastewater (typically around 1 mL) was directly injected after simple filtration (Chiaia et al., 2008). Although LVI could generate results in less than 1 h with acceptable quantification limits for common illicit drugs and metabolites in wastewater, only a limited number of research groups used this technique, likely because quantification limits of this injection mode were higher than those achieved using solid phase extraction.

High performance liquid chromatography (HPLC) and ultra performance liquid chromatography (UPLC) has been used to separate target analytes. Separation was usually achieved using reversed-phase columns (e.g., Phenomenex Gemini C18 column) and moderately polar mobile phases that consisted of a mixture of water and an organic solvent. Organic solvents used in the mobile phases included methanol and acetonitrile. The aqueous mobile phases consisted of water or ammonium formate or acetate that were acidified with formic or acetic acids (e.g., Du et al., 2015; Li et al., 2014). Acidification improves the ionization of the compounds monitored in the positive ionization mode. Cannabinoids were separated using different aqueous mobile phases, such as a basic solution of 0.05% triethylamine (Castiglioni et al., 2006) or a slightly acidic solution of 0.05% ammonium formate (Hogenboom et al., 2009), as analysis of cannabinoids was performed in negative ionization mode.

Hydrophilic interaction liquid chromatography (HILIC) has also been used to separate cocaine and its metabolites (Gheorghe et al., 2008; van Nuijs et al., 2009a), and amphetamine-type substances (such as amphetamine, methamphetamine) (Li et al., 2014), and opioids (such as 6-monoacetylmorphine) (van Nuijs et al., 2009a). The HILIC separation typically used a polar stationary phase (e.g., porous silica microspheres) and a highly organic mobile phase consisting of organic solvents (e.g., methanol, acetonitrile) and water. HILIC has been shown to better resolve some analytes than the reversed-phase columns (Gheorghe et al., 2008; van Nuijs et al., 2009a).

Illicit drugs and their metabolites except cannabinoids are ionized in the positive mode by means of electrospray. Cannabinoids has shown good response in both positive (Bijlsma et al., 2009; Boleda et al., 2007) and negative ionization modes (Castiglioni et al., 2006; Hogenboom et al., 2009; Postigo et al., 2008). The electrospray interface (ESI) is susceptible to matrix effects (suppression or enhancement of the analyte ionization signal). Matrix effects have been observed to increase notably as the complexity of the matrix increases (e.g. from effluent to influent wastewater). Matrix effects can be reduced by optimizing the pretreatment process and accounted for using isotopically labeled internal standards (e.g. deuterated analogues). Deuterated analogues of common illicit drugs and their primary metabolites are commercially available.

Illicit drugs and their metabolites were commonly quantified using triple quadrupole mass spectrometers (QqQ) in the selected reaction monitoring (SRM) mode (Bartelt-Hunt et al., 2009; Bijlsma et al., 2009; Boleda et al., 2007; Cas-

tiglioni et al., 2006; Chiaia et al., 2008; González-Mariño et al., 2009; Huerta-Fontela et al., 2007; Hummel et al., 2006; Kasprzyk-Hordern et al., 2008; van Nuijs et al., 2009a). The SRM mode yields good sensitivity and selectivity as at least two specific SRM transitions are monitored for each target analyte (Pozo et al., 2006). In addition to QqQ, hybrid instruments based on quadrupole-linear ion trap (QLIT) (Postigo et al., 2008) and ion trap (IT) (Bones et al., 2007; Gheorghe et al., 2008) have also been occasionally used for quantification. The QLIT is also operated in SRM mode, whereas IT mass spectrometers identify target analytes by recording structural information of the target analytes through collision induced dissociation (CID) analyses or mass spectra of the molecular ions. While high-resolution mass spectroscopy is a powerful in identification and confirmation of unknown drugs and metabolites in water samples, this technique has rarely been used, if used at all, in WEB studies, as quantification limits are not low enough to accurately determine the concentrations of illicit drugs and metabolites in wastewater samples.

Most analysis was performed to measure the DTR concentrations in aqueous phase. Using aqueous concentrations alone to estimate drug consumption would yield considerable errors if sorption of the DTRs to suspended solids in wastewater is significant. For the majority of DTRs, this practice is acceptable, as sorption of DTRs was negligible. Baker and Kasprzyk-Hordern (2011a) reported that sorption benzoylecgonine (DTR for cocaine), methamphetamine, morphine (potential DTR for heroin), ketamine, codeine, and MDMA was all less than 5%. The same authors reported amphetamine sorption ranged from 1.6–8.6%, whereas methadone and 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidin (EDDP) sorption was much higher, ranging from 8.1–18.6% and 12.1–34.5%, respectively. Subedi and Kannan (2014) reported similar results, except that even more sorption of amphetamine (40.1%), methadone (41.1%), and EDDP (38.5%) was observed. Based on these studies, it is clear that to accurately estimate methadone consumption, it is necessary to measure both concentrations of EDDP (as DTR) in both aqueous and sorbed phases. To estimate amphetamine consumption, it is also better to measure amphetamine concentrations in both phases. For heroin, cocaine, methamphetamine, MDMA, codeine, and ketamine consumption, measuring aqueous concentrations is adequate.

3.4 Load and consumption estimation

The daily mass load of each target residue at a specific WWTP can be derived by multiplying the concentration of the DTR with the wastewater flow of the sampling day. A high total load of a DTR at a WWTP does not necessarily mean that the drug abuse in the service area is severer than in the community served by another WWTP that has a lower load, as the high load may well be caused by a much greater population served by the former WWTP. Thus, to make comparison between different WWTPs, drug loads at the WWTPs are all normalized to 1000 inhabitants (unit: mg/(1000 inh·d):

$$\text{Drug load}\left(\frac{\text{mg}}{1000\text{inh}\cdot\text{d}}\right)=\frac{\text{Concentration of a chemical residue}\left(\frac{\text{ng}}{\text{L}}\right)\times\text{Influent flow}\left(\frac{\text{L}}{\text{d}}\right)}{\frac{\text{Population served}}{1000}}\times\frac{1}{10^6}\left(\frac{\text{mg}}{\text{ng}}\right).$$

The load of a DTR can be further converted into drug consumption by the following equation that accounts for the in-sewer transformation/adsorption and excretion of the DTR:

$$\text{Consumption}\left(\frac{\text{mg}}{1000\ \text{inh}\cdot\text{d}}\right)=\text{Load}_{\text{DTR}}\left(\frac{\text{mg}}{1000\ \text{inh}\cdot\text{d}}\right)\times\frac{1}{\text{Stability}_{\text{DTR}}}\times\frac{1}{\text{Excretion}_{\text{DTR}}}\times\frac{MW_{\text{Drug}}}{MW_{\text{DTR}}},$$

where $\text{Excretion}_{\text{DTR}}$ is the excretion rate of the DTR following the abuse of a drug of interest; $\text{Stability}_{\text{DTR}}$ is the ratio of DTR concentration after in-sewer transformation and adsorption (to biofilm and suspended solids) to initial concentration; MW_{Drug} is the molecular weight of the drug of interest; MW_{DTR} is the molecular weight of the DTR.

3.4.1 Stability of illicit drugs

Degradation and/or transformation of illicit drugs may occur to varying degrees during transport in sewers and storage of wastewater samples before analysis. Degradation of DTRs before analysis will lead to significant underestimation of drug use. On the contrary, production of DTRs (e.g., due to transformation of parent or other compounds during transport and storage) would cause overestimation of drug consumption. Around 50 WBE studies acknowledged the importance of drug stability and 24 included tests to examine stability in wastewater (McCall et al., 2016). Most studies focused on in-sample stability, i.e. stability during sample preservation, storage, and preparation (e.g., Baker and Kasprzyk-Hordern, 2011a; Östman et al., 2014). In general, these studies found that methamphetamine, MDMA, ketamine and methylenedioxypyrovalerone (MDPV) seemed to have high in-sample stability (McCall et al., 2016). Benzoylecgonine and amphetamine are most likely also substances of high stability, although they may be formed due to transformation of other substances. It has been commonly recognized that cocaine and 6-acetylmorphine are unstable in wastewater during storage. In contrast, stability of 11-nor-9-carboxy-delta-9-tetrahydrocannabinol (THC-COOH), cath-

inones, and most new psychoactive substances has not been well examined.

Hydrochemical and biological conditions of wastewater may be dramatically changed once it is removed from sewers. Hence, drugs stable during sample collection, storage, and preparation are not necessarily stable in sewers. Unfortunately, to our knowledge, there are only two studies in the literature that examined drug stability using laboratory-scale reactors mimicking different sewer conditions (Ramin et al., 2016; Thai et al., 2014). Thai et al. (2014) demonstrated that methamphetamine, MDMA, and benzoylecgonine were stable in the sewer reactors, whereas cocaine and 6-acetylmorphine degraded quickly. While this finding is in qualitative agreement with findings of studies examining in-sample stability, the authors found that cocaine and 6-acetylmorphine degradation rates were significantly higher than the values reportedly by in-sample stability studies. Furthermore, benzoylecgonine and morphine were found to form from the degradation of cocaine and 6-acetylmorphine. This observation indicates that using benzoylecgonine and morphine concentrations to back-calculate cocaine and heroin consumption may lead to overestimation. Ramin et al. (2016) reported that drug transformation significantly differed from aerobic to anaerobic conditions, and abiotic conversion was the dominant mechanism for many of the selected substances (e.g., cocaine, mephedrone, THC-COOH). Results obtained also demonstrated that redox conditions could have a significant impact on transformation kinetics of the selected substances. Both studies underscore the importance of accounting for in-sewer stability of drugs and metabolites.

3.4.2 Population estimation

Accurate estimation of populations in WWTP service areas is critical, as drug loads have to be normalized to population for comparison purposes. However, estimating population is not an easy undertaking. Census data and design capacity of WWTPs have typically been used for normalized load or consumption calculation. However, census data and design capacity may not reflect the population served by a particular WWTP for a number of reasons (Lai et al., 2011). First, actual number of people in the service area may be variable due to daily commuting, seasonal variability, holidays, and/or special events. Second, service area of a WWTP may change, as wastewater of some communities may be connected to other WWTPs. Third, census data is not always available and may be out of date. Fourth, floating population may not be fully accounted for in the census data. This is particularly true in China, as in many cities, floating populations even outnumber the registered residents.

Alternative approaches to estimate served population have been proposed. Biological oxygen demand, chemical oxygen demand, total phosphorus and nitrogen in wastewater has been proposed and used for back-calculation (van Nuijs et al. 2011a, 2011b; Castiglioni et al., 2013). However, these water quality parameters actually reflect human activities, rather than population size. More importantly, they may be significantly variable and influenced by other sources such as commercial and industrial discharges. Another potential approach is to use human biomarkers to estimate the number of people in a catchment. Chen et al. (2014) summarized that a population biomarker should at least meet the following criteria: (1) be quantifiable; (2) have little affinity to particulate matter in wastewater or to filter paper; (3) be stable in wastewater; (4) be constantly excreted, and (5) the total excretion should correlate with census population, meaning there should be no contribution other than human metabolism. The same authors evaluated the eligibility of eight previously proposed biomarker candidates (namely, creatinine, cholesterol, coprostanol and cotinine, cortisol, androstenedione and 5-hydroxyindoleacetic acid (5-HIAA)) using the above criteria. The authors concluded that both cotinine and 5-HIAA were eligible and that 5-HIAA may be more suitable for international comparisons. Senta et al. (2015) used cotinine to estimate population size in Milan and Como of Italy. The authors reported that the estimated population agreed well with census data. Rico et al. (2017) recently used the two substances to estimate population of three WWTPs in Valencia, Spain. However, although in-sample stability (if properly preserved) of the two biomarker has been demonstrated by Chen et al. (2014) and Rico et al. (2017), whether they are stable in sewers is highly questionable. Thus, whether these biomarkers are widely applicable for population estimation remains to be confirmed.

In addition to biomarkers suggested by Chen et al. (2014), other chemicals were also used to estimate population size. Senta et al. (2015) examined the feasibility to use caffeine to estimate population and concluded that additional information on caffeine metabolism was needed. O'Brien et al. (2014) developed a model to estimate population based on linear regressions of daily mass loads of high use pharmaceuticals and personal care products (e.g., acesulfame and gabapentin) against population size. The authors further used Bayesian inference to incorporate chemical markers into the model and validate this model on a leave-one-out basis.

3.4.3 Excretion rates of DTRs

Accurate excretion rates of DTRs are not readily available, due to scarce pharmacokinetic data, which normally can only be obtained from a limited number of studies that typically involve a small number of healthy volunteers (e.g., Kim et al., 2000). In addition, excretion may vary significantly, depending on the route of administration, the frequency of use, co-consumption with other drugs, and other factors such as ethnicity, gender, age, dose administered, and health conditions (Gracia-Lor et al., 2016). Pharmacokinetic data of

common illicit drugs have been systematically reviewed by Khan and Nicell (2011, 2012).

Castiglioni et al. (2013) developed a method to refine the excretion rate of benzoylecgonine following cocaine use by considering the percentages of urinary excretion after intranasal, intravenous, smoking and oral administration, and weighting these values by the number of subjects included in each study and the frequency of use by each route of administration. More recently, following the same method, Gracia-Lor et al. (2016) refined the excretion rates of parent compounds and metabolites following use of amphetamine, methamphetamine, MDMA, and Δ^9-tetrahydrocannabinol (THC). Also recently, Thai et al. (2016a) suggested an excretion rate of 30% codeine following codeine use that yielded consumption matching reasonably well with the sales statistics.

Table 1 summarizes the most updated excretion rates of drugs and metabolites. Note that the excretion rate of 6-acetylmorphine following heroin abuse is not listed, because this metabolite, although exclusively of heroin, is extremely unstable in wastewater and thus is not suitable for heroin consumption estimation. Heroin consumption in China can be estimated with reasonable accuracy using morphine loads, as contribution of therapeutic use of morphine and codeine is very low and can be subtracted from the total load in wastewater (Du et al., 2017). On the contrary, in many European countries, contribution of therapeutic morphine and codeine is much higher than that of street heroin (e.g., Baker et al., 2014; Vuori et al., 2014). In these countries, estimating heroin consumption using morphine loads in wastewater would involve huge uncertainties. It is also worth noting that huge discrepancy in the excretion rate of ketamine exists in the literature (Andrés-Costa et al., 2014; Yargeau et al., 2014).

3.5 Uncertainties of wastewater-based epidemiology

Every aspects involved in WBE studies, sample collection, flow measurement, in-sewer and in-sample stability, analysis, population estimation, and excretion rate, can all bring in large uncertainties in drug consumption estimation. Among these aspects, flow rate measurement usually involves least uncertainty, as long as the flow meter is properly installed and functioning. Sampling uncertainty can be reduced greatly, and sampling biases can be eliminated completely, through careful investigation of the sewer system and by choosing an appropriate sampling mode (i.e. the flow-proportional mode) and frequency (Ort et al., 2010b). However, only a small fraction of previous WBE studies provided justification for the choice of their sampling protocols; and in general, descriptions of the sampling protocols were very short and more implicit than explicit (Ort et al., 2010b). In these studies, sampling errors may lead to over-interpretation of measured data and even wrong conclusions. Most WBE studies devoted the largest efforts to sample analysis. Analytical uncertainties can be evaluated in the laboratory under controllable conditions. The precision of the analysis can be quantified using replicate samples. The accuracy of an analytical method can be greatly improved using isotopically labelled internal standards and verified via inter-laboratory comparison.

Largest uncertainties of WBE studies lie in the stability of drugs in wastewater, excretion rates, and population estimation. If collected samples are properly preserved and stored, in-sample degradation and transformation of drugs can be minimized. However, in-sewer stability of drugs and metabolites has been poorly investigated. No in-sewer transformation study has been performed in real sewers. Wastewater following toilet flushes goes first to septic tanks, where feces were decomposed. Designed residence time in septic tanks is typically over 24 h. Thus significant transformation of drugs in septic tanks is likely. However, stability of drugs in septic tanks has not been even mentioned in the literature. If using a parent compound as the DTR, degradation in septic tanks can lead to significant underestimation of drug consumption estimation. If a metabolite is used as the DTR, drug consumption may be overestimated if production of metabolite due to degradation of the parent compound is greater than the degradation of the metabolite itself.

Future research is urgently needed to quantify drug stability in sewers. Full-scale experiments to gain accurate results may be difficult, due to a number of factors, such as too many confluents to monitor and limited access to confined space. However, it is relatively easy to examine transformation in septic tanks, e.g., by continually monitoring drug concentrations at the inlets and outlets of septic tanks. Stability of drugs in wastewater ducts can be investigated by laboratory and pilot-scale experiments that simulate realistic sewer conditions. For example, in-sewer laboratory experiments should include biofilms that grow on sewer walls. Control experiments (e.g., abiotic controls) should also be performed to facilitate interpretation and comparison of experiments carried out in different laboratories and under different conditions.

Excretion rates in Table 1 were obtained from limited number of healthy volunteers and may not represent excretion rates of drug abusers. Furthermore, excretion rates of some routes of administration are not available. For example, "chasing" is the primary routes of methamphetamine use in China. Yet excretion rate of this route of use is missing in the literature. In addition, huge discrepancy in excretion rate of ketamine, a primary synthetic drugs of abuse in China and southeastern Asia, exists in the literature. Andrés-Costa et al. (2014) adopted an excretion rate of 2.3%, whereas Yargeau et al. (2014) used a value of 30%. For many other drugs, such

as cathinones and all new psychoactive substances, no excretion rate has ever been reported. Future research is needed to close this gap. Here we suggest to work closely with law enforcement to recruit drug abusers to obtain more representative excretion rates. For example, once a drug abuser is caught by police on spot, he/she can be consulted about the willingness to serve as a volunteer. He/she will also be asked to provide the amount of drug used and the purity of the drug residue they left can be measured. All his/her urine in the following days can be collected to get an excretion profile. The total excreted amount may be extrapolated from the profile and an excretion rate will be obtained. With drug abusers of different age, gender, and consumption pattern as volunteers, more representative excretion rates can be obtained.

Currently, estimating population using census data, design capacity, water quality parameters, and human biomarkers all has drawbacks. More efforts may be directed toward the in-sewer stability of human biomarkers. Another option is to work closely with police department who in some countries (e.g., China) have population information of individual communities. The sewer system connecting to a particular WWTP can be investigated to find out the communities served by the WWTP. Population information of individual communities from police departments may provide better constraints on population size estimation.

4. Application of wastewater-based epidemiology

Following its first implementation in Italy by Zuccato et al. (2005), WBE approach was soon applied in several other cities in West Europe and the United States (Banta-Green et al., 2009; Bones et al., 2007; Chiaia et al., 2008; Kasprzyk-Hordern et al., 2009a; van Nuijs et al., 2009b, 2009c, 2009d; Zuccato et al., 2008). Since 2010, WEB studies have been expanded to a much larger number of countries in Europe, North America, Asia, and Caribbean (Damien et al., 2014). To date, there have been 89 WBE studies reported in the literature. Table 2 presents the WBE studies that have been reported since 2005. WBE studies have been performed at different scales, from specific locations (schools, prisons) (Castiglioni et al., 2011), to national and international scale (e.g., Du et al., 2015; Ort et al., 2014; Thomas et al., 2012). Temporal trends in drug use have also been examined using WBE approaches in several countries (Lai et al., 2016b; Prichard et al., 2014; Zuccato et al., 2011). Following is a brief description of WBE researches carried out in different regions across the globe. More details of WBE investigations across the globe are available in recent review report of European Monitoring Center of Drugs and Drug Addiction (2016).

4.1 WBE studies of traditional illicit drugs

4.1.1 Europe

Europe is the region with the highest number of WBE studies. About 60% of WBE applications worldwide were implemented in Europe. This percentage has been decreasing in recently years as WBE is applied in more regions and countries outside Europe. Other than Italy, where WBE was applied for the first time (Zuccato et al., 2005), United Kingdom (Bones et al., 2007; Kasprzyk-Hordern et al., 2009b), Belgium (van Nuijs et al., 2009b, 2009c, 2009d), and Spain (Boleda et al., 2009; Huerta-Fontela et al., 2008) are the counties that witnessed the earliest WBE studies. Much more WBE work has been carried out in these countries in recent years (e.g., Boogaerts et al., 2016; Castiglioni et al., 2015; Mendoza et al., 2016; Rodríguez-Álvarez et al., 2015; van Wel et al., 2016; Zuccato et al., 2016). To date, WBE has also been applied in Switzerland (Been et al., 2015, 2016; Berset et al., 2010), France (Karolak et al., 2010; Nefau et al., 2013), Dutch (van der Aa et al., 2013), Norway (Bramness et al., 2015), Germany (Been et al., 2016; Meyer et al., 2015), Finland (Kankaanpää et al., 2014, 2016; Vuori et al., 2014), Sweden (Östman et al., 2014), Czech Republic (Baker et al., 2012), Croatia (Krizman et al., 2016; Terzic et al., 2010), Slovakia (Mackul'ak et al., 2014a, 2014b), Poland (Klupczynska et al., 2016), Greece (Gatidou et al., 2016), Portugal (González-Mariño et al., 2017). Some of these WBE studies examined spatial variation of drug use at national scale (e.g., Kankaanpää et al., 2014; Östman et al., 2014) and temporal trends (e.g., Zuccato et al., 2016).

In order to standardise WBE methodologies and coordinate international studies, a Europe-wide network (Sewage Analysis CORe group-SCORE) was set up in 2010. The group coordinated the first Europe-wide monitoring study in March 2011 that collected wastewater samples from 21 WWTPs of 19 cities in 11 countries (Thomas et al., 2012). This Europe-wide monitoring study was continued and expanded to more cities and countries. For example, the 2013 sampling campaign covered 47 WWTPs of 42 cities in 21 countries (total population 24.74 million) (Ort et al., 2014). These studies assessed and confirmed spatial differences in drug use in large cities across Europe. For example, cocaine use was found to be much higher in cities in West Europe relative to cities in Eastern Europe and Scandinavia. In contrast, highest methamphetamine use was found in cities in Norway and Czech Republic. In general, results of these studies were in agreement with traditional surveillance data. In addition, some data of the studies were adopted in annual European Drug Report (European Monitoring Center of Drugs and Drug Addiction 2015, 2016).

4.1.2 North America

United States is one of the countries that carried out earliest

Table 2 WBE studies that examined common illicit drug in wastewater across the globe[a)]

Continent	Country	Scale	Analyst	References
Europe	Italy	City	COC, BE	Zuccato et al., 2005
		City	COC, BE, MOR	Mari et al., 2009
		City	7 (COC, METH, MOR, etc.)	Zuccato et al., 2011
		City	6 (COC, METH, AMP, etc.)	Castiglioni et al., 2013
		City	6 (AMP, 6-AM, METH, etc.)	Repice et al., 2013
		Region	Nicotine, Cotinine, Hydroxycotinine	Castiglioni et al., 2015
		Nation	5 (COC, METH, MDMA, etc.)	Zuccato et al., 2016
	Italy, UK, Switzerland	City	8 (HER, AMP, METH, etc.)	Zuccato et al., 2008
	Spain	City	25 (MOR, AMP, METH, etc.)	Postigo et al., 2008
		Region	15 (AMP, METH, COC, etc.)	Huerta-Fontela et al., 2008
		Basin	11 (MOR, COD, THC-COOH etc.)	Boleda et al., 2009
		City	17 (6-AM, MOR, AMP, etc.)	Postigo et al., 2010
		Region	4 (MDA, MDMA, etc.)	Bijlsma et al., 2014
		City	8 (AMP, COC, KET etc.)	Andrés-Costa et al., 2014
		Region	16 (COC, METH, THC, etc.)	Mendoza et al., 2016
	Spain, Italy	City	3 (EtS, BE, COE)	Rodríguez-Álvarez et al., 2015
	Belgium	Nation	2 (COC, BE)	van Nuijs et al., 2009c
		Nation	2 (COC, BE)	van Nuijs et al., 2009d
		City	9 (COC, METH, 6-AM, etc.)	van Nuijs et al., 2011b
		City	5 (COC, MDMA, AMP, etc.)	van Wel et al., 2016
	UK	City	16 (COC, MOR, KET, etc.)	Bones et al., 2007
		Basin	54 (COD, AMP, COC, etc.)	Kasprzyk-Hordern et al., 2009b
		City	6 (AMP, METH, MDA, etc.)	Kasprzyk-Hordern and Baker, 2012
		City	64 (COC, AMP, METH, etc.)	Baker and Kasprzyk-Hordern, 2013
		City	80 (COC, MOR, COD, etc.)	Baker et al., 2014
	Norway	City	11 (MOR, AMP, COC etc.)	Harman et al., 2011
		City	3 (METH, COC, BE)	Reid et al., 2011
		City	2 (METH, AMP)	Bramness et al., 2015
	France	City	5 (COC, MDMA, AMP, etc.)	Karolak et al., 2010
		Nation	17 (COC, MOR, etc.)	Nefau et al., 2013
		City	17 (COC, METH, MET, etc.)	Damien et al., 2014
	Croatia	City	11 (MOR, COC, AMP, etc.)	Terzic et al., 2010
		Nation	13 (6-AM, MOR, COC, etc.)	Krizman et al., 2016
	Finland	City	11 (AMP, COD, METH, etc.)	Vuori et al., 2014
		Nation	8 (AMP, MDMA, METH, etc.)	Kankaanpää et al., 2014
		Nation	9 (AMP, COC, MET, etc.)	Kankaanpää et al., 2016
	Sweden	Nation	31 (AMP, COC, heroin, etc.)	Östman et al., 2014
	Czech	City	60 (COC, AMP, METH, etc.)	Baker et al., 2012

(*To be continued on the next page*)

(*Continued*)

Continent	Country	Scale	Analyst	References
Europe	Dutch	City	14 (AMP, METH, KET, etc.)	Bijlsma et al., 2012
		City	34 (AMP, METH, MDMA, etc.)	van der Aa et al., 2013
	Slovakia	Town	4 (METH, COC, THC-COOH, MDMA)	Mackul'ak et al., 2014a
		Nation	9 (AMP, COC, METH, etc.)	Mackul'ak et al., 2014b
	Germany	Region	2 (METH,AMP)	Meyer et al., 2015
	Germany, Switzerland	Nation	4 (BE, MDMA, AMP, METH)	Been et al., 2016
	Switzerland	City	11 (COD, AMP, METH, etc.)	Berset et al., 2010
		City	4 (MOR,6-AM, MET, EDDP)	Been et al., 2015
	Greece	City	158 (COC, AMP, MOR etc.)	Alygizakis et al., 2016
	Greek	City	5 (COC, BE, MDMA, etc.)	Gatidou et al., 2016
	Polish	Nation	6 (AMP, MDMA, COC, etc.)	Klupczynska et al., 2016
	19 European cities	International	6 (COC, AMP, METH etc.)	Thomas et al., 2012
South America	Brazil	City	2 (COC, BE)	Maldaner et al., 2012
	Colombia	City	8 (AMP, METH, MDMA, etc.)	Bijlsma et al., 2016
		City	2 (COC, BE)	Hernández et al., 2015
North America	Canada	City	2 (MDMA, COC)	Comtois-Marotte et al., 2017
		City	7 (METH, BE, COC, etc.)	Palardy et al., 2016
		City	14 (COC, BE, AMP, etc.)	Rodayan et al., 2016
		City	14 (COC, BE, AMP, etc.)	Yargeau et al., 2014
		City	6 (COC, BE, AMP, etc.)	Metcalfe et al., 2010
	USA	City	2 (METH, AMP)	Boles and Wells, 2016
		City	4 (AMP, COD, MET, MOR)	Vatovec et al., 2016
		Region	4 (METH, MDMA, BE, MET)	Banta-Green et al., 2016
		City	16 (AMP, METH, MDA, etc.)	Heuett et al., 2015b
		City	17 (COC, COD, MOR, etc.)	Heuett et al., 2015a
		City	13 (COC, MOR, MET, etc.)	Subedi and Kannan, 2014
		City	1 (BE)	Kinyua and Anderson, 2012
		City	1 (METH)	Chiaia-Hernandez et al., 2011
		City	10 (COC, BE, etc.)	Castiglioni et al., 2011
		City	14 (METH, AMP, COC, etc.)	Gerrity et al., 2011
		City	12 (COC, BE, MOR, etc.)	Bisceglia et al., 2010
		City	2 (MDMA, METH)	Loganathan et al., 2009
		City	14 (METH, AMP, EP, etc.)	Chiaia et al., 2008
Oceania	Australia	City	4 (COC, BE, MDMA, METH)	Lai et al., 2016b
		Nation	3 (BE, MDMA, METH)	Lai et al., 2016a
		City	9 (COD, MOR, KET, etc.)	van Dyken et al., 2014
		City	3 (MDMA, METH, BE)	Chen et al., 2011
		City	3 (MDMA, METH, BE)	Irvine et al., 2011
Asia	Mainland China	City	7 (MDMA, MDA, BE, etc.)	Yao et al., 2016
		Nation	METH, AMP, KET, NK	Du et al., 2015
		City	METH, AMP	Li et al., 2014
		City	12 (COC, AMP, METH, etc.)	Khan et al., 2014
	China Taiwan	City	7 (AMP, METH, COC, etc.)	Jiang et al., 2015
	Korea	City	14 (COC, AMP, METH, etc.)	Kim et al., 2015

a) MDA, 3,4-methylenedioxyamphetamine; THC-COOH, 11-nor-9-carboxy-delta-9-tetrahydrocannabinol; EtS, ethyl sulfate

WBE studies. The first WBE study was performed by Chiaia et al. (2008) at seven WWTPs to examine occurrence of several classes of illicit drugs (cocaine, amphetamines, opioids, ketamine, etc.) in wastewater. Banta-Green et al. (2009) evaluated cocaine, methamphetamine and MDMA use in 96 municipalities across the state of Oregon. WBE studies were also conducted to examine illicit drug use during sporting events (Gerrity et al., 2011) and in small communities, such as schools (Burgard et al., 2013; Castiglioni et al., 2011) and prisons (Brewer et al., 2016). Since 2014, more WBE studies have been performed to examine illicit drug use at city and regional scales (Banta-Green et al., 2016; Boles and Wells, 2016; Heuett et al., 2015a, 2015b; Subedi and Kannan, 2014; Vatovec et al., 2016) (Table 2).

WBE was applied in Canada first by Metcalfe et al. (2010), followed by Yargeau et al. (2014). Both studies found that cocaine was the most used illicit substance tested, with the highest levels of consumption in a large city. The high levels of methamphetamine use were also found in the large city. More recently, Palardy et al. (2016) examined occurrence of illicit drugs and pharmaceutical residues in the wastewaters of an eastern Canadian city.

4.1.3 Australia

WBE was first applied in Australia in 2009 to examine cocaine, MDMA, and methamphetamine use in metropolitans and regional areas (Irvine et al., 2011). Cocaine use was found to be much lower than in Europe, United States, and Canada, whereas MDMA and methamphetamine use were at the similar levels. Following this study, a number of WBE studies were performed to examine the same group of drugs in Queensland (Lai et al., 2011; Prichard et al., 2014), during holidays and events (Lai et al., 2013a, 2013b), in prison (van Dyken et al., 2014). More recently, Lai et al. (2016b) examined temporal trends of cocaine, MDMA, and methamphetamine consumption (2009–2015) in South East Queensland. It was found that from 2009 to 2015, there had been no significant change in cocaine and MDMA abuse, whereas methamphetamine consumption in the urban and rural area had increased by five and three times, respectively. The same group also carried out a nationwide WBE study to examine spatial variations in the consumption of illicit stimulant drugs across Australia (Lai et al., 2016a). The authors reported that cocaine was the most prevalent illicit drug around Australia, and the consumption of cocaine and MDMA was greater in large cities relative to rural towns. On the contrary, methamphetamine abuse was remarkably similar across their study regions, except for a few large cities.

4.1.4 Asia

WBE studies were implemented relatively late in Asia and so far have been implemented in only two Asian countries, namely, China and Korea. Lai et al. (2013b) performed the first WBE study in Hong Kong. The largest WWTP of the city that served around 3.5 million people was sampled. The author found that ketamine was the predominant drug, followed by methamphetamine, cocaine, and MDMA (not detected). Khan et al. (2014) performed WBE study for the first time in mainland China. Wastewater was collected in four megacities, Beijing, Shanghai, Guangzhou, and Shenzhen. Loads of fourteen common illicit drugs and metabolites were examine. Li et al. (2014) examined methamphetamine and amphetamine loads in wastewater in the entire urban area of the Beijing. More recently, Du et al. (2015, 2017) carried out a nation-wide reconnaissance of methamphetamine, ketamine, and heroin use in China. Over 40 WWTPs were sampled in over 20 major cities across the country. The authors found that no clear geographical pattern was observed in METH use, although slightly lower use in north and east China relative to other regions can be suggested. In contrast, an overall increasing trend from the north to the south was evident for KET loads (Du et al., 2015). Highest heroin consumption was observed in northwestern and southwestern China (Du et al., 2017), likely because these two regions are closest to foreign sources of heroin (Myanmar and Afghanistan). Kim et al. (2015) collected wastewater from 15 WWTPs in 5 Korean cities. They found that methamphetamine was the primary drug of abuse, but its abuse was lower than in most other countries.

4.1.5 Other regions

WBE studies in regions other than Europe, North America, Australia, and East Asia are scarce. The first WEB study in these regions was carried out in Martinique, Caribbean (Damien et al., 2014). The first study in South America to examine occurrence of cocaine and benzoylecgonine in wastewater and surface waters was performed in Columbia in 2015 (Hernández et al., 2015). More recently, Bijlsma et al. (2016) estimated the use of amphetamine, methamphetamine, MDMA, cocaine, cannabis, heroin, and ketamine in the main cities of Columbia. Occurrence of cocaine and benzoylecgonine in sea water, surface water, and drinking water in Brazil has also been reported (Pereira et al., 2016; Campestrini and Jardim, 2017). However, occurrence of illicit drugs in wastewater in Brazil has not been examined. So far, no WEB study has been performed in Africa, Middle East, Central and Southeast Asia, and former Soviet Union countries.

4.2 WBE studies of new psychoactive substance

WBE studies of NPS have a major drawback: NPS consumption cannot be estimated, as excretion rates of NPS are missing in the literature. Baker and Kasprzyk-Hordern conducted the first WBE study to examine NPS occurrence in wastewater in England (Baker and Kasprzyk-Hordern,

2011b). To date, there have been only 17 WBE studies of NPS use in the literature (Table 3). Again, Europe is the region where most studies (13 out of 17) were performed. The remaining four WBE studies were carried out in Australia (3) and China (1), respectively. Methcathinone, methylenedioxypyrovalerone (MDPV), mephedrone, and benzylpiperazine (BZP) are the most examined NPSs in these WBE studies. The concentrations of NPS in wastewater were typically much lower than those of traditional illicit drugs or metabolites, such as cocaine, benzoylecgonine, methamphetamine, and morphine. Some NPS (e.g. mephedrone, MDPV, methylone) were detected in wastewater only sporadically (Andrés-Costa et al., 2016; Baker and Kasprzyk-Hordern, 2011b; Baz-Lomba et al., 2016; Borova et al., 2015; Castiglioni et al., 2015; Chen et al., 2013; Gao et al., 2016; González-Mariño et al., 2016a, 2016b; Kankaanpää et al., 2014; Kinyua et al., 2015; Mwenesongole et al., 2013; Reid et al., 2014; Styszko et al., 2016; Thai et al., 2016b; Tscharke et al., 2016; van Nuijs et al., 2014). However, a few NPS were widely detected at noticeable concentrations in some countries. For example, Gao et al. (2016) reported that BZP was detected at all the 36 WWTPs in 18 cities across China.

5. Suggestions on applying wastewater-based epidemiology in China

WBE is an objective, quick, and inexpensive method to monitor drug abuse. It can potentially play important roles in drug control. This methodology can be particularly useful in China, as China is a country with centralized governance. In the Chinese government system, every year police departments of the upper government levels review the performance of police departments of lower levels based on drug seizure, drug criminals caught, and the drug abusers found. Undoubtedly, these numbers depend not only on the efforts the lower level police departments make, but also on some sort of luck. In addition, the police departments of upper levels set task targets of the next year for their counterparts of lower levels. The task assignment is largely based on the numbers of the previous year (typically by an increase). The outcome of such a working framework is that the harder the police departments of lower levels work, the higher their task target will be. Apparently, such a working framework is unfair and discourages the local police departments who make more efforts in drug control. In contrast, task assignment based on WBE would be much more objective and fair.

In the past two decades, infrastructure of wastewater treatment has been greatly improved. In provincial capitals and equivalent cities (in terms of population and economic development), typically over 95% of municipal wastewater is collected and treated by treatment plants). In some areas in the Yangtze River delta and Pearl River Delta, every town has its own centralized wastewater treatment plants. This wastewater treatment infrastructure sets a firm foundation to apply WBE to monitor drug abuse in China, especially in urban areas. In fact, WWTPs that treat wastewater of urban

Table 3 WBE studies that examined new psychoactive substance use[a)]

Continent	Country	Scale	Number of drugs of metabolites analyzed	References
Europe	UK	City	65 (BZP, TFMPP, et al.)	Baker and Kasprzyk-Hordern, 2011a
		City	25 (MEPH, BZP,etc.)	Mwenesongole et al., 2013
	Belgium	City	6 (MDPV, MEPHe, etc.)	van Nuijs et al., 2014
	Belgium Switzerland	Nation	7 (ethylone, methylone, etc.)	Kinyua et al., 2015
	Norway	City	14 (MEPH, PMMA, etc.)	Reid et al., 2014
		City	51 (MEPH, etc.)	Baz-Lomba et al., 2016
	Finland	Nation	9 (methylone, MDPV, etc.)	Kankaanpää et al., 2014
	Italy	Nation	2 (MEPH, KET)	Castiglioni et al., 2015
		City	35 (MEPH, methylone, etc.)	González-Mariño et al., 2016a
	Greece	Region	10 (MEPH, BZP, etc.)	Borova et al, 2015
	Spain	Nation	42 (MDPV, mCPP, TFMPP, etc)	Andrés-Costa et al., 2016
	Poland	City	5 (MEPH, MDPV, mCPP, etc.)	Styszko et al., 2016
	UK, Spain, Italy, Norway	Nation	17 (MEPH, MDPV, etc.)	González-Mariño et al., 2016b
Oceania	Australia	City	7 (MEPH, MDPV, BZP, etc.)	Chen et al., 2013
		Region	2 (methylone, MEPH)	Thai et al., 2016b
		City	21 (BZP, TFMPP, MEPH, etc.)	Tscharke et al., 2016
Asia	China	Nation	5 (BZP, TFMPP, MEPH, etc.)	Gao et al., 2016

a) MEPH, mephedrone; MDPV, methylenedioxypyrovalerone; BZP, benzylpiperazine; TFMPP, trifluoromethylphenylpiperazine; mCPP, 1-(3-Chlorophenyl)piperazine; PMMA, para-methoxy-N-methylamphetamine

districts of some forty provincial capitals and equivalent cities total about 350 WWTPs and serve a population size of 270 million people (based on 2010 census data). A nation-wide drug use monitoring network involving all these WWTPs is not difficult to build. Likewise, every province can build its own province-wide wastewater-based monitoring network.

It is worth noting that for such nation or province-wide monitoring networks, excretion rates of DTR are no longer an issue of that importance. Although inaccuracy in excretion rates lead to uncertainties in absolute drug consumption estimation, the errors it causes are systematic and does not affect relative comparison between different cities and regions. In addition, although population size of a particular WWTP cannot be accurately estimated at this stage, total population sizes of cities in China can be better constrained (e.g. by per capita water consumption). Thus, as long as all the WWTPs of a city are sampled, the total drug loads of the city can be calculated with relatively low uncertainties (based on accurate flow and drug concentration measurement) and normalized to the entire population of the city. Normalized loads derived this way would allow reliable comparison between different cities across the country. The only possible obstacle for reliable spatial comparison is the potential variability in in-sewer stability of drugs in different geographic regions. Therefore, in the nearest future, in-sewer stability of drugs in wastewater, especially its possible spatial variability, is the top priority of WBE research in China.

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