REVIEW ARTICLE

Biomarker selection strategies based on compound stability in wastewater-based epidemiology

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Abstract

The specific compositions of human excreta in sewage can be used as biomarkers to indicate the disease prevalence, health status, and lifestyle of the population living in the investigated catchment. It is important for guiding and evaluating public health policies as well as promoting human health development. Among several parameters of wastewater-based epidemiology (WBE), the decay of biomarkers during transportation in sewer and storage plays a crucial role in the back-calculation of population consumption. In this paper, we summarized the stability data of common biomarkers in storage at different temperatures and in-sewer transportation. Among them, cardiovascular drugs and antidiabetic drugs are very stable which can be used as biomarkers; most of the illicit drugs are stable except for cocaine, heroin, and tetrahydrocannabinol which could be substituted by their metabolites as biomarkers. There are some losses for part of antibiotics and antidepressants even in frozen storage. Rapid detection of contagious viruses is a new challenge for infectious disease control. With the deeper and broader study of biomarkers, it is expected that the reliable application of the WBE will be a useful addition to epidemiological studies.

Keywords Wastewater-based epidemiology · Biomarker · Stability · Excretion rate · Illicit drugs · Therapeutic drugs · Pathogens

Introduction

Based on the analysis of human excretion products (biomarkers) in municipal wastewater, wastewater-based epidemiology (WBE) was proposed to estimate the amount of specific drugs used by the population in a given catchment of a wastewater treatment plant (WWTP) (Daughton 2001).

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It has been successfully applied to estimate the consumption of illicit drugs (Du et al. 2020a) and therapeutic drugs (van Nuijs et al. 2015). The drug would be lost during the transportation after the excretion from human body to the WWTPs due to hydrolysis, absorption, repartition, and microbial decay. According to the substance balance, the per capita mass load can be back-calculated based on the drug concentration in the influent of the WWTP by Eq. (1) (Zuccato et al. 2008):

$$M_C = \frac{C \times F}{P \times S \times E} \times \frac{1}{10^6} \tag{1}$$

where M_c is the daily per capita consumption of the target drug, mg· (day·1000 people)$^{-1}$; C is the concentration of the drugs measured in the influent, ng·L^{-1}; F is the daily influent flow of the WWTP, L·day^{-1}; P is the population of the area served by the WWTP, 1000 people; S is the stability of drugs, %; E is excretion rate of the drugs, %.

In addition to the back-calculation of drugs consumption, the research of WBE has gradually expanded to other aspects, such as using endogenous (produced naturally in the body) or exogenous metabolites (substances produced by intentional

consumption or by accidental exposure) to assess the health status of the population (Gracia-Lor et al. 2017a). Therefore, the drug in Eq. (1) can be collectively referred to as biomarker.

In the WBE study, the uncertainty is the combined results of all parameters in Eq. (1). Among them, F is provided by the operator of WWTP and it is related to the maintenance of the flow meter. As for C and P, there are a lot of studies have been carried out to increase the accuracy of the analysis of compounds (Félix et al. 2016) and population estimation (Rico et al. 2017). Compared to other parameters, there are still many blanks in the study of the human excretion rate of the drugs (E) and their stability (S). A suitable biomarker must meet the following criteria: (1) It must be excreted primarily in urine and the concentration level in urine should be at least in the µg/L level to ensure that it can be detected after dilution by raw sewage (Chen et al. 2014). (2) It must be sufficiently stable in sewage, and substances with low decay ($< 20\%$) are generally considered suitable as biomarkers (McCall et al. 2016; Daughton 2012b). (3) Preferably, it should be specific to human metabolism, ensuring that its presence originates only from human metabolism and not from external emissions (Daughton 2012a). (4) The excretion is consistent across populations (gender, age, ethnicity, etc.). According to the above criteria, some of the easily decay substances (through absorption, hydrolysis, and/or degradation process) are not suitable as biomarkers for back-calculation while their stable metabolite may be an alternative. Thus, a correction factor (CF) is added to revise Eq. (1) as Eq. (2):

$$M_C = \frac{C \times F \times CF}{P \times S} \times \frac{1}{10^6} \qquad (2)$$

where C, S are the parameters for metabolites, and CF is calculated by Eq. (3):

$$CF = \frac{\frac{MW_{\text{parent drug}}}{MW_{\text{metabolite}}}}{E_{\text{metabolite}}} \qquad (3)$$

where $MW_{\text{parent drug}}$ is the molecular mass of the parent drug; $MW_{\text{metabolite}}$ is the molecular mass of the metabolite; $E_{\text{metabolite}}$ is the excretion rate of the metabolite, %.

In this paper, we summarize the human excretion rates and stability in-sewer and in-sample of biomarkers reflecting drug use, lifestyle, and disease transmission based on the available WBE papers, expecting to lay the foundation for biomarker selection in the application of WBE.

Data sources

The literature search was conducted in the Web of Science database for the period: January 1, 2000–April 2, 2022; search content: subject = (sewage epidemiology) or (wastewater-based epidemiology), literature type = article and review. Papers that reported substance stability data were manually screened and summarized according to their study objects and experimental conditions.

Biomarkers of addictive substances

Illicit drugs

Initially, WBE was used to investigate cocaine use in the community (Zuccato et al. 2005), and has now been well applied to various illicit drugs. We evaluated the storage stability, and stability in-sewer for each substance according to high stability (decay 0–20%), medium stability (decay 20–60%), and low stability (decay 60–100%) (McCall et al. 2016) based on the available papers (Table 1).

Only 1–5% of cocaine entering the body is excreted as prototype, and it is unstable in sewage (Gheorghe et al. 2008), with about 29.2% being excreted as the metabolite benzoylecgonine (BEG) (Ambre et al. 1988). Cocaine consumption is mainly back-calculated through BEG because of its unique source, stability, and qualitative and quantitative match to human urine (Thai et al. 2014). Castiglioni et al. obtained a correction factor (CF) of 3.59 for cocaine

Table 1 Stability and correction factors of illicit drug biomarkers[1]

Parent/metabolite	Storage and in-sewer stability	CF
BEG	High	3.59
THC-COOH		152
Morphine		3.1
EDDP		2.04
Amphetamine		3.3
Methamphetamine		2.3–4.06
MDMA		1.5—6.7
Nor-ketamine		NA
Ketamine	Medium	50
Methadone		NA
Heroin	Low	NA
6-MAM		86.9
Cocaine		NA
THC		NA

[1]The stability classification of the substances in-sewer and in the storage period is: high stability (decay < 20%), medium stability (decay 20–60%), and low stability (decay > 60%). Data in the Table 1 are from Li et al. 2020; Baker and Kasprzyk-Hordern 2011; Causanilles et al. 2017; Du et al. 2020b; Gao et al. 2017; Gracia-Lor et al. 2016; Heuett et al. 2015; Li et al. 2021; Lin et al. 2021b; McCall et al. 2017; Ramin et al. 2017; Ramin et al. 2018; Senta et al. 2014; Thai et al. 2014; van Nuijs et al. 2011a, b; Zheng et al. 2021; and Zuccato et al. 2008. Storage condition stability study at pH = 2 for 72 h–196 days; in-sewer stability study for 6–8 h. NA: not available

by dividing the molecular weight ratio COC/BEG (303/289) by 0.292 (Castiglioni et al. 2013).

The main active ingredient of cannabis is Δ-9-Tetrahydrocannabinol (THC), but it cannot be used as a biomarker for WBE due to its instability in sewage (Heuett et al. 2015). THC-COOH, a metabolite of THC, is highly stable in sewage compared to other metabolites and is therefore used as a biomarker for cannabis consumption (Causanilles et al. 2017). However, cannabis is very extensively metabolized in the human body and the excretion percentage of THC-COOH is only 0.5% with its CF of 182 (Gracia-Lor et al. 2016). In addition, due to the low polarity of THC-COOH, the recovery rate of THC-COOH in the sample after acidification treatment is only 52% (Causanilles et al. 2017). The low excretion and low recovery rate are two difficulties for accurate estimation of cannabis consumption. Future research can be conducted for the screening of more suitable biomarkers or the optimization of sample pretreatment methods.

Opioids are still one type of abused drugs in Europe. Heroin is rapidly hydrolyzed to 6-acetyl morphine (6-MAM) in the body and further hydrolyzed to morphine in the liver (Smith 2009), so 42% of the heroin taken is excreted as morphine and 1.3% as 6-MAM (van Nuijs et al. 2011b). Heroin and 6-MAM are unstable in sewage and sewer (McCall et al. 2016; Thai et al. 2014), and back-calculation based on their concentrations may underestimate heroin consumption. Morphine is highly stable with a CF of 3.1 (Zuccato et al. 2008). However, the amount of morphine produced by therapeutic use and codeine metabolism should be deducted (Du et al. 2019). This poses new challenges to the back-calculation and more studies are needed to find eligible biomarkers for opioids consumption.

Amphetamine-type stimulants (ATS) are the most used stimulants (UNODC 2015), mainly including amphetamine, methamphetamine, and MDMA (3,4-Methylenedioxy-N-methylamphetamine). The three illicit substances currently use the parent compounds as biomarkers, and all are chiral enantiomers. Differences in the metabolic conversion rate of chiral enantiomers change the ratio of enantiomers. So, verifying their chiral characteristics in sewage allows to distinguish between direct disposal and consumer use of the substance (Xu et al. 2017). For example, methamphetamine-producing plants or police raids may result in direct disposal and thus affect the back-calculation result. The two can be effectively distinguished using enantiomeric profiling, with enantiomeric fractions (EFs) > 0.5 indicating illegal use and EFs equal to 0.5 indicating direct disposal (Eq. 4). However, pure enantiomer will be produced in the synthesis of methamphetamine using ephedrine, at which point enantiomeric profiling cannot distinguish between processing events. Bade et al. recently found that direct disposal and consumption of methamphetamine could be distinguished when

benzidine was used as the biomarker for methamphetamine. Moreover, it showed high stability (decay rate < 20%) for up to 1 week at both 4 °C and 20 °C. They obtained reliable results when they used the method to back-calculate methamphetamine consumption in samples taken from Australian WWTPs (Bade et al. 2021).

$$EF = \frac{MDMA(-)}{MDMA(-) + MDMA(+)} \tag{4}$$

Ketamine and methadone are two of the more studied illicit drugs. Although both ketamine and its metabolite nor-ketamine are very stable in sewage (Lin et al. 2021b), ketamine is generally chosen as the biomarker because of its higher concentration and detection frequency in sewage. Du et al. revised the ketamine excretion factor (E = 20%) and the consumption data estimated using the new excretion factor matched better with the official data (Du et al. 2020b). The main metabolite of methadone is EDDP and they both show high stability in sewage (van Nuijs et al. 2012). Five to fifty percent of methadone is excreted in urine as prototype and 27.5% is generally used as the excretion rate (Postigo et al. 2011); the excretion rate of EDDP is 55% (Thai et al. 2016) and CF is 2.04 (Zheng et al. 2021). It has been reported that methadone is completely undetectable after 72 h at 4 °C (González-Mariño et al. 2010). So, it is more appropriate to use EDDP as biomarker for drug consumption back-calculation based on the current study.

New psychoactive substances

New psychoactive substances (NPS) are substances that are not controlled by the Convention on Psychotropic Substances of 1971 but are abused and can pose a threat to public health (UNODC 2013). These substances are produced by slightly altering the functional groups of controlled substances (e.g., cocaine, amphetamines) and have psychoactive effects as traditional drugs. Over the past few years, sales of NPS in the illicit drug market have grown rapidly, and new categories continue to emerge. Their high toxicity and adverse health effects have raised concerns because some death cases were reported (Krotulski et al. 2018), and its consumption estimation is gradually being carried out.

The prevalence of NPS and the detected concentrations in sewage are low (pg·L^{-1}-ng·L^{-1}) compared to other illicit drugs due to a large number of species and constant new synthesis (Bijlsma et al. 2020). Synthetic cathinones and phenethylamines are the two main types of NPS that are used more frequently. Among them, methcathinone, mephedrone, and methylone were the substances detected more frequently in Europe in the past 2 years (Castiglioni et al. 2021). In 2017–2018, the most detected in Australia were pentylone, N-ethylpentanone, and ethylone, and the popular

substances in 2019–2020 became eutylone, mephedrone, and methylone (Bade et al. 2020). The current studies on NPS focus on qualitative determinations using parent compounds as biomarkers to detect temporal differences in prevalence across regions and determine potential outbreaks in their use. However, the lack of pharmacokinetic data and drug stability data in the sewer and sewage may hinder accurate back-calculation of NPS drug consumption. We summarize the decay of some NPS biomarkers in different states (in Table 2), which are stable substances and are used for reliable results of consumption back-calculation.

Drugs for common diseases or exogenous biomarkers

The effectiveness of community health programs, which play an important role in public health, depends on routine monitoring and assessment of the health status of community populations, and the current challenge is mainly the timeliness and reliability of the assessments. Exogenous biomarkers refer to specific classes of drugs, such as antibiotics, antidepressants, and β-blockers, which are mainly used in the treatment of specific diseases.

Antibiotics

The antibiotics can be classified into macrolides, tetracyclines, fluoroquinolones, quinolones, penicillins, cephalosporines, lincosamides, sulfonamides, and nitroimidazoles according to their chemical structures. Parent compounds are now commonly used as biomarkers for the detection of antibiotics. Among them, penicillins, cephalosporins, sulfonamides, nitroimidazoles, and lincosamides are considered relatively stable (decay < 20% after 3 months of storage at − 20 °C); In contrast, fluoroquinolones, quinolones, tetracyclines, and macrolides are unstable (Llorca et al. 2014). It was reported that the metabolites of sulfapyridine, sulfamethoxazole, trimethoprim, and azithromycin were stable

(decay < 20%) in sewage at 4 °C (48 h) and − 20 °C (30 days) (Han et al. 2021).

However, the assessment of the consumption of antibiotics has significant limitations. In addition to the therapeutic use by humans, a large portion of the antibiotics in sewage originates from the sewage discharges from pharmaceutical plants and breeding. It was reported that about one-third of unused antibiotics were disposed directly in landfills (Zhang et al. 2020) and the antibiotics may enter the WWTPs with the landfill leachate (Yu et al. 2020), leading to an overestimation of human antibiotic consumption. The stability of antibiotics and their metabolites in-sewer is also unknown, and more relevant studies are needed to propose more targeted human's biomarkers to improve the accuracy of antibiotic consumption estimation.

Antidepressants

Major depressive disorder (MDD) is the 11th most important factor in disability-adjusted life years globally (Murray et al. 2012). Selective 5-HT reuptake inhibitors (SSRIs) are commonly prescribed antidepressants, including fluoxetine (FLU), sertraline (SER), paroxetine (PAR), and citalopram (CTP). Another class of antidepressants are selective 5-hydroxytryptamine and norepinephrine reuptake inhibitors (SSNRIs), represented by venlafaxine (VEN). These antidepressants have been widely marketed since the mid-1980s, mainly for patients diagnosed with clinical depression (Schultz and Furlong 2008).

Lin et al. investigated the stability of antidepressants in sewage and sewer under different conditions. It was found that VEN, FLU, and CTP showed high stability after 72 h at 4–35 °C (Table 3). The biodegradation rates of PAR and SER increased with temperature, with decay rates of about 26% and 36% at 35 °C, respectively, and were medium stable substances. When the temperature was below 15 °C, the decay of drugs was mainly due to the adsorption of suspended solids (SS), and their adsorption basically followed the K_{ow} order, indicating that K_{ow} can be used to predict

Table 2 Stability of NPS biomarkers under different conditions[1]

Parent/metabolite	Storage stability	In-sewer stability
Methylenedioxypyrovalerone (MDPV)	High	High
Methoxetamine (MTO)		
4-Methoxy-methamphetamine (PMMA)	NA	
4-Methoxy-amphetamine (PMA)	NA	
Methiopropamine (MPA)	High	Medium
Mephedrone (MEPH)	High	Low
Methylone (METL)	High	Low

[1]The stability classification of the substances in-sewer and in the storage period is: high stability (decay < 20%), medium stability (decay 20–60%), and low stability (decay > 60%). Data in Table 2 are from Gao et al. 2017; Bade et al. 2020; Kinyua et al. 2018; Mardal and Meyer 2014; and McCall et al. 2017. Storage condition stability study at pH = 2 for 24 h–14 days; in-sewer stability study for 6–24 h. NA: not available

Table 3 Stability of common drugs in different states[1]

Category	Parent/metabolite	Storage stability[a]			In-sewer stability[b]
		Room temperature (22 ± 2 °C)	Refrigeration (4 ± 2 °C)	Frozen (−20 ± 2 °C)	
Antidepressants	Citalopram (CTP)	High	High	High	High
	Fluoxetine (FLU)			NA	Medium
	Venlafaxine (VEN)			Medium	Medium
	Paroxetine (PAR)	Medium	High	Medium	Low
	Sertraline (SER)			NA	
Sedative and hypnotic drugs	Flunitrazepam (FLUN)	Low	Low	High	NA
	Nitrazepam (NZP)	Medium	High	High	High
	Diazepam (DZP)				Medium
	Oxazepam (OXA)	High	High	High	High
	Clonazepam (CLON)				
	Temazepam (TEM)				NA
	7-Amino clonazepam (7ACLON)				
	7-Amino flunitrazepam (7AFLUN)			NA	
Cardiovascular drugs	Bezafibrate (BZB)	High	High	High	Medium
	metoprolol (MET)			NA	High
	Gemfibrozil (GFB)			NA	High
	Atorvastatin (ATO)			Medium	High
	Atenolol (ATE)			Medium	High
Antidiabetic drugs	Metformin (MEF)	High	High	High	High
	Glipizide (GLP)			NA	
Asthma and antihistamines	Salmeterol (SX)	High	High	NA	Medium
	Salbutamol (SAL)				High
	Loratadine (LOR)	Low	High	NA	Medium
	Desloratadine (DLOR)			Medium	
	Cetirizine (CER)	High	High	NA	Medium
	Fexofenadine (FEX)			High	High

[1]The stability classification of the substances in-sewer and in the storage period is: high stability (decay < 20%), medium stability (decay 20–60%), and low stability (decay > 60%). Data in Table 3 are from Baker and Kasprzyk-Hordern 2011; Fedorova et al. 2014; Gao et al. 2019; Lin et al. 2021a; Liu et al. 2022; Racamonde et al. 2014; Richard et al. 2020; and Yan et al. 2019. NA: not available

[a]Room temperature stability study for 72 h–7 days; refrigeration stability study for 72 h–7 days; frozen stability study for 27–120 days

[b]In-sewer stability study for 6–24 h

the adsorption of SS on antidepressants. The decay rates of VEN, FLU, and CTP in the aerobic and anaerobic sewer with biofilms ranged from 20 to 60% after 24 h. They are medium-stable substances and can be used as biomarkers of WBE within a certain range. While PAR and SER decayed > 80% (Lin et al. 2021a), they are low stable substances with large errors in back-calculation and are not suitable as biomarkers for WBE.

WBE can be an important tool for assessing the mental health of the population, but in developing areas where people often do not seek treatment because of the lack of understanding of mental health problems or forced by economic and social pressures. Thus, back-calculating drug consumption to determine the mental health of the area may lead to optimistic results.

Sedative and hypnotic drugs

Benzodiazepines are commonly prescribed psychotropic drugs used to treat various psychiatric disorders such as anxiety, stress, and insomnia. However, they have a high dependence potential and are frequently abused (Richard et al. 2020), which have been detected in ng L^{-1} levels in sewage (Asimakopoulos and Kannan 2016). Since many benzodiazepines share common metabolites (for example, oxazepam is a metabolite of temazepam and diazepam) (Griffin et al. 2013), the parent compound is mainly used as biomarker. Although clonazepam and flunitrazepam decayed < 20% in sewage after 24 h, their metabolites, 7-aminoclonazepam, and 7-aminoflunitrazepam, are more stable (decay rates < 5%) (Table 3). Therefore, it is more

appropriate to use their metabolites as biomarkers (Liu et al. 2022; Racamonde et al. 2014). The excretion rate of 7-aminoflunitrazepam is about 90% and CF is 0.81 (Shu et al. 2021).

Currently, most of the research on benzodiazepines is limited to the detection of drugs, and only a few drugs have reported stability data (Liu et al. 2022). The link between drug use levels and population health has not been established, so more research is needed to promote the use of WBE for this class of drugs.

Cardiovascular drugs

Cardiovascular diseases are common in middle-aged and elderly people over 50 years old, with high prevalence, disability rate, and mortality rate, and ranking the first among various causes of death (McAloon et al. 2016). In recent years, cardiovascular diseases are trending younger due to obesity and unbalanced diets, so the use of WBE to monitor the use of cardiovascular drugs in specific regions is important to understand the health level of the population.

One of the more used classes of drugs is the β-adrenergic receptor blockers (β-blockers), such as atenolol, metoprolol, nadolol, and propranolol, the parent compound is now mostly used as biomarker. Ninety-two percent of atenolol is excreted in the urine as prototype, whereas propranolol is almost completely absorbed in humans and only 0.5% is excreted in the urine as prototype (Thomaidis et al. 2016). Metabolism of metoprolol is similar to it, with 5% excreted as prototype (Alder et al. 2010). Typical cardiovascular drugs (metoprolol, bezafibrate, gemfibrozil, and atenolol) are highly stable in sewage and sewer (Lin et al. 2021a) (Table 3), and therefore they are considered suitable biomarkers for WBE.

Antidiabetic drugs

Diabetes has serious complications, such as stroke, heart disease, kidney disease, peripheral artery disease, and vision impairment. Its prevalence has been increasing worldwide over the past decades (Rafael et al. 2013), and therefore the prevalence of diabetes needs to be continuously monitored to reflect the health status of the population. Metformin is a synthetic antidiabetic drug with no natural origin or known analogs (Yao et al. 2018) and is the most widely used drug in the treatment of diabetes (Viollet et al. 2012). It is not metabolized in humans and is mainly excreted in urine and feces (80%), with high stability in both sewage and sewer (Lin et al. 2021a; Yan et al. 2019) (Table 3), and thus meets the criteria for biomarker. In addition, glipizide is one of

the most widely used oral antidiabetic drugs. It is rapidly absorbed in the intestine and metabolized by the liver, and about 97% is excreted in the urine. Glipizide exhibits high stability in both sewage and sewer from 4 to 35 °C (Lin et al. 2021a) and meets the criteria of biomarker that can be used as a complement to metformin to jointly assess the prevalence of diabetes.

Asthma drugs and antihistamines

The global prevalence of asthma and allergic rhinitis is increasing, with children and young adults being the most prevalent groups (Pawankar 2014). Detecting the prevalence of asthma and allergy in the population and discovering its relationship with specific environmental factors are new applications of WBE. The three common antihistamines loratadine, fexofenadine, and cetirizine, as well as the asthma drugs salbutamol and salmeterol, are excreted in the urine after administration. And their consumption is now mainly back-calculated using the parent drug as biomarker (Choi et al. 2018; Liu et al. 2022). The decay rate of salbutamol is < 20% after 24 h in sewage and sewer (Table 3), which is a highly stable substance. While salmeterol is stable in sewage, its decay rate in the aerobic sewer is > 40% (Lin et al. 2021a), making it a medium stable substance and therefore salbutamol is more suitable as a biomarker for asthma drugs. Loratadine and its metabolite desloratadine are unstable (decay rate > 50%) in sewage and sewer. Cetirizine is stable in sewage (decay rate < 20% after 72 h at room temperature), but decayed > 20% in aerobic sewer. Whereas, Fexofenadine is stable in both sewage and sewer with a decay rate < 20% (Table 3) (Fedorova et al. 2014; Liu et al. 2022), making it more suitable as biomarker for antihistamines. In addition to this, more asthma, allergy, and other respiratory medications should be detected, as consumers may use many different medications based on need and preference.

Anticancer drugs

Cancer is now considered to pose a serious threat to global development and according to surveys globally, the chances of developing cancer during the lifetime (0–79 years) are 1 in 3 for men and 1 in 4 for women (Rafael et al. 2013). Due to the increased use of various anticancer drugs, they were also frequently detected in sewage (ng/L) (Victoria et al. 2014). Most anticancer drugs are structurally stable to maximize their reach to the part of the body that needs treatment. Hence, they are highly chemically stable (Janssens et al. 2019) and not biodegradable (Kummerer et al. 1997).

However, data on stability studies in sewer and sewage are not yet available. The main source of anticancer drugs in sewage is excretion by patients, so suitable anticancer drugs should be selected as biomarkers for the assessment of the prevalence of different types of cancer in a specific regional population.

Endogenous biomarkers

Endogenous compounds are associated with human metabolism and can avoid the problems of exogenous compounds, e.g., differentiating the origin of substances in sewage, when used as biomarkers. However, their main limitation is the intra and inter individually excessive variability in excretion. Creatinine, 5-HIAA, cortisol, androstenedione, and isoprostane are currently the main endogenous biomarkers used for population assessment and oxidative stress levels.

Creatinine is a decomposed product of creatine phosphate in muscle and is usually produced at a constant rate. But it is unstable in sewage (Rico et al. 2017) (Table 4) and cannot be used as a biomarker. 5-HIAA, cortisol, and androstenedione have been reported to have high urinary excretion rates and are therefore considered to have potential as population biomarkers. However, cortisol and androstenedione are unstable in sewer, whereas 5-HIAA exhibits high stability (Thai et al. 2019), with a level of approximately 3.44 mg·(day·person)$^{-1}$ in urine (Tisha et al. 2008), therefore is the most promising biomarker available. The potential of catecholamine metabolites—homovanillic acid (HVA) and vanillylmandelic acid (VMA)—as biomarkers were recently evaluated and both were found to be stable in sewage, with < 20% decay after 14 days of storage at − 20 and 4 °C. Their excretion factors are 1.24 ± 0.07 and 1.07 ± 0.05 mg·(day·person)$^{-1}$ for HVA and VMA, respectively, and are suitable for monitoring relative population size changes in the short term (Pandopulos et al. 2021).

Table 4 Stability of other biomarkers in different states[1]

Endogenous metabolites	Storage stability		In-sewer stability
	Room temperature (22 ± 2 °C)	Refrigeration (4 ± 2 °C)	
Creatinine	Low	Medium	Low
5-HIAA	High	High	High
8-iso-PGF2α	High	NA	High

[1]The stability classification of the substances in-sewer and in the storage period is: high stability (decay < 20%), medium stability (decay 20–60%), and low stability (decay > 60%). Data in Table 4 are from O'Brien et al. 2019; Rico et al. 2017; Ryu et al. 2015; and Thai et al. 2019. Stability study for 4–30 h. NA: not available

8-iso-prostaglandin F$_{2\alpha}$ (8-iso-PGF$_{2\alpha}$) and prostaglandin E$_2$ (PGE$_2$), which are associated with oxidative stress, are stable in sewage and sewer (O'Brien et al. 2019; Ryu et al. 2015), and thus may serve as reliable biomarkers of oxidative stress in the community.

Pathogens

Monitoring the presence of pathogens in sewage from entire communities is important for preventing the spread of infectious diseases. The use of WBE for this detection is an emerging field that shows more potential with the rapid development of technologies such as high-throughput sequencing and bioinformatics.

Conventional pathogens

Human enteroviruses are responsible for many stomachs and intestinal infections, respiratory infections, conjunctivitis, hepatitis, and other serious infectious agents with high mortality rates in immunocompromised individuals. Patients with viral gastroenteritis or viral hepatitis excrete approximately 10^5 to 10^{11} viral particles/g stool (Bosch 1998), including adenovirus, astrovirus, norovirus, hepatitis E virus, and enterovirus. Therefore, routine monitoring of pathogenic enteroviruses in sewage is important to understand the abundance and transmission of viruses in a certain area. It provides valid information for the development and evaluation of health intervention programs.

Enteroviruses can survive over a wide pH range (pH 3–10) and at low temperatures for 4–6 months (Kocwa-Haluch 2001). Moreover, due to their host-specific requirements (infecting only humans), they have no regenerative capacity in the environment and can be used to assess the risk of virus transmission (Fong and Lipp 2005). For example, high concentrations of the virus were detected in sewage during norovirus outbreaks (5×10^3–2×10^4 copies·mL^{-1}) (Thongprachum et al. 2018). The sewage analysis has proved to be a very sensitive method for monitoring poliovirus transportation (Ivanova et al. 2019), with a detection limit of 20 CCID$_{50}$ (Delogu et al. 2018), can help track wild and vaccine-derived poliovirus, and has played a major role in the global elimination of poliovirus.

In the follow-up of eight pathogenic viruses (norovirus, astrovirus, astrovirus, rotavirus, adenovirus, Aichi virus, paralysis virus, hepatitis A virus, and hepatitis E virus) in sewage, it was found that Ct thresholds of 24.3–45 for viruses could be used as an early warning of outbreaks (Hellmér et al. 2014).

SARS-CoV-2

The recent outbreak of corona virus disease 2019 (COVID-19) has become a global public health event, with the number of infections continuing to increase in many countries, causing unprecedented harm to human health and safety. Several recent studies have demonstrated the presence of live SARS-CoV-2 in the stool and urine of both symptomatic and asymptomatic populations (Jeong et al. 2020; Xiao et al. 2020). In addition, viral RNA continued to be detected in stool, rectal swabs, and urine for 5 weeks after patients tested negative for respiratory samples and resolution of symptoms (Ling et al. 2020; Xu et al. 2020), and survived for 4 days in urine, but only 2 days in the stool (Liu et al. 2021).

The steps to detect SARS-CoV-2 RNA concentration in sewage include virus concentration, extraction, and quantification using RT-qPCR (Kumblathan et al. 2021). However, SARS-CoV-2 RNA may be broken down into smaller fragments during sewer transportation as well as in sewage, so there is a large uncertainty for the detection of viral RNA in sewage. Boogaerts et al. tested the stability of N2 and E gene fragments and found that after 10 days of storage in 4 °C sewage, the N2 gene showed high stability and the E gene was relatively less stable (Boogaerts et al. 2021a); However, Hokajärvi et al. experimentally found that the E gene remained stable after 84 days of storage in sewage at 4 °C (Hokajärvi et al. 2021). The results of current studies on viral RNA stability are highly variable, so more studies on viral RNA stability under standardized and uniform conditions are needed. Additionally, the viral RNA load in sewage is substantially reduced in the -20 °C storage (Boogaerts et al. 2021a), so sewage samples should be transported and stored at 4 °C to reduce the decay of viral RNA. More studies are also needed to explore the effects of sewer transportation on viral RNA.

Studies have shown that the slow decay of SARS-CoV-2 RNA in sewage supports its detection and quantification, with detection limits of 3.99, 5.52, and 5.74 copies/L for E gene, RNA, and N gene, respectively (Monteiro et al. 2022), which can be used as a feasible tool to monitor the presence of the virus in the community. Currently, most of the diagnostic criteria for positive novel coronaviruses worldwide are Ct values $\leq$ 35. There are no criteria for determining whether there is an outbreak from the concentration of virus in sewage, and more research is needed to propose uniform criteria to enable WBE to better monitor the development of the outbreak.

Other applications of biomarkers

Exogenous biomarkers to back-calculate population size

In addition to assessing the use of illicit substances and common drugs, some biomarkers are stable and have low fluctuations in per capita emissions that can be used to assess the size of the population in the investigated area, and Eq. (5) is obtained by deforming Eq. (1)

$$P = \frac{C \times F}{DDD \times E} \times \frac{1}{10^3} \tag{5}$$

where C is the biomarker concentration, ng·L^{-1}; F is the daily flow rate of the WWTP, L·day^{-1}; and DDD is the daily dose required per 1000 inh, mg· (day·1000 people) $^{-1}$; E is the excretion rate, %.

The artificial sweetener acesulfame (ACE) is not metabolized in humans and is completely excreted after consumption with a DDD of 0.057 mg· (day·1000 people) $^{-1}$ (Rico et al. 2017). ACE is highly stable (decay < 20%) in sewage and sewer (Lin et al. 2021b; O'Brien et al. 2019) and has therefore been proposed as a potential biomarker. Fourteen exogenous compounds, including atenolol, carbamazepine, codeine, norfloxacin, and paracetamol, were quite reliable because of their strong correlation between population and mass load ($R^2 > 0.8$) (O'Brien et al. 2014). They can be used as potential biomarkers for population estimation, but the respective mass loadings within different regions or at different population sizes need to be obtained based on specific consumption characteristics.

Exogenous markers to assess population lifestyle habits

Alcohol

After consumption of alcohol-containing beverages, 95–98% of ethanol is metabolized primarily in the liver, where ethanol is first converted to acetaldehyde, further oxidized to acetic acid, and the rest is excreted through urine, breathing, and sweat (Jones 1990). Ethyl sulfate (EtS) and ethyl glucuronide (EtG) can be detected in urine 1 h after alcohol ingestion (Helander and Beck 2005) and they are good indicators of alcohol consumption. Among them, EtG is less stable in sewage and sewer (Banks et al. 2018) (Table 5), while EtS shows high stability and originates only from the human metabolism of alcohol. It is therefore considered as the best biomarker for estimating alcohol consumption (López-García et al. 2020). Thai et al. obtained a CF of 4000 by directly comparing alcohol sales data with EtS concentrations measured in sewage (Thai et al. 2021), overcoming the low excretion rate of EtS (0.010–0.016%) (Reid et al. 2011) and effectively improving the accuracy of alcohol consumption estimates for the Australian population. However, the use of the CF in different regions will bring uncertainty due to the large variation in the excretion rate of EtS in the population and the different levels of decay of EtS in different sewers.

Table 5 Stability of alcohol, tobacco, and caffeine and their metabolites in different states and their correction factors[1]

Category	Parent/metabolite	Storage stability		In-sewer stability		CF
		Room temperature (22 ± 2 °C)	Refrigeration (4 ± 2 °C)	GS	RM	
Alcohol	EtS	High	NA	High	Medium	4000
	EtG	Low		Low		NA
Tobacco	Nicotine	High		High		7.69
	Cotinine					3.13
	Anabasine			High	Medium	NA
	Anatabine			Medium		NA
	Trans-3′-hydroxycotinine			High		2.31
Caffeine	Caffeine	Medium		Medium	Low	41.67
	Paraxanthine	NA			Medium	NA

[1]The stability classification of the substances in-sewer and in the storage period is: high stability (decay < 20%), medium stability (decay 20–60%), and low stability (decay > 60%). Data in Table 5 are from Banks et al. 2018; Gao et al. 2019; Lai et al. 2017; Nguyen et al. 2018; Tscharke et al. 2016; and Zheng et al. 2020. Storage condition stability study at pH = 2 for 12 h–14 days; in-sewer stability study for 3–12 h. NA: not available

Tobacco

Nicotine, the main alkaloid in tobacco, is addictive and is the main reason for the continued use of tobacco products (Hukkanen et al. 2005). Nicotine is extensively metabolized in humans and excreted in the urine as parent and metabolites (mainly cotinine and trans-3′-hydroxycotinine). All three compounds show high stability in sewers and sewage (Castiglioni et al. 2014) (Table 5), so they can be used as biomarkers for the back-calculation of tobacco consumption. From the available literature, we determined the mean percentage excretion of cotinine and trans-3′-hydroxycotinine after nicotine absorption (Benowitz and Jacob 1994; Jacob et al. 1988) (Table 6). Since more than 90% of the conjugated form of the metabolite can be converted to the free state by β-glucuronidase in sewage (Rodríguez-Álvarez et al. 2014), the CF is calculated to be 3.13 and 2.31 using 30% and 44% as the excretion rates of cotinine and trans-3′-hydroxycotinine, respectively (Boogaerts et al. 2021b).

It is noted that the use of teas, nicotine replacement therapies (NRTs), and e-cigarettes also excrete nicotine and may lead to an overestimation of tobacco consumption. Two specific biomarkers of tobacco, anabasine, and anatabine, are found stable in sewage (Tscharke et al. 2016) and can be

Table 6 Nicotine excretion rate in human urine

Compound	Nicotine	Cotinine	Trans-3′-hydroxycotinine
Excretion rate(%)-free form	10	13	35
Excretion rate(%)-conjugated form	3	17	9
Total excretion rate (%)	3	30	44

used as biomarkers of tobacco consumption (Zheng et al. 2020). However, information on excretion rates for anabasine and anatabine is not available and only mass load changes can be used to assess tobacco use.

Caffeine

Caffeine is the most widely used stimulant in the world and is widely found in a variety of teas, energy drinks, coffee, and some medications, but coffee is its primary source. Caffeine is extensively metabolized in the liver, with 1.7% of caffeine excreted in the urine as prototype and the rest as metabolites: 3,7-dimethylxanthine (1.5%), 1,7-dimethylxanthine (4.6%), and 1,3-dimethylxanthine (0.6%), further decomposition to 1,7-dimethyl uric acid (6.7%) (Heckman et al. 2010). By comparing the metabolic profiles of each metabolite in sewage and urine, only 1,7-dimethyluric acid was found to be a suitable biomarker with a CF of 14.8 (Gracia-Lor et al. 2017b).

Other foods

Some specific foods are also used as biomarkers to assess the standard of living of the population in a given area. N-methyl-2-pyridone-5-carboxamide (2PY) and N-methyl-4-pyridone-3-carboxamides (4PY) are the major metabolites of vitamin B3 (Tsuji et al. 2011). α-carboxyethyl-hydroxychromans (α-CEHC) is the main metabolite of vitamin E (Lodge et al. 2001). 4-Pyridoxic acid accounts for 40–60% of dietary vitamin B6 intake and is a marker of short-term vitamin B6 intake (Tsuji et al. 2010). There is a dose-dependent relationship between citrus consumption and urinary excretion of proline betaine (Lang et al. 2017).

Enterodiol and enterolactone are abundant in cereals, legumes, and fruits (Potischman and Freudenheim 2003). Therefore, these substances are considered appropriate biomarkers for a healthy diet. Vitamin B, cereals, fruits and vegetables, and other high-fiber foods were found to be positively associated with higher socioeconomic status. The correlation between α-CEHC and sociodemographic indicators was not significant, which may be because the prevalence of vitamin E in readily available foods (e.g., cooking oils) (Choi et al. 2019). However, the data on the stability of these biomarkers in sewer and sewage are not available yet, so it is unable to analyze the quantitative consumption characteristics of these substances and only qualitative analysis can be performed.

Conclusion and prospect

Conclusions

Municipal sewage is a reservoir of biological and chemical information that reflects the health status of populations. Through the detection of relevant biomarkers, WBE can provide a range of information about the dietary consumption and health of the population. Importantly, as an emerging subject, the capabilities of WBE are rapidly expanding as detection methods and technologies improvement. It can also be used as a tool to detect human exposure to emerging contaminants.

As we discussed previously, with information on biomarker excretion, sewage and in-sewer conversion, WBE has the potential to provide information on illicit and therapeutic drugs consumption, and other health aspects of the population. The main conclusions obtained in this paper are as follows:

(1) Most of the illicit drugs showed high stability in sewage and sewer, only cocaine, heroin, and THC are unstable and their stable metabolites BEG, morphine, and THC-COOH are used as biomarkers for consumption back-calculation, respectively.
(2) Among the common drugs, the cardiovascular drugs metoprolol, bezafibrate, gemfibrozil, atenolol, and the antidiabetic drugs metformin are stable in sewage and sewer and can accurately back-calculate the per capita consumption of drugs by WBE.
(3) Other common drugs such as antibiotics and antidepressants are degraded to some extent in both sewage and sewer, which may have large errors in back-calculation and require more reliable biomarkers.
(4) For alcohol, tobacco, and caffeine, reliable biomarkers have also been found as EtS, nicotine, cotinine, trans-3′-hydroxycotinine, and 1,7-dimethyluric acid, respectively.

(5) Acidification (except THC-COOH) and low temperature (−20–4 °C) are effective in the long-term storage of samples. But for some drugs such as sulfonamides and macrolactone antibiotics, the stability is significantly reduced after a week of frozen storage. For benzodiazepines, acidification can also significantly reduce the stability of the drug, and it is recommended to use sodium metabisulfite (0.5 g/L) to preserve sewage samples (the drug can be stable at −20 °C, 4 °C, and room temperature for 1 week) and analyze them as soon as possible.

Prospect

For the better application of WBE in various fields, some suggestions for the needs and trends in its development are presented here:

(1) Enhancement of human pharmacokinetic studies. The selection of biomarkers that are excreted mainly through urine and metabolized in high proportions is essential for the back-calculation of substance consumption. And it helps to distinguish the number of substances from human or other sources.
(2) Stability studies of substances within samples and in the sewer need to be refined. Stability studies have been conducted for more studied substances such as cocaine, cannabis, venlafaxine, and metformin, but stability studies have yet to be conducted for more drugs. In addition, the stability data reported in available studies for the same substance vary greatly due to different experimental conditions and the number of microorganisms in sewers in different regions. So, there is a need to establish uniform and standardized stability experimental conditions to produce accurate data.
(3) Regular virus monitoring of sewage using DNA-based biosensors may provide an early warning of an impending outbreak. Molecular biology techniques also play an important role in the development and application of endogenous biomarker analysis and virus monitoring in WBE.

In the future, more biomarkers that can reflect human diet and health need to be explored to provide a more direct measure of population health. At the same time, the relationship between WBE detection and socio-economic or environmental indicators should be explored and established, so that it can show more application value for public health. The development of WBE would rely on the development of analytical and detection technologies, such as mass spectrometry and metabolomics technologies. More research is also needed on biomarkers' pharmacokinetics, sewer migration transformation, and stability in sewage, which are key

information for translating measurements in sewage into population-level consumption values.

Data availability The authors ensure that all data, materials, software applications, and custom code support the statements published in this study.

Declarations

Compliance with ethical standards This study did not involve human participants and animals.

Consent to publish All of the authors have read and approved the paper and it has not been published previously nor is it being considered by any other peer-reviewed journal.

Competing interests The authors declare no competing interests.

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