Trends in Analytical Chemistry 105 (2018) 453–469

Contents lists available at ScienceDirect

Trends in Analytical Chemistry

journal homepage: www.elsevier.com/locate/trac

Wastewater-based epidemiology biomarkers: Past, present and future

Phil M. Choi [a, 1], Ben J. Tscharke [a, 1], Erica Donner [b], Jake W. O'Brien [a], Sharon C. Grant [a], Sarit L. Kaserzon [a], Rachel Mackie [a], Elissa O'Malley [a], Nicholas D. Crosbie [c], Kevin V. Thomas [a, *], Jochen F. Mueller [a]

[a] Queensland Alliance for Environmental Health Sciences (QAEHS), The University of Queensland, 20 Cornwall Street, Woolloongabba, QLD 4102, Australia
[b] Future Industries Institute (FII), University of South Australia, University Boulevard, Mawson Lakes, SA 5095, Australia
[c] Melbourne Water, 990 La Trobe Street, Docklands, VIC 3008, Australia

ARTICLE INFO

Article history:

Keywords:
Wastewater based epidemiology
Illicit drugs
Pharmaceuticals and personal care products
Population health
Antibiotic resistance
Microbiome

ABSTRACT

Wastewater is a complex matrix containing a wide range of chemical and biological markers of human activity. Relating concentrations of these "waste" materials in wastewater influent streams to population-scale use, consumption, or rates of exposure, can provide important qualitative or quantitative information on the activity of inhabitants within a given wastewater catchment. Many publications in this field of study have focussed on the usage of pharmaceuticals, illicit drugs, tobacco and alcohol. However, many other potential applications are emerging which can contribute useful knowledge on human health, exposure to industrial chemicals, infectious diseases or pathogens and antibiotic resistance. This review summarises the established wastewater based epidemiology (WBE) biomarkers, and presents a critical review of the current capabilities of WBE. We further discuss possible future strategies and challenges anticipated in analysing wastewater to measure chemical markers of population health as well as biological markers of microbial exposure and disease.

1. Introduction

The analysis of wastewater for concentrations of human-use substances, pharmaceuticals, chemicals, exogenous contaminants, and nutrient concentrations has been employed for decades. The main focus of analyses has generally been to determine substance concentrations entering the wastewater treatment process, to monitor removal efficiencies of wastewater treatment processes, and to evaluate wastewater effluent as a point source for environmental contamination. More recently, Wastewater-Based Epidemiology (WBE), defined here as the normalisation of analyte influent concentration to per capita mass loads using the daily flow and wastewater treatment plant (WWTP) population, provides population-scale information on human activity within catchment boundaries. This allows for community-level assessments on per-capita consumption, use, exposure, or release of chemical or biological agents. Therefore, the amount of a target chemical or biological agent in the wastewater stream can provide qualitative or quantitative information on the total and mean population exposure to the agent in a given sewer catchment. Assessments of spatial or temporal trends, or response(s) to events within catchments can also be conducted. The method is non-invasive and is done on a population-scale level, so individuals are not targeted and privacy is respected [1]. In addition, the population normalisation of data allows for direct comparisons to be made between catchments of different population sizes.

After entering the sewer network, these excreted agents arrive at a wastewater treatment plant (WWTP) where wastewater samples can be collected over a defined sampling period, Fig. 1.

To obtain a quantitative estimate of a given agent with WBE, representative samples are collected over a given period (typically 24 h) using autosamplers that collect time or flow proportional samples of wastewater influent entering a WWTP [3]. One important consideration in WBE studies is the solubility and partitioning of analytes of interest into the aqueous phase, which allows for adequate and representative sampling within wastewater samples. For small molecules such as drugs, per capita daily consumption of a parent compound in a given catchment is calculated using an equation such as Equation (1) [4].

* Corresponding author.
E-mail address: Kevin.Thomas@uq.edu.au (K.V. Thomas).
[1] Co-first authors.

https://doi.org/10.1016/j.trac.2018.06.004
0165-9936/

$$Daily\ chemical\ consumption_i \left(\frac{mass}{\frac{day}{1000\ people}} \right) = \frac{C_i \cdot F \cdot \frac{R_i}{E_i}}{P} \quad (1)$$

here, C_i is the concentration of a given drug residue i (parent drug or metabolite) measured in raw wastewater samples, F is the total wastewater volume during the sampling period (typically 24 h), P is the number of people in the catchment, R_i is the ratio of molar mass of parent drug to its metabolite and E_i is the average excretion rate of a drug residue i.

Compared to wastewater analysis, WBE is a relatively young and growing field with new advancements being made continuously, as evidenced by the growing number of new publications in this field (Fig. 2). A number of recently published reviews also summarise important advancements in aspects of the field, in terms of specific biomarkers [5–7] or analytical techniques and challenges [8,9]. The present review focuses on WBE studies, critically presenting progress made to date, and offers insights into new areas where WBE could be applied in future and shows explorative promise, such as pathogen and health indicator monitoring. In this review, each class of biomarkers are discussed as follows: (i) Illicit and licit drugs; (ii) pharmaceuticals and personal care products, (iii) population markers, (iv) industrial chemical exposure markers, (v) stress, food and diet markers, and (vi) biological markers. Each topic of discussion outlined in this review is shown as a branch in the mind-map (Fig. 3).

2. Illicit and licit drugs

Drugs can be broken down into several groupings and classes, including licit and illicit substances. Here, we briefly summarise the previous and ongoing WBE efforts. Each class will be discussed in turn, beginning with the illicit drugs: established stimulants, heroin, cannabis, ketamine, and finally, the New Psychoactive Substances (NPS). A more comprehensive list of biomarkers can be found in SI1.

2.1. Illicit drugs

2.1.1. "Established" drugs – MDMA, methamphetamine, amphetamine, cocaine

Methamphetamine, MDMA (3,4-methylenedioxymethamphetamine), and cocaine have been widely analysed in WBE since the key studies in the field were published in 2005 and 2008 [4,10]. Subsequently, hundreds of publications have focussed on illicit drug use due to their ubiquitous prevalence across the globe. MDMA and methamphetamine have been predominantly monitored through their parent drugs, and not their metabolites. This is mainly driven by the high elimination of the parent drugs in urine, and the non-selective nature of many of the dominant metabolites. For example, methamphetamine also metabolises to amphetamine, and so confounds amphetamine load estimates. Therefore in regions where methamphetamine consumption is much higher than amphetamine (e.g. Australia, United States of America, and some Eastern European countries), estimating consumption of amphetamine can be problematic due to the contribution from methamphetamine metabolism, whereas for areas where methamphetamine consumption is low to non-existent (e.g. much of Western Europe), then the effect is not as pronounced [2]. Similarly, MDMA metabolises to MDA (3,4-methylenedioxyamphetamine), an illicit drug in its own right,

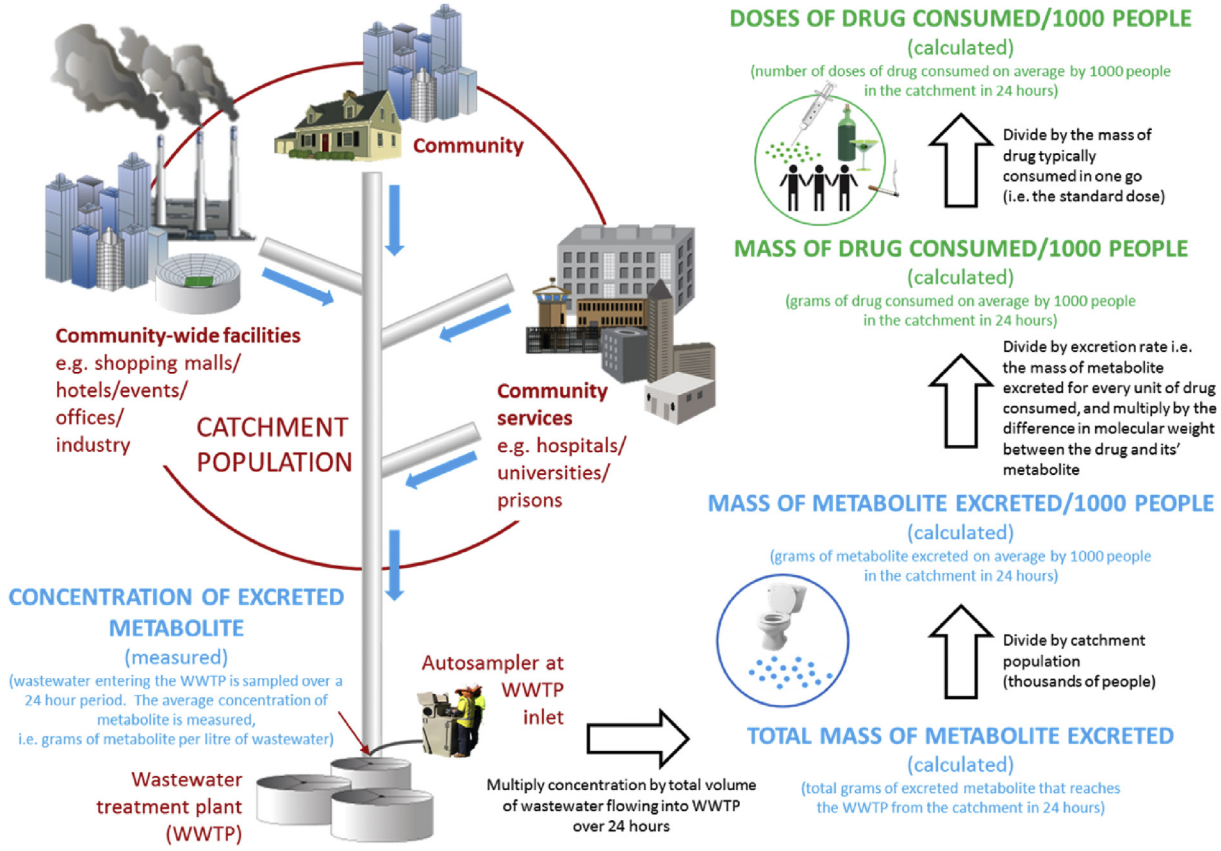

Fig. 1. Schematic of the population catchment area and methodology employed to convert measured concentration of substances in wastewater to mass loads or doses consumed per day per estimated number of contributing population, adapted from Ref. [2].

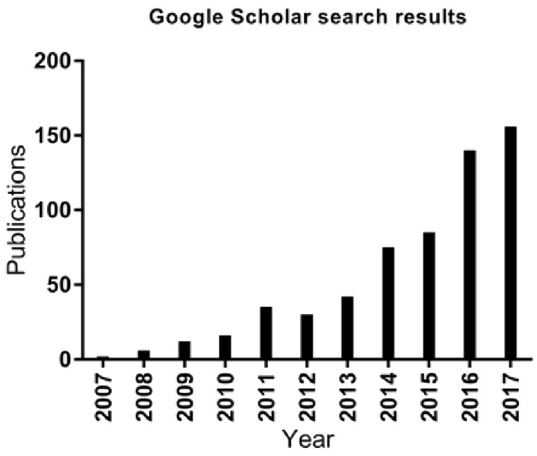

Fig. 2. New publications in the field of wastewater based epidemiology per year. Generated using Google Scholar, accessed March 2018. Search term used: "Wastewater epidemiology" OR "sewage epidemiology" OR "wastewater based epidemiology" OR "Sewage based epidemiology".

which subsequently confounds estimates of MDA consumption [11,12].

Cocaine is generally monitored by its selective metabolite, benzoylecgonine, to ensure that it has been consumed [4], while the ratio between parent/metabolite has been suggested as a way of identifying dumping events [13]. Cocaine itself has been found to be somewhat unstable in wastewater between sample collection and analysis, while benzoylecgonine is relatively stable, furthering the study focus on benzoylecgonine [14]. Co-consumption of cocaine and ethanol (alcohol) generates cocaethylene, which can be used to monitor the co-consumption of the two [15]. However, most WBE studies that have measured cocaine have not co-analysed cocaethylene or considered the effect of co-consumption of alcohol to the cocaine estimate. The influence of alcohol co-consumption should not be excluded from the interpretation of benzoylecgonine WBE results, as it has been found to decrease the urinary excretion of the metabolite by up to 48% [16].

MDMA, amphetamine, and methamphetamine are chiral compounds. Chirality can influence the potency, as well as the pharmacokinetic profile of the excretion from different administration forms (injection, oral, intranasal, etc.), and can potentially reveal the nature of the source. Amphetamine from licit or illicit sources can be distinguished through its chiral profile (ratio of enantiomers) due to the chiral nature of illicit versus licit (e.g. ADHD medication) sources [17–20]. Hence, when the ratio of the S(+) and R(−) enantiomers are compared, one can estimate the extent to which measured amphetamine levels could be reflective of licit or illicit sources. A study in China suggested the technique could be utilised to determine regions where clandestine manufacture of methamphetamine or amphetamine occurred, and by what synthetic process [21]. Similar enantiomeric analyses could be made for MDMA as it also has a chiral centre, although there are no significant licit uses of this compound at present. Despite this, there are other sources of methamphetamine, such as the metabolism of selegiline (Parkinson's Disease drug), which metabolises to methamphetamine, although in negligible amounts unlikely to affect WBE [11]. Specific metabolites or intermediate metabolites of MDMA and methamphetamine could be co-analysed with the parent drugs to evaluate if high consumption events were caused by increased use or the dumping of drugs into the sewer.

2.1.2. Heroin

Heroin metabolises predominantly to morphine and, in minute amounts of approximately 1.3%, to 6-monoacetylmorphine (6-MAM) [12]. For the selective monitoring of heroin, 6-MAM has been used to estimate heroin consumption [22–24]; however, the addition of preservatives such as sodium metabisulfite or acidification to pH 2 is necessary to prevent degradation of 6-MAM post-sampling [14]. This raises stability issues in locations where sewer residence times are high or unknown, and increases the uncertainties around the in-sewer degradation of 6-MAM pre-

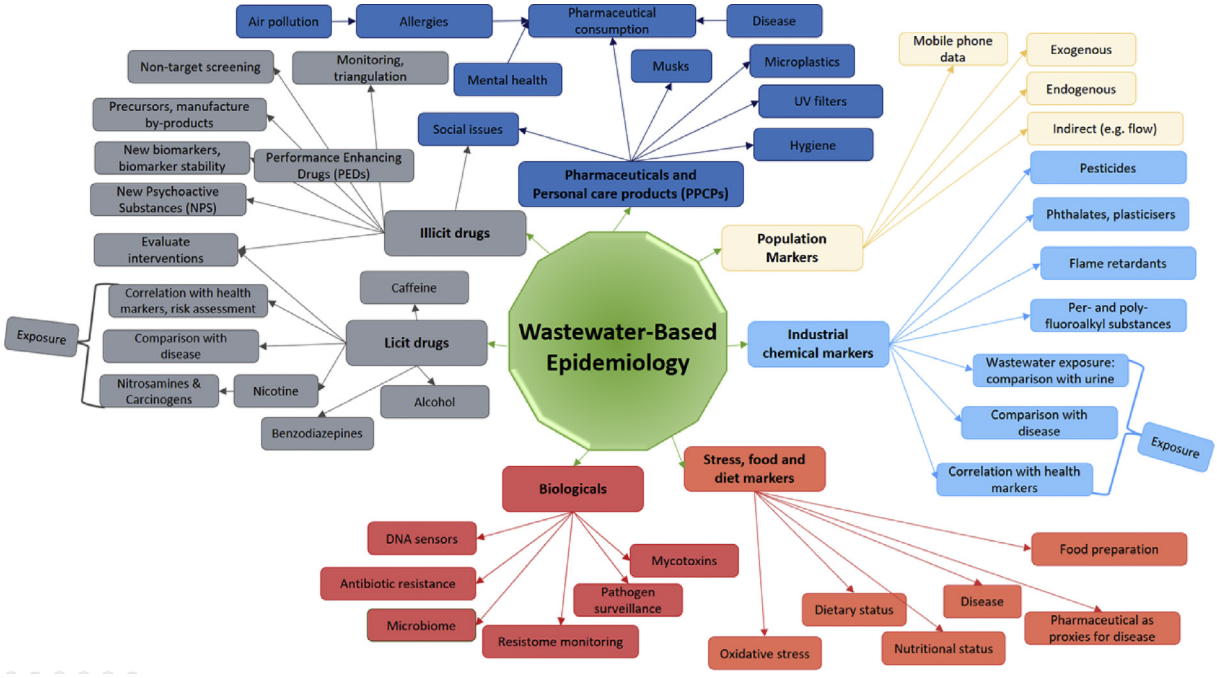

Fig. 3. Mind-map of current and future possibilities of wastewater-based epidemiology. Each branch of the WBE mind-map is a subheading (biomarker classification) as explored in this review. Refer to SI1 for a more comprehensive list of individual biomarkers.

sampling, as further discussed by McCall, Bade [9]. In addition to 6-MAM, heroin use has also been estimated through the subtraction of codeine and morphine prescriptions from the measured amounts of morphine and codeine present in wastewater (as codeine also metabolises to morphine) [25]. Collating data on morphine and codeine consumption can be problematic in some jurisdictions due to over-the-counter sales of codeine in some locations, and difficulties faced in collecting data from different layers of the healthcare system where drugs may be dispensed (hospitals, pharmacies, general practitioners etc.).

2.1.3. Cannabis

Cannabis has been measured through the carboxylic acid (THC-COOH) and hydroxylated (THC-OH) metabolites of the active ingredient, tetrahydrocannabinol (THC) [25,26]. Analysis of the THC metabolite THC-COOH has been the most reported marker of cannabis consumption in WBE publications, and has been assessed by direct injection and extraction techniques in wastewater [4,23,25,27–29]. The hydrophobic nature of THC-COOH is less compatible with common preservation techniques (i.e. adjusting to pH 2), as the compound has high affinity for plastics at low pH in its neutral form [29]. The very low nature of the urinary excretion (less than 1%) and potential simultaneous faecal route of elimination means the exact elimination profile into sewers requires further investigation [29]. Current sampling techniques may not adequately represent load in the sewer, as in-sewer loss due to sorption to particulates and surfaces is largely unknown. Although there are significant difficulties accurately determining cannabis use via WBE, it remains the most used illicit drug globally and therefore will continue to be of interest. WBE monitoring of the drug could be especially helpful to monitor changes in consumption in jurisdictions experiencing legalisation or decriminalisation of the drug.

2.1.4. Ketamine

Ketamine has legal uses as a sedative, although it is also used illegally as a recreational drug. Ketamine can be detected through its metabolite, norketamine, and both are quite stable in wastewater [13,30–32]. Ketamine use has been assessed in many countries [13,24,30,31,33,34] and other smaller areas such as a hospital and prison [35]. Du and colleagues found a 100-fold variation in ketamine loads across 36 wastewater treatment catchments across China, indicating a strong geographic association with consumption which could be further investigated by health authorities [24]. Similarly, Castiglioni et al. found increasing consumption of ketamine in several regions of Italy, demonstrating a higher rate of use in larger cities, with significantly lower rates in Southern Italy [30].

2.1.5. New psychoactive substances

The NPS cover many different classes of drugs, which can be generally broken down into synthetic cathinones, piperazines, synthetic cannabinoids, phencyclidine-type substances, synthetic tryptamines (psychedelics), phenethylamines, and synthetic benzodiazepines, among others. Many of these classes of drugs have been exploited to contravene laws, and were marketed as “legal highs”, “designer drugs”, “plant feed” or “bath salts” [36]. Previously, these emerging drugs were not yet considered illegal as the drugs were not specifically scheduled as illicit drugs in many jurisdictions [37]. This has since changed, with many jurisdictions employing analogue clauses to allow prosecution of NPS offences (e.g. UK Psychoactive Substances Act 2016). Up to 80 NPS have been regularly available globally, with 739 new NPS discovered in seizures between 2009 and 2016 [38]. The bulk of these NPS fulfil the stimulant (36%) and the synthetic cannabinoid niches (33%), with remaining NPS classification of hallucinogens (16%), opioids (4%), dissociatives (3%), sedatives (3%) and other (5%), contributing to the remaining NPS that have been available on the market [38]. At least one study, for example, has targeted the metabolites of selected synthetic cannabinoids and found certain metabolites to occur in wastewater, with greater loads in rural areas [39]. However as many of these drugs are emerging compounds, the human pharmacokinetic profile of their excretion is not well known in most instances, leading to a lack of knowledge of their main metabolites. Therefore, the parent drug has been chosen as the target analyte for a large proportion of wastewater studies [23,40–42]. One limitation of targeting the parent drug is that it may fall below detection thresholds if the urinary excretion of the parent is in much lower abundance than its metabolites. Reliable human pharmacokinetic elimination profiles of these drugs would enable normalisation to consumption, and therefore allow direct comparisons to other stimulants. Such comparisons would reveal important insights into the overall significance and scale of NPS use, while also allowing for a better interpretation of the whole drug market as assessed by WBE. Emerging compounds pose an additional challenge in sourcing pure analytical standards for these compounds for use with targeted methods. Furthermore, in many instances, the stability of parent compounds and/or metabolites of NPS have not been explored for the purposes of WBE.

Many of the synthetic cathinones are close structural analogues of methamphetamine, amphetamine, and MDMA. Consumption of some cathinones and piperazines have appeared to decline in some locations after peaking in 2009–2011 [23] and show different spatial distributions [43]. If this decrease is consistent in other jurisdictions, it may be difficult to detect these compounds or their metabolites in wastewater at the present time. Consumption levels of these compounds, while unknown in many instances, is likely to be very low in comparison to the established stimulants of amphetamine, methamphetamine, cocaine, and MDMA, as comparative mass loads of the drugs are significantly lower. Hence, their detection in wastewater, even with large volume extraction (e.g. Refs. [23,40,41]), may not reach detection thresholds.

Detection of the synthetic cannabinoids has experienced similar issues regarding selection of the parent compound. The *in vitro* metabolism of these compounds by liver microsomes has shown significant metabolism of the parent drug to hydroxylated, carboxylated, and N-dealkylated forms on various functional groups [44]. Despite this, some studies have been able to detect the synthetic cannabinoid and/or their metabolites in wastewater samples, albeit sporadically [41,45]. The 2-(4-Iodo-2,5-dimethoxyphenyl) ethan-1-amine (2C-I) derivatives and analogues present similar analytical hurdles. However, the very low dose size (some down to sub mg level) and low prevalence of these substances and largely unknown human metabolism, means that WBE studies of 2C-I compounds are less likely to be successful at the present time.

To our knowledge, no wastewater studies have detected phencyclidine-type substances, synthetic tryptamines, or synthetic benzodiazepines in wastewater. As for other NPS, the overall consumption of these compounds in the population may sit well below the required threshold for detection, as they represent a smaller proportion of the NPS market. Also, considering the high potency of some of these compounds, single users would consume low amounts, and hence potentially insignificant amounts may enter the sewer network via human metabolism.

Overall, the interest in NPS detection in wastewater is not necessarily a function of the prevalence, but perhaps stems from the high toxicity and adverse health implications resulting from use of some of these compounds [46]. If NPS are substituted in preparations sold as MDMA, methamphetamine, heroin or other mainstream drugs without user knowledge, naïve or unaware users may be disproportionally affected compared to regular users of other illicit drugs [47]. A suspect, i.e. non-target, screening approach to

identify NPS in wastewater may be the most effective strategy to overcome the analytical hurdles of these compounds [48]. Current and future WBE studies are investigating wastewater screening to identify NPS via non-target, high resolution LC-MS/MS detection techniques [49–51].

2.1.6. Performance enhancing drugs

Use of performance enhancing drugs (PEDs) are a pervasive issue not only for elite athletes, but also among amateur athletes who may not understand the health complications that may arise from its abuse [52]. PED usage was as high as 8.4% in a study of 718 Dutch fitness centre members using randomised response technique, indicating significant usage among amateur athletes [53]. This is unsurprising considering a range of androgens (used as growth promoters by bodybuilders and competitive athletes) have been detected in wastewater influent and effluent [54–56]. More recently, Causanilles, Nordmann [57] monitored wastewater from three different catchments hosting various professional and amateur sporting events. For all three events, loads of weight loss stimulants (namely ephedrine, norephedrine, methylhexanamine and 2,4-dinitriphenol) were increased around the time of the sporting events. Additionally, androgens such as nandrolone and metandienone were commonly detected. Enhanced understanding of urinary metabolites of PEDs and improvements in sensitivity of detection methods will increase confidence in WBE methods for measuring and understanding population PED use, which may be greater than previously thought [57].

2.2. Licit drugs

2.2.1. Caffeine

Caffeine intake has been measured in wastewater via the parent drug caffeine and its metabolites paraxanthine (1,7-dimethylxanthine), 1-methylxanthine, 7-methylxanthine and 1,7-dimethyluric acid [58,59]. Of these, 1-methylxanthine and 7-methylxanthine may not be optimal biomarkers of caffeine intake as they are also metabolites of theophylline and theobromine respectively, which are present in some foods, drinks and pharmaceutical formulations [60]. Good correlation has been found between caffeine intake as measured through WBE using 1,7-dimethyluric acid to individual survey data, and, for some catchments, statistics of coffee trade [59]. The stability of some caffeine markers have been established as satisfactory for the duration of wastewater testing [58]. However, cultural factors surrounding consumption of caffeinated products (e.g. tea, coffee, energy drinks) may impair the utility of WBE comparability between locations, and internationally.

2.2.2. Alcohol

Alcohol (ethanol) can be measured through its metabolites, ethyl sulfate (EtS) and ethyl glucuronide (EtG) [15,61,62]. Most studies have used EtS to estimate consumption of ethanol from wastewater, as it is much more stable in wastewater than EtG [61,62]. EtS is very hydrophilic and requires separate chromatographic conditions during LC-MS/MS detection, and therefore is generally analysed separately to other analytes. Despite the very low excretion of EtS after ethanol consumption (approx. 0.012% of initial dose), the large quantities of ethanol consumed on a community level affords its detection by direct injection LC-MS/MS. Some studies have converted amounts of EtS in wastewater to amounts of ethanol consumed in litres per 1000 people per day, which could also be converted into units of standard drinks [15,45,63–66]. Alcohol abuse results in many adverse health outcomes and causes up to 5.9% of all deaths globally [67]. This therefore presents an important opportunity for WBE monitoring of alcohol in areas aiming to assess consumption and associated burden of disease.

2.2.3. Tobacco

Tobacco has been measured using nicotine as the main biomarker as the pharmacokinetics are relatively well known [68,69]. Some sources of nicotine consumption, such as nicotine gum, patches and E-cigarettes, will contribute to this measure. Nicotine has been monitored using its main metabolites, cotinine and hydroxycotinine. Using these metabolites, studies have back-calculated the amounts of these analytes in wastewater to cigarettes consumed on a per 1000-person basis [2,68–72]. Other biomarkers of tobacco, anabasine and anatabine, have also been assessed in wastewater to monitor tobacco consumption [73]. Although their pharmacokinetics are not well understood, they may be more reflective of tobacco use as they are only present in tobacco leaves and not in other sources of nicotine such as nicotine cessation therapies.

3. Pharmaceuticals and personal care products

3.1. Benzodiazepines

Benzodiazepines are prescription pharmaceuticals with sedatory effects used to treat anxiety, seizures, insomnia and related illnesses. These compounds can be prone to misuse, which can lead to dependence [74]. Several benzodiazepines have been measured in wastewater [75–84]. To our knowledge, no formal in-sewer stability or post-collection studies have been carried out to determine the validity of these assessments. Nevertheless the presence of benzodiazepines in effluent wastewater also suggests these compounds may be somewhat stable during the sewer treatment processes [76,85,86]. Additionally, studies exploring connections between benzodiazepine levels to other population health issues have yet to be published. Understanding any such relationship could add further utility and meaning to WBE measurement of benzodiazepines.

3.2. Antidepressants

Major depressive disorder (MDD, or depression) is the 11th greatest contributor to disability-adjusted life years globally [87]. Antidepressant drugs are generally effective at treating MDD [88]. Antidepressants and their metabolites have frequently been detected in wastewater influent and effluent as environmental pollutants [89–91] and as a general pharmaceutical marker in WBE studies [48,92]. Meanwhile, studies focussing on antidepressants specifically as a marker of mental health have been limited [93]. With accurate wastewater catchment population data, it would be possible to calculate per capita use of certain antidepressants in wastewater catchments. Future efforts involving antidepressants in WBE should attempt to validate per capita antidepressant measurements with depression-related data from the medical community. This would be crucial in using WBE as a tool for cost-effectively assessing or comparing the mental health of populations, especially in areas where dispensing data is difficult or expensive to attain. These methods could be particularly beneficial for understanding mental health issues in rural communities of developed nations, where health outcomes often stagger behind their metropolitan counterparts [94].

3.3. Antibiotics and other antimicrobials

Antibiotics are common-use drugs for the treatment of bacterial disease. They are one of the major classes of antimicrobial drugs,

along with antifungals, antivirals, and antiprotozoals. It is recognised that prescriptions based solely on symptoms are often unnecessary or inappropriate [95], and broad spectrum agents (acting against both gram negative and gram positive bacteria) are also too often prescribed [96]. Moreover, antibiotic use has steadily increased in many countries [97] and this has led to problems of increasing antibiotic resistance in pathogens. Due to the high percentage of antibiotics with incomplete human metabolism [98] and their potential for adverse effects on non-target organisms and humans, studies on antibiotics in wastewater have mostly focused on fate and removal during treatment (for reviews see Refs. [99,100]). Nevertheless a limited number of WBE studies have been published regarding antibiotics. A 2006 study in Italy found antibiotic excretion, which was higher in winter than in summer, to be as high as 614 mg/day/1000 inhabitants for ofloxacin [98]. A recent study of two Indian WWTPs found loads of antibiotics ranging from 0.76 to 35.4 mg/day/1000 inhabitants [101], suggesting that antibiotic consumption differs considerably from population to population. Jelic et al. assessed degradation of biomarkers using a stretch of anaerobic, pressurised sewer pipe with a hydraulic retention time around 19 h [102]. They showed that the antibiotics clarithromycin and ciprofloxacin were removed in the sewer, whereas negative removal was apparent for sulfamethoxazole and erythromycin. This highlights the need for a thorough understanding of antibiotic transformation in sewer systems for reliable quantitative measurements with WBE. Similarly, WBE measurements of antibiotics will benefit from use of non-parent biomarkers which are specific for consumption (as opposed to disposal into the sewer).

3.4. Pharmaceutical opioids

The primary therapeutic application of many opioids is analgesia, in addition to other uses in anaesthesia (e.g. fentanyl derivatives) or treatments for opioid dependence (e.g. methadone). In general, pharmaceutical opioids are of concern due to the potential for sustained abuse [103], while the relatively narrow therapeutic windows can lead to overdose [103,104]. Numerous publications have tested for opioids in wastewater; in influent or effluent, (for example [23,31,75,77,78,105–109]), during sporting events [110] or in a prison [111]. For the latter two scenarios, testing smaller populations may compromise the anonymity provided by large catchments, which could be problematic from an ethics perspective [1]. Other studies have assessed the removal of some opioids and other pharmaceuticals from pharmaceutical manufacturing wastewater [112,113] and through the wastewater treatment processes [108,114,115]. Wastewater analyses have converted measured concentrations to community consumption estimates to reveal spatial [116] or temporal trends [23,117], while others have compared these estimates against other data sources [22,66]. For example, Thai et al. aimed to increase the accuracy of WBE back-estimates for codeine, methadone, and methadone metabolite, EDDP, by contrasting large wastewater datasets with prescription statistics and an excretion rate meta-analysis – finding that many excretion rates in other studies may have significantly over or underestimated consumption due to the excretion rate used [118].

3.5. Asthma medicines and antihistamines

Asthma and allergic rhinitis (hay fever) are two common and often co-existing diseases which are prevalent in both developed and developing countries [87,119]. WBE has shown potential for use as a method to not only monitor the asthma and allergy burden of a population, but also for finding environmental factors correlated with its use. A study in Oslo found that major increases in the concentration of the antihistamine cetirizine in wastewater coincided with seasonal pollen events [120]. Similarly, a separate study in Milan found significant associations between levels of airborne particulate matter and the bronchodilator medicine salbutamol in wastewater [121]. WBE can therefore be used to monitor population response to pollen and pollution cues. Investigating additional environmental or pollution parameters which correlate with asthma medicines and antihistamines in wastewater may provide novel insights into factors influencing the asthma and allergy burden of populations. Effort should also be directed towards reliably measuring a broader spectrum of asthma, allergy and respiratory medicines, as consumers have access to multiple different drugs based on need and preference.

3.6. Other pharmaceuticals

Whilst data on the prescription and dispensing of pharmaceuticals may be available in many instances, WBE offers the opportunity to assess consumption of pharmaceuticals, and hence compliance of consumption or indication of potential misuse, in near real-time. It could also allow for locality-based health assessments, using pharmaceuticals as proxies for disease prevalence.

To date, many of the hundreds of publications on pharmaceuticals in wastewater has focused on developing multi-residue methods for determination of the parent active compounds [77,89,101,122–124], whilst other studies have also assessed their metabolites [125,126]. From the WBE perspective, only limited research has been conducted on pharmaceuticals where the main focus is using them as: markers of population size and correlating sewer loads with expected consumption [127]; to correlate pharmaceutical loads with environmental stressors, and hence associated health outcomes in a given community [128]; and to evaluate whether or not a particular pharmaceutical is being abused within a community [129].

Petrie and colleagues conducted a review on detection of pharmaceuticals and some of their metabolites in wastewater influent and effluent [130]. This review clearly indicates that while some pharmaceuticals are present in trace amounts in wastewater influent, others such as acetaminophen may be detected at high levels of up to 500 μg/L. While this review also touches on their removal during wastewater treatment, it also illustrates that there are a limited number of studies conducted to determine the in-sewer stability of many pharmaceuticals [102,131], research which enables better interpretation of the limitations of consumption estimates.

Other pharmaceuticals such as the non-prescription analgesics ibuprofen and paracetamol (acetaminophen) may be of interest for WBE monitoring as they are cheap, high use chemicals that have a broad number of intended uses. Other drugs such as atenolol or the statins may also be useful as markers of cardiovascular disease. There is already one study suggesting that the analysis of atenolol in wastewater may be useful to identify links between consumption and environmental stressors such as ambient air temperature [128]. Drugs such as carbamazepine, which are not degraded by many wastewater treatment processes, may be an appropriate indicator compound to monitor and evaluate the efficacy of wastewater treatment [101,132–134].

3.7. Personal care products

Personal care products (PCPs) are regularly measured in wastewater, but the possibilities of using PCP influent concentrations to assess human exposure or risk to human health has been scarcely investigated. Commonly measured PCPs that present a potential risk to human and environmental health include ultraviolet (UV) filters, synthetic musks, parabens and disinfectants such

as triclosan [135]. However, data on excretion rates, persistence in sewer and product source information for these compounds is severely lacking. For UV filters, only the most commonly investigated UV filter (benzophenone 3) has an estimated excretion rate (1%) [136]. Several dozen studies published in the last decade investigating UV filters in wastewater considered environmental implications or removal efficiencies but have not addressed UV filters from a specifically epidemiological approach [137]. Mass loadings and environmental emissions have been estimated for benzophenone derivatives in the USA where loads up to 81.4 mg/d/1000 people were estimated [138].

This trend in research continues for other PCPs including synthetic musks, parabens and triclosan where wastewater analyses have mainly reported concentrations, investigating fate and removal efficiency [139–142]. A few recent studies have reported population based loads for PCPs investigated parabens and their metabolites [143,144]. Population based loads have also been estimated for benzotriazoles, benzothiazoles, benzophenones and bisphenols in Indian and American WWTPs. Comparatively high (up to 7500 mg d^{-1} 1000-$people^{-1}$) loads of benzothiazole was found in Indian WWTPs [145] The most notable challenge in linking PCP concentrations in influent to product use and human health is the variety of sources of PCPs and the difficulty in identifying the original source, and the extent of personal use and exposure to the substance.

4. Markers of population size

For WBE to be used for quantitative or per capita estimates of exposure to environmental agents or other health-related information (e.g. consumption of drugs), the size of the population in the catchment of interest needs to be understood. Previous reviews of the uncertainties involved in WBE found that the uncertainty of the collection-period-specific population size, the *de facto* population, is often the largest uncertainty [146,147].

Traditional methods to estimate the contributing population of each sample have relied on simple models to estimate the *de facto* population size, such as those based on flow; other wastewater parameters such as biological oxygen demand (BOD), chemical oxygen demand (COD), total nitrogen and total phosphorous; wastewater treatment plant estimates based on number of connected dwellings, projected plant capacities, number of municipal bins, or based on the sum of population within suburbs served from census data [147–149]. However, such methods may not be robust as they are susceptible to changes in flow, trade waste, and movements of populations over time.

Alternatively, small molecules present in wastewater have been validated and used as markers of population size. O'Brien et al. present criteria for the selection of 'best practice' wastewater-present population markers [131]. These are (1) ease of measurement (occurs at levels above the limit of quantitation (LOQ)), (2) in-sewer mass load which correlates with population size ($R^2 > 0.8$), (3) negligible degradation (<10% loss in 24 h under sewer conditions), and (4) mean residence time of the WWTP sewer network shorter than the time for 10% degradation to occur for the compound under sewer conditions. Importantly, inclusion of multiple markers of population size into a single model through Bayesian inference led to a more precise estimation of the population size, regardless of whether all of the above criteria were met.

4.1. Exogenous markers

Exogenous biomarkers whose excretion is consistent throughout different populations have been assessed as population markers. For ten WWTP catchments in Australia, 14 exogenous chemicals were evaluated against the aforementioned best practice criteria. Acesulfame, atenolol, carbamazepine, gabapentin and ibuprofen all met these criteria [131].

Nicotine and caffeine are widely used recreationally. Both of these chemicals and their metabolites have been proposed as potential population markers [58,150,151]. In some WWTP catchments both chemicals and their respective metabolites have been found to be good markers, but there is still concern when applying these markers to other catchments, particularly internationally, where differing caffeine consumption or disposal habits are known to prevail [58,59,66,101].

Pharmaceuticals have also been proposed as markers of population size but so far this research is limited to four studies and only covers atenolol (beta-blocker), carbamazepine (antiepileptic), codeine, gabapentin (anticonvulsant), hydrochlorothiazide (diuretic), ibuprofen, paracetamol (analgesic), furosemide (diuretic), iopromide (contrast medium), naproxen (antiinflammatory), salicylic acid (metabolite of acetylsalicylic acid) and venlafaxine (antidepressant) [127,147,151]. A limitation of pharmaceuticals is that they may be used by small sub-sets of the population and hence provide skewed population estimates for some catchments. By their nature, exogenous markers may only be representative of particular catchments, and their consumption patterns. Ideally, reliable endogenous markers for estimating population size would be found by assessing candidate markers against the same best practice criteria.

4.2. Endogenous markers

Endogenous chemicals specific to human metabolism with homogenous excretion throughout the community and low variance would be ideal markers of *de facto* population size. As yet, only 5-hydroxyindoleacetic acid (5-HIAA) and ammonia have been assessed as endogenous markers that fulfil most criteria for wastewater-based epidemiology [150,152].

Some endogenous chemicals have been assessed as potential population markers, though with little success. Creatinine, a breakdown product of creatine phosphate in muscle is usually produced at a constant rate by the body. Although regularly used to normalise urine in clinical contexts, its stability in wastewater is too low to be used as a population marker [153]. Coprostanol, the major metabolite of cholesterol in humans, is entirely excreted via faeces but it substantially sorbs to particulate matter and thus has been considered unsuitable as a population marker for wastewater-based epidemiology [150]. Current autosamplers, even when setup using the best practice method (i.e. constant flow proportional sampling), are only capable of representatively sampling the liquid phase. Therefore, the concentration of a given analyte is dependent on the partitioning coefficient between the water and the organic phase, as well as the partitioning kinetics. As the amount of particulate matter in the wastewater at any given time or across different WWTP catchments is not expected to be consistent, the concentration and mass loads calculated from the concentration in wastewater could be highly uncertain [150]. 1-amino-propan-2-one, a precursor to vitamin B-12, is thought to be relatively unique to human metabolism, is highly water soluble and has been detected in wastewater [154], but further investigation as a population marker has yet to be conducted. 5-HIAA, has potential as a population marker as it has consistent excretion rates in urine of approximately 3.44 μg/person/day [155]. Both Chen, Kostakis [150] and Rico, Andrés-Costa [151] have found relatively good agreement between mass load and nominal population size for their investigated WWTPs. To date, however, 5-HIAA has not been calibrated with accurate population figures, and in-sewer stability has yet to be assessed. Other neurotransmitter metabolites may have potential such as

vanillylmandelic acid (VMA) and homovanillic acid (HVA). Androstenedione, an intermediate in the formation of sex hormones including testosterone, oestrogen and oestradiol, has been proposed as a population marker. However concerns have been raised over its stability and as such further evaluation as a population marker has not been conducted [150,151]. Ammonium, an indirect marker of urea, has also been evaluated for use in population normalisation of WBE, yielding consistent, albeit slightly higher mass loads when compared to census-normalised loads. The marker shows promise as it allows for real-time monitoring using in-sewer ion electrodes [152]. However, as ammonium exists in equilibrium with volatile ammonia, levels may be influenced by sewer network setup as headspace, pH and temperature could affect the equilibrium and cause loss of the target compound. A recent study has used ammonia population normalisation for the estimation of nicotine consumption, finding good agreement with wastewater treatment plant estimates on the service population [156]. Unlike many other potential population markers, DNA is extremely stable in the environment and may persist for hundreds of years. Although it has not been assessed against the other population marker criteria, research has begun on detecting it in wastewater and on quantitating it in near real-time using DNA specific sensors in wastewater [157].

4.3. Validators of population markers

Whilst attempts have been made to validate population markers against nominal WWTP population estimates [151], population counts from previous census [150], or from back-calculating consumption based on expected excretion of a given chemical [127], another approach is to validate population markers using wastewater samples collected during a population census [147]. Through screening of a range of chemicals in wastewater samples collected during a population census, it may be possible to identify both endogenous and exogenous chemicals which were previously not considered as potential population markers and additionally may rule out some already proposed. This technique has proved successful in a study using Australian wastewater and census data [131]. At least one attempt was made to evaluate some of these validated population biomarkers in a variety of Chinese wastewater catchments, but due to a lack of robust population figures to validate against, only moderate correlations could be found [158]. An alternate (but more difficult) approach to population census is to use dynamic population estimation based on cellular communication data [159]. Recently, a study was able to account for population flux between wastewater catchments in Oslo and used it to calculate per capita consumption of drugs and pharmaceuticals [160]. The study demonstrated that mobile device data can be used to quantify the high degree of intraday, week and month variability within a catchment, and quantitatively confirmed previous assessments that population estimates can account for uncertainties of up to 55% in static normalised data [146]. Mobile device-based population activity patterns allow for dynamic normalisation that yields much improved temporal and spatial trend analysis, but its utility will be limited to populations with a high usage of mobile technology.

5. Industrial chemicals

5.1. Pesticides

Pesticides are chemicals that either kill, deter or incapacitate pests such as animals, insects, plants microbes and pathogens. While they are important chemicals, particularly in regard to their use in agriculture, there is concern regarding adverse effects on both human and environmental health from exposure to pesticides. There are established links between pesticide exposure and higher risk of birth defects, chronic diseases (e.g. cancer and diabetes), neurodegenerative disorders (e.g. Alzheimer's and Parkinson's disease) and reproductive diseases [161,162]. For humans, exposure to pesticides is usually through diet and household application [163]. This is usually assessed through human biomonitoring studies focussing on measuring parent pesticides and/or their metabolites in body fluids (e.g. blood and urine) or within tissues [164]. Pesticide and pesticide metabolite measurement in wastewater has been occurring for more than a decade [165] but from the WBE perspective the research so far is limited to just a few studies [166,167]. These studies have focused on urinary metabolites of triazine herbicides, pyrethroids and organophosphates and indicate that the levels and profiles observed are in agreement with human biomonitoring data [167] and that spatial differences can be observed [166]. As some pesticides are structurally similar to other high use chemicals such as organophosphorus flame retardants and organophosphate pesticides, caution must be taken to identify suitable metabolites which are unique to pesticide exposure [168]. Additionally, some metabolites are non-specific to a particular pesticide and as such may only be representative of exposure to a group of pesticides such as in the case of some triazine metabolites [169]. As of yet, the stability of pesticides in wastewater is not well defined but initial laboratory stability tests in wastewater stored in amber glass bottles at room temperature, 4°C and at −20°C, showed little degradation over a 24 h period [167]. Further investigation is required to investigate potential influences of real-world in-sewer processes.

5.2. Flame retardants

Organophosphate ester flame retardants (OPFRs) are used in several commercial applications including as plasticizers, anti-foaming agents and additives for lubricants and hydraulic fluids. Their use has increased after the ban of some brominated flame retardants. A handful of studies describing the measurements of OPFRs in wastewater streams have been reported and used as a tool for assessing changes in the consumption, exposure to and use of these chemicals [170–172]. Only limited initial data have been published on the use of OPFRs in WBE, with two studies suggesting their use as population markers [147,171,172]. A study on eleven wastewater treatment plants with Australian Census population estimates found per capita mass loads of PFRs to be similar when compared with European and U.S. WWTPs and has estimated that approximately 2.1 mg person^{-1} day^{-1} of PFRs were received into Australian wastewater equating to 16 tonnes per year [173]. An additional study conducted at a WWTP in New York State, USA, investigated the occurrence of 14 triester organophosphate flame retardants (OPFRs) and plasticizers as well as two of their diester metabolites [172]. Here, a mass load range of 0.02 mg person^{-1} day^{-1} (TPP) to 28.7 mg person^{-1} day^{-1} (TBOEP) of OPFRs was estimated. While these studies informed per capita exposure to parent compounds and metabolites, a similar approach has been suggested considering the addition of the analysis of OPFR urinary metabolites [174,175]. By targeting OPFR urinary excretion metabolites, a WBE approach could be undertaken for a better understanding of actual population exposure and derivation of population specific excretion factors. Until recently, however, while methods for the analysis of these chemicals are well established, a key analytical challenge was the typically very low levels of these metabolites expected in urine samples [176]. In 2017, Been et al. presented the development of an analytical method for both parent and OPFR metabolites in wastewater, and measured for the first time these biomarkers of exposure to OPFRs in wastewater influent samples from Belgium [171]. Reported concentrations were in the

range of <LOD to 1072 ng L^{-1}. The study highlighted spatial differences in per capita loads of the target analytes, suggesting the potential for a WBE approach to investigate OPFR flame retardants and their metabolites as biomarkers of population-exposure.

5.3. Plasticizers

Phthalates are a class of chemicals with widespread general population exposure due to their prevalent use and omnipresence in the environment. A number of studies show the prevalence of these chemicals in the environment including in wastewater systems [177]. Di (2-ethylhexyl) phthalate (DEHP) is the most studied with reported levels in wastewater from 10 countries ranging from 0.716 to 122 mg/L [178]. Recently, wastewater analysis has been proposed as an alternative to urine analysis for estimating human exposure to phthalates [179]. The metabolites of six phthalate esters in wastewater from the NW region of Spain were shown to occur at concentrations of up to 1.6 μg/L that were converted into exposure levels. Exposure of four of the six phthalates was found to be over the daily exposure thresholds recommended by the U.S. Environmental Protection Agency and the European Food Safety Authority [179].

Data exists on excretion rates [180,181] and half-lives [182] for these analytes. However, WBE applications have only recently been trialled, in a study in which phthalate metabolites have been investigated to estimate population exposure [179]. Six phthalate diester metabolites and three monoester phthalate metabolites were analysed in wastewater and excretion factor values of between 1.5 and 12 have been reported. 24-hour excretion factors were reported relative to the parent diester doses, and a correction factor was applied to convert monoester excretion loads to phthalate diesters (e.g. the three monoester metabolites, MEHHP, MEOHP and MECPP, were used to estimate an excretion factor for the diester phthalate DEHP). The study proposed WBE as a rapid and inexpensive tool to compare temporal and spatial exposure trends and to potentially identify regions where people may be most at risk, and to complement assessments such as individual urinary metabolite analysis [179]. As analytical methods for the determination of phthalate metabolites in biological and environmental matrices are established [183,184], and a proof of concept for WBE has been presented [179], future work could apply this approach to assess its performance when used on larger population sizes. A better understanding of in-sewer stability and transformation potential of phthalates and metabolites would provide confidence in the use of these chemicals as biomarkers. In the case of phthalates, given the low dose–response relations in humans even with low phthalate levels detected in urine [185], the identification of biomarkers could be quite an important tool within WBE and population health risk assessments.

5.4. Per-and poly-fluoroalkyl substances

Per- and polyfluoroalkyl substances (PFASs) are emerging environmental contaminants with a global environmental distribution due to their industrial scale manufacturing for over 50 years [186]. Due to their moderate water solubility, the majority of the PFAS environmental burden is assumed to be in the water phase. The perfluoroalkyl carboxylates (PFCAs) and sulfonates (PFSAs) have surfactant properties, are anionic at the pH of environmental waters, and resist both chemical and metabolic degradation [187,188]. The persistence of these compounds in the environment, their consistent detection in monitoring studies [188–190], and possible impact on organisms [191] have prompted growing concern. Perfluorooctanesulfonic acid (PFOS) and some of its derivatives has been listed on the United Nation's Stockholm Convention on Persistent Organic Pollutants since 2009, while perfluorooctanoic acid (PFOA) was more recently nominated for listing in October 2015 [192]. Since then agencies such as the US EPA, Europe's REACH, the Canadian and Australian governments, have taken measured to further regulate and/or eliminate the use of these substances [193–195].

A growing body of literature describes the occurrence of some PFAS in environmental matrices and WWTPs [170,196–199]. Multiple studies have shown that PFAS are not effectively removed in WWTPs and in some cases can be enriched in the effluent [199,200]. Therefore WWTPs may act as potential outfalls and point sources in the environment [201,202]. Numerous studies have also found low level (ng/L) concentrations in municipal drinking waters [203,204] with contaminated water supplies providing significant contribution to daily intakes in some areas, raising serum concentrations in affected residents [191,205,206]. Data on PFAS monitoring in environmental media and its relevance to human exposure has been investigated with PFAS concentrations in indoor and ambient air, house dust, drinking water and food reported [207].

However, the use of PFAS to assess human exposure in WBE is limited as PFAS do not metabolise in the body and therefore human excretion cannot be differentiated from other environmental sources in the wastewater. Furthermore, different chain length PFAS have different elimination routes. For example urinary excretion was identified as the major elimination route for short PFCAs ($C \leq 8$), but for longer PFCAs, PFOS and PFHxS, other routes of excretion such as nail growth, lactation, and sweat are also likely to contribute to overall elimination [208].

The complexity of epidemiological and biomonitoring studies (i.e. due to different behaviours of the various PFAS) and their resistance to degradation in the human body means it is challenging to distinguish between and identify the source of these chemicals in WWTPs. Furthermore, although methods for the analysis of a large suite of PFAS are well established [209], they seem to represent only a small fraction of the thousands of PFAS and precursor chemicals that exit [210]. An added complexity is that PFAS are likely transformed or formed prior to and during wastewater treatment processes as evident by the increase of some species at the outfall of treatment plants in comparison with influent levels [211,212]. Nevertheless, the monitoring of PFAS in WWTPs may provide a rapid and inexpensive tool to compare temporal and spatial exposure trends in catchment areas and to potentially identify regions where people may have elevated risk, and to complement other direct assessments such as blood and or urine analysis of individuals. There is the possibility to investigate ratios and formulation of PFAS occurrence to try and elucidate potential precursors and sources through chemical fingerprinting to identify polluters. With the introduction of restrictions and/or bans on some PFAS there is also opportunity to assess the effectiveness of government led regulatory strategies by examining long term trends of PFAS and identifying whether use patterns are decreasing/increasing in given catchments.

6. Stress, food and diet markers

Biochemical markers of stress, food and diet can be measured by WBE to measure aspects of population health. Developments in this space may lead to the acceptance of WBE as a tool to monitor and inform various aspects of community health beyond drug and pharmaceutical consumption.

6.1. 8-Iso prostaglandin F2α as an endogenous marker of stress

Endogenous chemicals generated by human metabolism and excreted in urine can be used to diagnose specific health effects. A

number of reviews have proposed several (small molecule or protein) biomarkers for determining the prevalence of disease or health (or lack thereof) in a particular community [213–215]. Numerous other protein and small-molecule biomarkers of health have been reported in metabolomics studies. This is a broad topic which has remained largely untapped in the WBE field, with the exception of 8-iso-prostaglandin F2α (8-iso-PGF2α), a biomarker of oxidative stress first proposed for WBE in 2012 [214]. Ryu and colleagues subsequently used 8-iso-PGF2α as a marker for oxidative stress of specific catchments in 11 European cities [65]. The estimated per capita daily loads of 8-iso-PGF2α in the 11 cities ranged between 2.5 and 9.9 mg/day/1000 inhabitants with a population-weighted mean of 4.8 mg/day/1000 inhabitants. While no temporal trends were reported, spatial inter-city differences were found that correlated to the degree of urbanization. Furthermore, 8-iso-PGF2α mass load was found to be strongly associated with that of *trans*-3′-hydroxycotinine but not with ethyl sulfate. The study showed the potential for 8-iso-PGF2α as a wastewater biomarker for the assessment of community public health. The current published method for measuring 8-iso-PGF2α in wastewater relies on an expensive immunoaffinity purification step [216], and improvements to the cost-effectiveness of this method will be a welcome advancement.

6.2. Potential endogenous markers of stress

Many other biomarkers exist which may potentially serve as population health markers in the manner of 8-iso-PGF2α. A broader understanding of oxidative stress may be achieved by complementing 8-iso-PGF measurements with that of 8-hydroxy-2′-deoxyguanosine (8-OHdG), which is associated with oxidative DNA damage as a result of diseases or exposure to carcinogens such as through smoking behaviour [217,218]. Given that smoking behaviour can be measured by WBE using other biomarkers (See Section 2.2.3), it would be useful to consider stress biomarkers whose excretion is not significantly influenced by smoking, and thus provides insights into other physiological stresses. Ortho-tyrosine is a marker of protein oxidation stress, whose excretion is elevated in conditions such as diabetes mellitus and renal failure [219]. Other biomarkers are more reflective of specific physiological states, such as bromotyrosine and chlorotyrosine, whose excretion is increased following eosinophil and neutrophil activation in asthma patients [220]. Protein biomarkers, such as vitamin D binding protein have also been suggested for WBE applications [215]. While urinary proteins (and fragments of proteins) represent an unexploited pool of biomarkers for WBE, routine quantification of specific proteins in wastewater has yet to be achieved. Reliable quantification of proteins in wastewater are therefore a major bottleneck, and advances in this space are expected to overcome major hurdles in the field of community biomonitoring [215]. To our knowledge evaluations of the stability of many of these biomarkers in wastewater have yet to be carried out to establish their WBE suitability.

6.3. Markers of food consumption

WBE has the potential to serve as a platform to measure aspects of food consumption at a population scale [213]. Metabolomics studies have established numerous urinary biomarkers of specific foods, such as grapes [221], wheat [222], cruciferous vegetables [223], citrus fruits [224,225], apples [226,227] and meats [228,229]. These biomarkers have been studied to various degrees, often with the aim of using them to assess subjects' compliance to dietary regimes in nutrition studies. These could therefore serve as semi-quantitative indicators of consumption of specific foods within a wastewater catchment. However, confident application of these biomarkers in wastewater will depend on several areas which require further understanding. For example, urinary biomarkers of food and nutrition which are not metabolised or transformed by humans may not be suitable biomarkers in wastewater catchments which serve agricultural or food processing industries [230]. Potential food biomarkers or their progenitors may be present at different levels in the food, depending on factors such as seasonal variation, cultivar, transport and storage conditions and food preparation methods [231,232]. Similarly, the stability of potential food biomarkers in wastewater should also be evaluated, especially for biomarkers (e.g. polyphenols) which are known to be transformed by gut microflora and other microbes which likely exist in the sewer system [233]. Selecting a stable, well-defined biomarker is therefore a key priority for mining objective information regarding population food consumption. Once identified, a suitable biomarker or a panel of biomarkers could provide objective insights into food consumption profiles and patterns in communities, complementing traditional dietary assessment methods such as self-reporting which is inherently inaccurate and suffers from pervasive under-reporting [234].

6.4. Markers of diet

Similarly, biomarkers reflecting dietary characteristics could provide additional information about the foods eaten in a population. These biomarkers would reflect a more general outcome of dietary habits. Such an approach would avoid uncertainties associated with differences in levels of food biomarkers or their progenitors in food products. Examples of such biomarkers include metabolites and conjugates of water-soluble vitamins whose 24 h urinary excretion correlates with dietary intake [235–238]. Also of potential interest are plant-derived polyphenols such as the isoflavones and enterodiol, whose urinary excretion inversely correlated with body mass index (BMI) in American cohorts [239,240]. If viable in WBE, biomarkers of diet could be used to complement established techniques for assessing population-level food consumption and dietary habits.

7. Biologicals

Sanitation plays a key role in preventing the spread of infectious disease by concentrating the enteric microbial load discharged from a community into a single waste stream for treatment. This makes wastewater an ideal matrix for disease monitoring and surveillance as it represents the entire enteric pathogen load from the local catchment area and captures both the clinical and community disease burden. WBE for infectious disease monitoring is a nascent field generating increasing global interest and its potential continues to grow as rapid advances in high throughput sequencing, bioinformatics and biostatistics support increasingly powerful methodologies. Current efforts focus on evaluating the potential to use raw wastewater and WWTP influents to (i) monitor population disease burden, (ii) evaluate the diversity and abundance of antibiotic resistance genes in circulation, (iii) provide early warning of impending risks, and, (iv) evaluate antibiotic stewardship and health intervention programs.

7.1. Pathogen monitoring

Historically, wastewater pathogen monitoring has been focussed predominantly on effluents and, due to conventions, practicalities and methodological constraints (e.g. reliance on culture-based techniques), has usually been confined to a small number of microbial indicators used for compliance monitoring

such as faecal coliforms and *Escherichia coli*. For research purposes, investigations have been extended to a wider range of medically relevant pathogens and other potential health indicators, such as *Klebsiella pneumonia, Pseudomonas aeruginosa, Acinetobacter* spp., and various bacteriophage, but these have typically been monitored as individual research projects over defined periods of time and hence used a variety of different approaches in the absence of standardised methods [241–244]. With respect to WWTP influent communities, only a very narrow range of species has been examined in any detail to date.

Research has shown that microbial communities in sewer infrastructures contain a discernible human faecal signature including taxonomic groups from the *Bifidobacteriaceae*, *Coriobacteriaceae, Bacteroidaceae*, *Lachnospiraceae* and *Ruminococcaceae*. Use of 454 pyrosequencing showed that even though the faecal signature made up a relatively small fraction of the overall taxa present in sewage, their relative abundance mirrored the population structures of human faecal samples [245]. This supports the premise that wastewater influents may be informative for epidemiological surveillance of gut microbiota, particularly enteric pathogens. Nevertheless, the complex and dynamic nature of WWTP influent microbial communities presents some obvious challenges for wastewater-based epidemiology. For example, 10^5–10^{10} *E. coli* CFU/ml are reportedly present in wastewater, but only a small proportion of these are pathogenic [246]. Furthermore, interspecies competition, predation, and other selective pressures within the sewer may impact the abundance of target pathogens and as these are often likely to be minor species some may be particularly challenging to detect and enumerate. McLellan et al. showed that some dominant taxa in WWTP influent samples were relatively rare in faecal samples, suggesting that these organisms are of environmental origin and/or that they proliferate within the sewer system [245]. Further research is needed to understand the role of competition, selection, and weather related fluctuations in wastewater microbial community structure. Combined sewers (stormwater + sewerage) will present particular challenges in this regard but weather effects can also be an issue in cities with separated systems as fluctuating groundwater infiltration can be significant, especially in older networks.

Methodological decisions are of key importance for wastewater-based infectious disease monitoring as a wide variety of methods (including culture-based, culture-independent, and mixed approaches) can be used to investigate infectious disease agents and associated resistomes. Aside from WBE of bacterial, fungal, and protozoan pathogens, it may also be possible to use wastewater to improve the routine surveillance of pathogenic enteric viruses such as norovirus, enterovirus, adenovirus, astrovirus, and rotavirus. These are found frequently in municipal wastewaters worldwide [247] and previous attempts have been made to assess the effects of intervention programs by tracking changes in the abundance and circulation of virus strains in defined geographic regions (e.g. Ref. [248]. Norovirus outbreaks impart a significant global economic burden to public healthcare systems [249]. Wastewater is an ideal source for molecular epidemiological studies of norovirus because infected individuals excrete high levels of virus and continue to shed for up to 8 weeks, even after symptom resolution [250]. By screening both clinical and wastewater samples, Lun and colleagues could obtain a more complete representation of norovirus epidemiology at a city-based population level [251], whilst Fioretti and co-workers used this approach to document the occurrence of Norovirus GIV in the State of Rio de Janeiro, Brazil, which was detected in 52% of raw sewage samples [252]. Such information may eventually be used to design or evaluate control strategies, including vaccines.

In the context of the global effort to eliminate poliovirus, sampling from wastewater treatment plants represents a large (53% of samples from studies reviewed by Ref. [253] and important component of the environmental surveillance programs which supplement clinical surveillance. It plays a role in documenting the elimination of wild poliovirus, detecting re-introductions, and detecting vaccine-derived poliovirus, however more information on site-specific detection probability and surveillance costs will be required to formally assess the effectiveness of this form of surveillance [253].

7.2. Antimicrobial resistance markers

Antimicrobial resistance (AMR) is one of the greatest human health challenges of the 21st century [254–258]. Of the various challenges falling under this umbrella, antibiotic resistance has been the most widely explored in wastewater to date. Bacteria have developed resistance to all known classes of antibiotics [259] and research has shown that antibiotic resistance genes are frequently carried on mobile genetic elements such as plasmids, transposons, and integrons which can be exchanged both within and across taxonomic boundaries [260,261]. Microorganisms continue to evolve and transfer new mechanisms and combinations of resistance, some of which culminate in the ultimate challenge of multidrug-resistant 'superbugs' [259]. Resistant microorganisms and their genes are present in virtually all environments [262,263] but wastewater has been highlighted as a key environment of interest. Different countries and regions have different antibiotic prescribing patterns and this will likely be reflected in the wastewater microbial resistome. Wastewater resistomes are extremely diverse [264,265], and in addition to the direct link to resistant pathogens excreted from human hosts, wastewater also contains many chemical and abiotic stressors that may select for the evolution and spread of ABR [99,266–268]. This is of interest from a disease epidemiology perspective as new resistance gene combinations may potentially undermine therapeutic antimicrobials if transferred to human communities through the water cycle and/or food chain.

An extremely large number of resistance genes can potentially be targeted for quantification but they are not all of equal importance from a human health perspective [269]. To date, researchers quantifying resistance genes in wastewater have not only chosen different genes to study but also different primer/probe sequences to target the same genes. This, together with variations in DNA extraction and PCR protocols means that results cannot easily be compared across different studies. Moving forward, the selection of genes for wastewater-based monitoring should be based on clear criteria such as those identified by Lhat et al. and Berendonk et al., who selected: (i) clinically relevant genes posing a risk to human health; (ii) genes found in mobile elements, thus demonstrating potential for transfer; (iii) genes conferring resistance to high consumption antibiotics; (iv) genes conferring resistance to antibiotics that have been in use for a long time (e.g. tetracycline, sulfonamides); and (v) genes conferring resistance to newer, clinically relevant antibiotics such as the extended spectrum betalactams (carbapenems and 3rd and 4th generation cephalosporins) [267,270]. Sulfonamide (*sul*) and tetracycline (*tet*) resistance genes are among the most commonly studied antibiotic resistance genes in wastewater to date. Both groups have been in use as antibiotics for a very long time and resistance via multiple mechanisms is widespread [271–273]. Reported values for *sul1* and *sul2* in WWTP influents range from 10^4 to 10^{10} copies per ml, while reported values for individual tetracycline resistance genes (of which there are dozens) range from 10^3 to 10^{10} copies per ml [270,274–281]. Priority surveillance of genes conferring resistance

to more recently introduced and/or 'last resort' antibiotics of high medical relevance could assist in determining the speed and pattern by which these less abundant resistances spread within both human and environmental microbial communities. A short list, including genes conferring resistance to key beta-lactam and carbapenemase antibiotics (e.g. *blaTEM*, *blaCTX-M*, *blaNDM-1*, *blaKPC*, *blaVIM*) was proposed for future wastewater-based monitoring [267]. Research to date indicates that genes conferring resistance to last resort/restricted antibiotics are generally less abundant in both healthcare and WWTP environments than genes conferring resistance to more broadly consumed antibiotics [282], but resistance can potentially spread rapidly in the absence of appropriate antibiotic stewardship [283]. Seasonal trends in resistance may also be apparent in wastewater, although additional data is needed to further explore and confirm this. For example in Caucci et al., a seasonal trend was indicated, with higher AR gene abundances in autumn and winter also coinciding with higher outpatient antibiotic consumption [279], but other studies have only detected minor or no differences between seasons [270,284]. Seasonal trends may be obscured by climatic events, an issue requiring further assessment for the purposes of method validation.

Significant research is needed to realise the potential value of WBE for pathogen and antibiotic resistance monitoring. Carefully established wastewater pathogen/resistome baselines and profiles could provide useful measures against which to compare the success or otherwise of future disease intervention and management approaches (e.g. changes in antibiotic prescription regimes, improved pollution source control to decrease AMR selective pressure etc.).

8. Conclusions and future outlook

Wastewater is a treasure trove of biological and chemical information which reflects population health. With information regarding biomarker excretion, in-sewer transformation and population catchment size, WBE has potential to inform on a range of aspects of population chemical exposure, consumption and other aspects of health. Importantly, WBE capabilities are expanding far beyond monitoring illicit and pharmaceutical drug use. A diversity of pesticides, flame retardants, plasticizers and PFAS have been measured in wastewater, and there is great potential for such markers to be addressed in full-fledged WBE programs. With better understanding of the biomarkers that can be employed for these applications, WBE could serve as a cheap and efficient method of monitoring not only the ubiquity, but human exposure to these emerging contaminants.

In the future, WBE may be able to provide more direct measures of population health, by measuring biomarkers of food, diet and health. These methods would depend greatly on future advances in fields such as metabolomics to inspire potential WBE biomarkers which can provide meaningful information on population health. Advances in this field will be assisted by validating WBE measurements with pharmaceutical sales data. Exploring and establishing relationships between WBE measurements and socioeconomic or environmental metrics should serve to further add meaning and value to these methods, and make them more attractive as practical tools to achieve previously inaccessible insights into public health.

Additionally, WBE is currently at a prime position to be utilised for population microbiome research and monitoring. In this context, wastewater is an obvious but complex and relatively underexplored matrix. As such, careful consideration and some degree of standardisation of methods for measuring aspects of microbial factors in wastewater will be fundamental for future research to evaluate wastewater as a tool to monitor aspects of population microbial health and antimicrobial resistance.

Inter-disciplinary collaborations between analytical chemists and other agencies are providing fruitful data that inform key decision makers and impact on policy. For this there are some key inter-disciplinary success stories. For example, the collaboration and sharing of data between the analytical teams of the Sewer Analysis CORe group Europe (SCORE), an inter-laboratory collaboration focussed on wastewater testing across several European cities, informs epidemiologists at the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) on illicit drug trends in Europe [285]. Similarly, the National Wastewater Drug Monitoring Program conducted by the analytical research teams at the University of South Australia and the University of Queensland, informs the public and the Australian Criminal Intelligence Commission (ACIC) on illicit drug trends, opioids, nicotine and alcohol consumption across Australia [2]. As programs like these gather momentum, It is likely that such collaborations will become commonplace in future with the acceptance and advancement of the wastewater-based epidemiological approach.

Hardware and analytical improvements, such as in characteristics of mass spectrometers will remain important for traditional WBE techniques [286]. Improvements to the field are also being made by the annual SCORE inter-laboratory testing regime which enables participants to refine and evaluate their analytical accuracy and precision [287]. In many instances, the expansion of WBE applications hinges upon advances from peripheral fields to WBE. Information regarding the specificity, excretion kinetics, or in-sewer transformation of biomarkers will be principal in translating measurements in wastewater to population-normalised measures of exposure or consumption. Triangulating data from such sources with WBE measurements of multiple biomarkers may be helpful in elucidating and confirming relationships between WBE phenomena and "real-world" phenomena.

Acknowledgements

This work was supported by Melbourne Water. The authors thank A/Prof Barbara Drigo (University of South Australia) for her fruitful discussion and feedback.

References

[1] W. Hall, et al., An analysis of ethical issues in using wastewater analysis to monitor illicit drug use, Addiction 107 (10) (2012) 1767–1773.
[2] Australian Criminal Intelligence Commission, National Wastewater Drug Monitoring Program Report, ACIC, Canberra, 2017.
[3] C. Ort, et al., Sampling for PPCPs in wastewater systems: comparison of different sampling modes and optimization strategies, Environ. Sci. Technol. 44 (16) (2010) 6289–6296.
[4] E. Zuccato, et al., Estimating community drug abuse by wastewater analysis, Environ. Health Perspect. 116 (8) (2008) 1027–1032.
[5] E. Gracia-Lor, et al., Measuring biomarkers in wastewater as a new source of epidemiological information: current state and future perspectives, Environ. Int. 99 (2017) 131–150.
[6] A.G. Asimakopoulos, K. Kannan, Neuropsychiatric pharmaceuticals and illicit drugs in wastewater treatment plants: a review, Environ. Chem. 13 (4) (2016) 541–576.
[7] A.L. van Nuijs, et al., Illicit drug consumption estimations derived from wastewater analysis: a critical review, Sci. Total Environ. 409 (19) (2011) 3564–3577.
[8] M.J. Andrés-Costa, V. Andreu, Y. Picó, Liquid chromatography–mass spectrometry as a tool for wastewater-based epidemiology: assessing new psychoactive substances and other human biomarkers, TrAC Trends Anal. Chem. 94 (2017) 21–38.
[9] A.-K. McCall, et al., Critical review on the stability of illicit drugs in sewers and wastewater samples, Water Res. 88 (2016) 933–947.
[10] E. Zuccato, et al., Cocaine in surface waters: a new evidence-based tool to monitor community drug abuse, Environ. Health 4 (1) (2005) 14.

[11] U. Khan, J.A. Nicell, Sewer epidemiology mass balances for assessing the illicit use of methamphetamine, amphetamine and tetrahydrocannabinol, Sci. Total Environ. 421 (2012) 144–162.
[12] U. Khan, J.A. Nicell, Refined sewer epidemiology mass balances and their application to heroin, cocaine and ecstasy, Environ. Int. 37 (7) (2011) 1236–1252.
[13] F.Y. Lai, et al., Estimating daily and diurnal variations of illicit drug use in Hong Kong: a pilot study of using wastewater analysis in an Asian metropolitan city, Forensic Sci. Int. 233 (1) (2013) 126–132.
[14] C. Chen, et al., Evaluation of pre-analysis loss of dependent drugs in wastewater: stability and binding assessments, Drug Test. Anal. 5 (8) (2012) 716–721.
[15] N. Mastroianni, M. Lopez de Alda, D. Barcelo, Analysis of ethyl sulfate in raw wastewater for estimation of alcohol consumption and its correlation with drugs of abuse in the city of Barcelona, J. Chromatogr. A 1360 (2014) 93–99.
[16] D.S. Harris, et al., The pharmacology of cocaethylene in humans following cocaine and ethanol administration, Drug Alcohol Depend. 72 (2) (2003) 169–182.
[17] B. Kasprzyk-Hordern, V.V. Kondakal, D.R. Baker, Enantiomeric analysis of drugs of abuse in wastewater by chiral liquid chromatography coupled with tandem mass spectrometry, J. Chromatogr. A 1217 (27) (2010) 4575–4586.
[18] B. Kasprzyk-Hordern, D.R. Baker, Enantiomeric profiling of chiral drugs in wastewater and receiving waters, Environ. Sci. Technol. 46 (3) (2012) 1681–1691.
[19] B. Petrie, et al., Critical evaluation of monitoring strategy for the multi-residue determination of 90 chiral and achiral micropollutants in effluent wastewater, Sci. Total Environ. 579 (2017) 569–578.
[20] S.E. Evans, et al., Determination of chiral pharmaceuticals and illicit drugs in wastewater and sludge using microwave assisted extraction, solid-phase extraction and chiral liquid chromatography coupled with tandem mass spectrometry, Anal. Chim. Acta 882 (2015) 112–126.
[21] Z. Xu, et al., Tracing methamphetamine and amphetamine sources in wastewater and receiving waters via concentration and enantiomeric profiling, Sci. Total Environ. 601–602 (Suppl. C) (2017) 159–166.
[22] F. Been, et al., Data triangulation in the context of opioids monitoring via wastewater analyses, Drug Alcohol Depend. 151 (2015) 203–210.
[23] B.J. Tscharke, et al., Temporal trends in drug use in Adelaide, South Australia by wastewater analysis, Sci. Total Environ. 565 (2016) 384–391.
[24] P. Du, et al., Estimating heroin abuse in major Chinese cities through wastewater-based epidemiology, Sci. Total Environ. 605 (2017) 158–165.
[25] M.R. Boleda, M.T. Galceran, F. Ventura, Monitoring of opiates, cannabinoids and their metabolites in wastewater, surface water and finished water in Catalonia, Spain, Water Res. 43 (4) (2009) 1126–1136.
[26] C. Postigo, M.J. Lopez de Alda, D. Barceló, fully automated determination in the low nanogram per liter level of different classes of drugs of abuse in sewage water by on-line solid-phase extraction-liquid chromatography–electrospray-tandem mass spectrometry, Anal. Chem. 80 (9) (2008) 3123–3134.
[27] L. Bijlsma, et al., Simultaneous ultra-high-pressure liquid chromatography–tandem mass spectrometry determination of amphetamine and amphetamine-like stimulants, cocaine and its metabolites, and a cannabis metabolite in surface water and urban wastewater, J. Chromatogr. A 1216 (15) (2009) 3078–3089.
[28] T. Mackulak, et al., National study of illicit drug use in Slovakia based on wastewater analysis, Sci. Total Environ. 494 (2014) 158–165.
[29] A. Causanilles, et al., Improving wastewater-based epidemiology to estimate cannabis use: focus on the initial aspects of the analytical procedure, Anal. Chim. Acta 988 (2017) 27–33.
[30] S. Castiglioni, et al., Wastewater analysis to monitor spatial and temporal patterns of use of two synthetic recreational drugs, ketamine and mephedrone, in Italy, Environ. Sci. Technol. 49 (9) (2015) 5563–5570.
[31] V. Yargeau, et al., Analysis of drugs of abuse in wastewater from two Canadian cities, Sci. Total Environ. 487 (2014) 722–730.
[32] A.Y.-C. Lin, W.-N. Lee, X.-H. Wang, Ketamine and the metabolite norketamine: persistence and phototransformation toxicity in hospital wastewater and surface water, Water Res. 53 (2014) 351–360.
[33] M. Huerta-Fontela, M.T. Galceran, F. Ventura, Ultraperformance liquid chromatography–Tandem mass spectrometry analysis of stimulatory drugs of abuse in wastewater and surface waters, Anal. Chem. 79 (10) (2007) 3821–3829.
[34] A.L. Nuijs, et al., Optimization, validation, and the application of liquid chromatography-tandem mass spectrometry for the analysis of new drugs of abuse in wastewater, Drug Test. Anal. 6 (7–8) (2014) 861–867.
[35] E. van Dyken, et al., Monitoring substance use in prisons: assessing the potential value of wastewater analysis, Sci. Justice 54 (5) (2014) 338–345.
[36] J. Jerry, G. Collins, D. Streem, Synthetic legal intoxicating drugs: the emerging 'incense' and 'bath salt' phenomenon, Cleve. Clin. J. Med. 79 (4) (2012) 258–264.
[37] J.B. Zawilska, J. Wojcieszak, Designer cathinones—an emerging class of novel recreational drugs, Forensic Sci. Int. 231 (1–3) (2013) 42–53.
[38] United Nations Office on Drugs and Crime, World Drug Report 2017, United Nations, Vienna, 2017.
[39] M.J. Reid, L. Derry, K.V. Thomas, Analysis of new classes of recreational drugs in sewage: synthetic cannabinoids and amphetamine-like substances, Drug Test. Anal. 6 (1–2) (2013) 72–79.
[40] J. Kinyua, et al., Sewage-based epidemiology in monitoring the use of new psychoactive substances: validation and application of an analytical method using LC-MS/MS, Drug Test. Anal. 7 (9) (2015) 812–818.
[41] V.L. Borova, et al., Multi-residue determination of 10 selected new psychoactive substances in wastewater samples by liquid chromatography–tandem mass spectrometry, Talanta 144 (2015) 592–603.
[42] I. Senta, et al., Multiresidual analysis of emerging amphetamine-like psychoactive substances in wastewater and river water, J. Chromatogr. A 1425 (2015) 204–212.
[43] I. González-Mariño, et al., Wastewater-based epidemiology to monitor synthetic cathinones use in different European countries, Environ. Sci. Technol. 50 (18) (2016) 10089–10096.
[44] A. Wintermeyer, et al., In vitro phase I metabolism of the synthetic cannabimimetic JWH-018, Anal. Bioanal. Chem. 398 (5) (2010) 2141–2153.
[45] F.Y. Lai, et al., An exploratory wastewater analysis study of drug use in Auckland, New Zealand, Drug Alcohol Rev. 36 (5) (2017) 597–601.
[46] E. Tyrkkö, M. Andersson, R. Kronstrand, The toxicology of new psychoactive substances: synthetic cathinones and phenylethylamines, Ther. Drug Monit. 38 (2) (2016) 190–216.
[47] C.V. Giné, I.F. Espinosa, M.V. Vilamala, New psychoactive substances as adulterants of controlled drugs. A worrying phenomenon? Drug Test. Anal. 6 (7–8) (2014) 819–824.
[48] M.J. Reid, et al., Using biomarkers in wastewater to monitor community drug use: a conceptual approach for dealing with new psychoactive substances, Sci. Total Environ. 487 (2014) 651–658.
[49] M. Ibáñez, et al., Comprehensive analytical strategies based on high-resolution time-of-flight mass spectrometry to identify new psychoactive substances, TrAC Trends Anal. Chem. 57 (2014) 107–117.
[50] S. Castiglioni, et al., Testing wastewater to detect illicit drugs: state of the art, potential and research needs, Sci. Total Environ. 487 (2014) 613–620.
[51] I. González-Mariño, et al., Screening new psychoactive substances in urban wastewater using high resolution mass spectrometry, Anal. Bioanal. Chem. 408 (16) (2016) 4297–4309.
[52] J.P. Caplan, et al., Neuropsychiatric effects of prescription drug abuse, Neuropsychol. Rev. 17 (3) (2007) 363–380.
[53] J.H. Stubbe, et al., Prevalence of use of performance enhancing drugs by fitness centre members, Drug Test. Anal. 6 (5) (2014) 434–438.
[54] E.P. Kolodziej, J.L. Gray, D.L. Sedlak, Quantification of steroid hormones with pheromonal properties in municipal wastewater effluent, Environ. Toxicol. Chem. 22 (11) (2003) 2622–2629.
[55] H. Chang, et al., Trace analysis of androgens and progestogens in environmental waters by ultra-performance liquid chromatography–electrospray tandem mass spectrometry, J. Chromatogr. A 1195 (1) (2008) 44–51.
[56] L. Sun, et al., Trace analysis of fifteen androgens in environmental waters by LC-ESI-MS-MS combined with solid-phase disk extraction cleanup, Chromatographia 71 (9) (2010) 867–873.
[57] A. Causanilles, et al., Wastewater-based tracing of doping use by the general population and amateur athletes, Anal. Bioanal. Chem. 410 (6) (2018) 1793–1803.
[58] I. Senta, et al., Wastewater analysis to monitor use of caffeine and nicotine and evaluation of their metabolites as biomarkers for population size assessment, Water Res. 74 (2015) 23–33.
[59] E. Gracia-Lor, et al., Estimation of caffeine intake from analysis of caffeine metabolites in wastewater, Sci. Total Environ. 609 (2017) 1582–1588.
[60] D.S. Senchina, et al., Alkaloids and athlete immune function: caffeine, theophylline, gingerol, ephedrine, and their congeners, Exerc. Immunol. Rev. 20 (2014) 68–93.
[61] T. Rodríguez-Álvarez, et al., Ion-pair reversed-phase liquid chromatography–quadrupole-time-of-flight and triple-quadrupole–mass spectrometry determination of ethyl sulfate in wastewater for alcohol consumption tracing, J. Chromatogr. A 1328 (2014) 35–42.
[62] M.J. Reid, et al., Analysis and interpretation of specific ethanol metabolites, ethyl sulfate, and ethyl glucuronide in sewage effluent for the quantitative measurement of regional alcohol consumption, Alcohol Clin. Exp. Res. 35 (9) (2011) 1593–1599.
[63] T. Boogaerts, et al., Spatial and temporal trends in alcohol consumption in Belgian cities: a wastewater-based approach, Drug Alcohol Depend. 160 (2016) 170–176.
[64] M.J. Andrés-Costa, et al., Estimation of alcohol consumption during "Fallas" festivity in the wastewater of Valencia city (Spain) using ethyl sulfate as a biomarker, Sci. Total Environ. 541 (2016) 616–622.
[65] Y. Ryu, et al., Comparative measurement and quantitative risk assessment of alcohol consumption through wastewater-based epidemiology: an international study in 20 cities, Sci. Total Environ. 565 (2016) 977–983.
[66] J.A. Baz-Lomba, et al., Comparison of pharmaceutical, illicit drug, alcohol, nicotine and caffeine levels in wastewater with sale, seizure and consumption data for 8 European cities, BMC Publ. Health 16 (1) (2016) 1035.
[67] World Health Organization, Global Status Report on Alcohol and Health 2014, World Health Organization, Luxembourg, 2014.
[68] S. Castiglioni, et al., A novel approach for monitoring tobacco use in local communities by wastewater analysis, Tobac. Contr. 24 (1) (2015) 38–42.
[69] T. Rodríguez-Álvarez, et al., Assessment of local tobacco consumption by liquid chromatography–tandem mass spectrometry sewage analysis of nicotine and its metabolites, cotinine and trans-3′-hydroxycotinine, after enzymatic deconjugation, Anal. Chem. 86 (20) (2014) 10274–10281.

[70] D.G. Wang, et al., Using Monte Carlo simulation to assess variability and uncertainty of tobacco consumption in a city by sewage epidemiology, BMJ Open 6 (2) (2016) e010583.
[71] T. Mackulak, et al., National monitoring of nicotine use in Czech and Slovak Republic based on wastewater analysis, Environ. Sci. Pollut. Res. 22 (18) (2015) 14000–14006.
[72] A. Lopes, et al., Analysis of cocaine and nicotine metabolites in wastewater by liquid chromatography–tandem mass spectrometry. Cross abuse index patterns on a major community, Sci. Total Environ. 487 (2014) 673–680.
[73] B.J. Tscharke, J.M. White, J.P. Gerber, Estimates of tobacco use by wastewater analysis of anabasine and anatabine, Drug Test. Anal. 8 (7) (2015) 702–707.
[74] M. Lader, A. Tylee, J. Donoghue, Withdrawing benzodiazepines in primary care, CNS Drugs 23 (1) (2009) 19–34.
[75] D.R. Baker, B. Kasprzyk-Hordern, Multi-residue analysis of drugs of abuse in wastewater and surface water by solid-phase extraction and liquid chromatography–positive electrospray ionisation tandem mass spectrometry, J. Chromatogr. A 1218 (12) (2011) 1620–1631.
[76] T. Kosjek, et al., Environmental occurrence, fate and transformation of benzodiazepines in water treatment, Water Res. 46 (2) (2012) 355–368.
[77] D.R. Baker, L. Barron, B. Kasprzyk-Hordern, Illicit and pharmaceutical drug consumption estimated via wastewater analysis. Part A: chemical analysis and drug use estimates, Sci. Total Environ. 487 (2014) 629–641.
[78] V.L. Borova, et al., Highly sensitive determination of 68 psychoactive pharmaceuticals, illicit drugs, and related human metabolites in wastewater by liquid chromatography–tandem mass spectrometry, Anal. Bioanal. Chem. 406 (17) (2014) 4273–4285.
[79] E. Castrignanò, A. Lubben, B. Kasprzyk-Hordern, Enantiomeric profiling of chiral drug biomarkers in wastewater with the usage of chiral liquid chromatography coupled with tandem mass spectrometry, J. Chromatogr. A 1438 (2016) 84–99.
[80] P. Fernández, et al., Optimization of ultrasound-assisted dispersive liquid–liquid microextraction for ultra performance liquid chromatography determination of benzodiazepines in urine and hospital wastewater, Anal. Methods 6 (20) (2014) 8239–8246.
[81] D. Hummel, et al., Simultaneous determination of psychoactive drugs and their metabolites in aqueous matrices by liquid chromatography mass spectrometry, Environ. Sci. Technol. 40 (23) (2006) 7321–7328.
[82] I. Racamonde, et al., Determination of benzodiazepines, related pharmaceuticals and metabolites in water by solid-phase extraction and liquid-chromatography–tandem mass spectrometry, J. Chromatogr. A 1352 (2014) 69–79.
[83] I. Racamonde, et al., Application of polypropylene tubes as single-use and low-cost sorptive extraction materials for the determination of benzodiazepines and zolpidem in water samples, Microchem. J. 119 (2015) 58–65.
[84] L. Bijlsma, et al., Investigation of drugs of abuse and relevant metabolites in Dutch sewage water by liquid chromatography coupled to high resolution mass spectrometry, Chemosphere 89 (11) (2012) 1399–1406.
[85] T. Ternes, M. Bonerz, T. Schmidt, Determination of neutral pharmaceuticals in wastewater and rivers by liquid chromatography–electrospray tandem mass spectrometry, J. Chromatogr. A 938 (1) (2001) 175–185.
[86] M.J. Martínez Bueno, et al., Application of liquid chromatography/quadrupole-linear ion trap mass spectrometry and time-of-flight mass spectrometry to the determination of pharmaceuticals and related contaminants in wastewater, Anal. Chem. 79 (24) (2007) 9372–9384.
[87] C.J.L. Murray, et al., Disability-adjusted life years (DALYs) for 291 diseases and injuries in 21 regions, 1990–2010: a systematic analysis for the Global Burden of Disease Study 2010, Lancet 380 (9859) (2012) 2197–2223.
[88] J.C. Fournier, et al., Antidepressant drug effects and depression severity: a patient-level meta-analysis, JAMA J. Am. Med. Assoc. 303 (1) (2010) 47–53.
[89] K.H. Langford, M. Reid, K.V. Thomas, Multi-residue screening of prioritised human pharmaceuticals, illicit drugs and bactericides in sediments and sludge, J. Environ. Monit. 13 (8) (2011) 2284–2291.
[90] K. Langford, K.V. Thomas, Input of selected human pharmaceutical metabolites into the Norwegian aquatic environment, J. Environ. Monit. 13 (2) (2011) 416–421.
[91] O. Golovko, et al., Seasonal changes in antibiotics, antidepressants/psychiatric drugs, antihistamines and lipid regulators in a wastewater treatment plant, Chemosphere 111 (2014) 418–426.
[92] D.R. Baker, et al., Drugs of abuse in wastewater and suspended particulate matter — further developments in sewage epidemiology, Environ. Int. 48 (2012) 28–38.
[93] L.-H. Sheng, et al., Simultaneous determination of 24 antidepressant drugs and their metabolites in wastewater by ultra-high performance liquid chromatography–tandem mass spectrometry, Molecules 19 (1) (2014) 1212.
[94] E.J. Hauenstein, et al., Rurality and mental health treatment, Adm. Pol. Mental Health Mental Health Serv. Res. 34 (3) (2007) 255–267.
[95] O.J. Dyar, et al., How can we improve antibiotic prescribing in primary care? Expert Rev. Anti-infect. Ther. 14 (4) (2016) 403–413.
[96] P. Sarkar, I.M. Gould, Antimicrobial agents are societal drugs: how should this influence prescribing? Drugs 66 (7) (2006) 893–901.
[97] T.P. Van Boeckel, et al., Global antibiotic consumption 2000 to 2010: an analysis of national pharmaceutical sales data, Lancet Infect. Dis. 14 (8) (2014) 742–750.
[98] S. Castiglioni, et al., Removal of pharmaceuticals in sewage treatment plants in Italy, Environ. Sci. Technol. 40 (1) (2006) 357–363.
[99] I. Michael, et al., Urban wastewater treatment plants as hotspots for the release of antibiotics in the environment: a review, Water Res. 47 (3) (2013) 957–995.
[100] A. Christou, et al., The potential implications of reclaimed wastewater reuse for irrigation on the agricultural environment: the knowns and unknowns of the fate of antibiotics and antibiotic resistant bacteria and resistance genes–A review, Water Res. 123 (2017) 448–467.
[101] B. Subedi, et al., Mass loading and removal of pharmaceuticals and personal care products including psychoactives, antihypertensives, and antibiotics in two sewage treatment plants in southern India, Chemosphere 167 (2017) 429–437.
[102] A. Jelic, et al., Impact of in-sewer transformation on 43 pharmaceuticals in a pressurized sewer under anaerobic conditions, Water Res. 68C (2014) 98–108.
[103] R. Wightman, et al., Likeability and abuse liability of commonly prescribed opioids, J. Med. Toxicol. 8 (4) (2012) 335–340.
[104] United Nations Office on Drugs and Crime, Opioid Overdose: Preventing and Reducing Opioid Overdose Mortality, United Nations, Vienna, 2013.
[105] K.J. Ottmar, L.M. Colosi, J.A. Smith, Development and application of a model to estimate wastewater treatment plant prescription pharmaceutical influent loadings and concentrations, Bull. Environ. Contam. Toxicol. 84 (5) (2010) 507–512.
[106] P. Guerra, et al., Occurrence and fate of antibiotic, analgesic/anti-inflammatory, and antifungal compounds in five wastewater treatment processes, Sci. Total Environ. 473 (2014) 235–243.
[107] K.J. Bisceglia, et al., Quantification of drugs of abuse in municipal wastewater via SPE and direct injection liquid chromatography mass spectrometry, Anal. Bioanal. Chem. 398 (6) (2010) 2701–2712.
[108] D.R. Baker, B. Kasprzyk-Hordern, Spatial and temporal occurrence of pharmaceuticals and illicit drugs in the aqueous environment and during wastewater treatment: new developments, Sci. Total Environ. 454 (2013) 442–456.
[109] A. Jacox, et al., Quantitative analysis of opioids and cannabinoids in wastewater samples, Forensic Sci. Res. (2017) 1–8.
[110] W. Gul, et al., LC–MS-MS method for analysis of opiates in wastewater during football games II, J. Anal. Toxicol. 40 (5) (2016) 330–337.
[111] C. Postigo, M.L. de Alda, D. Barceló, Evaluation of drugs of abuse use and trends in a prison through wastewater analysis, Environ. Int. 37 (1) (2011) 49–55.
[112] P.J. Phillips, et al., Pharmaceutical formulation facilities as sources of opioids and other pharmaceuticals to wastewater treatment plant effluents, Environ. Sci. Technol. 44 (13) (2010) 4910–4916.
[113] Y. Lester, et al., Treating wastewater from a pharmaceutical formulation facility by biological process and ozone, Water Res. 47 (13) (2013) 4349–4356.
[114] A. Rodayan, P.A. Segura, V. Yargeau, Ozonation of wastewater: removal and transformation products of drugs of abuse, Sci. Total Environ. 487 (2014) 763–770.
[115] M. Kim, et al., Removal of pharmaceuticals and personal care products in a membrane bioreactor wastewater treatment plant, Water Sci. Technol. 69 (11) (2014) 2221–2229.
[116] E. Vuori, et al., Wastewater analysis reveals regional variability in exposure to abused drugs and opioids in Finland, Sci. Total Environ. 487 (2014) 688–695.
[117] I. Krizman, et al., Wastewater-based assessment of regional and temporal consumption patterns of illicit drugs and therapeutic opioids in Croatia, Sci. Total Environ. 566 (2016) 454–462.
[118] P.K. Thai, et al., Refining the excretion factors of methadone and codeine for wastewater analysis—combining data from pharmacokinetic and wastewater studies, Environ. Int. 94 (2016) 307–314.
[119] T. Ozdoganoglu, M. Songu, The burden of allergic rhinitis and asthma, Ther. Adv. Respir. Dis. 6 (1) (2011) 11–23.
[120] C. Harman, M. Reid, K.V. Thomas, In situ calibration of a passive sampling device for selected illicit drugs and their metabolites in wastewater, and subsequent year-long assessment of community drug usage, Environ. Sci. Technol. 45 (13) (2011) 5676–5682.
[121] E. Fattore, et al., Wastewater-based epidemiological evaluation of the effect of air pollution on short-acting beta-agonist consumption for acute asthma treatment, Environ. Res. 150 (2016) 106–111.
[122] K.H. Langford, K.V. Thomas, Determination of pharmaceutical compounds in hospital effluents and their contribution to wastewater treatment works, Environ. Int. 35 (5) (2009) 766–770.
[123] M.J. Gomez, et al., Determination of pharmaceuticals of various therapeutic classes by solid-phase extraction and liquid chromatography-tandem mass spectrometry analysis in hospital effluent wastewaters, J. Chromatogr. A 1114 (2) (2006) 224–233.
[124] B. Subedi, et al., Mass loading and removal of pharmaceuticals and personal care products, including psychoactive and illicit drugs and artificial sweeteners, in five sewage treatment plants in India, J. Environ. Chem. Eng. 3 (4, Part A) (2015) 2882–2891.
[125] B. Subedi, K. Kannan, Occurrence and fate of select psychoactive pharmaceuticals and antihypertensives in two wastewater treatment plants in New York State, USA, Sci. Total Environ. 514 (2015) 273–280.
[126] B. Kasprzyk-Hordern, R.M. Dinsdale, A.J. Guwy, The occurrence of pharmaceuticals, personal care products, endocrine disruptors and illicit drugs in surface water in South Wales, UK, Water Res. 42 (13) (2008) 3498–3518.

[127] F.Y. Lai, et al., Refining the estimation of illicit drug consumptions from wastewater analysis: co-analysis of prescription pharmaceuticals and uncertainty assessment, Water Res. 45 (15) (2011) 4437–4448.
[128] D. Phung, et al., Can wastewater-based epidemiology be used to evaluate the health impact of temperature? – an exploratory study in an Australian population, Environ. Res. 156 (2017) 113–119.
[129] P.K. Thai, et al., Monitoring temporal changes in use of two cathinones in a large urban catchment in Queensland, Australia, Sci. Total Environ. 545–546 (2016) 250–255.
[130] B. Petrie, R. Barden, B. Kasprzyk-Hordern, A review on emerging contaminants in wastewaters and the environment: current knowledge, understudied areas and recommendations for future monitoring, Water Res. 72 (2015) 3–27.
[131] J.W. O'Brien, et al., Impact of in-sewer degradation of Pharmaceutical and Personal Care Products (PPCPs) population markers on a population model, Environ. Sci. Technol. 51 (7) (2017) 3816–3823.
[132] M. Clara, B. Strenn, N. Kreuzinger, Carbamazepine as a possible anthropogenic marker in the aquatic environment: investigations on the behaviour of Carbamazepine in wastewater treatment and during groundwater infiltration, Water Res. 38 (4) (2004) 947–954.
[133] Y. Zhang, S.-U. Geißen, C. Gal, Carbamazepine and diclofenac: removal in wastewater treatment plants and occurrence in water bodies, Chemosphere 73 (8) (2008) 1151–1161.
[134] B. Kasprzyk-Hordern, R.M. Dinsdale, A.J. Guwy, The removal of pharmaceuticals, personal care products, endocrine disruptors and illicit drugs during wastewater treatment and its impact on the quality of receiving waters, Water Res. 43 (2) (2009) 363–380.
[135] S.A. Snyder, et al., Pharmaceuticals, personal care products, and endocrine disruptors in water: Implications for the water industry, Environ. Eng. Sci. 20 (5) (2003) 449–469.
[136] V. Sarveiya, S. Risk, H.A.E. Benson, Liquid chromatographic assay for common sunscreen agents: application to in vivo assessment of skin penetration and systemic absorption in human volunteers, J. Chromatogr. B 803 (2) (2004) 225–231.
[137] M.J. Gomez Ramos, et al., Concentrations of phthalates and DINCH metabolites in pooled urine from Queensland, Australia, Environ. Int. 88 (2016) 179–186.
[138] W. Wang, K. Kannan, Mass loading and emission of benzophenone-3 (BP-3) and its derivatives in wastewater treatment plants in New York State, USA, Sci. Total Environ. 579 (2017) 1316–1322.
[139] B. Kasprzyk-Hordern, R.M. Dinsdale, A.J. Guwy, Multiresidue methods for the analysis of pharmaceuticals, personal care products and illicit drugs in surface water and wastewater by solid-phase extraction and ultra performance liquid chromatography–electrospray tandem mass spectrometry, Anal. Bioanal. Chem. 391 (4) (2008) 1293–1308.
[140] E. Gracia-Lor, et al., Multi-class determination of personal care products and pharmaceuticals in environmental and wastewater samples by ultra-high performance liquid-chromatography-tandem mass spectrometry, Talanta 99 (2012) 1011–1023.
[141] M. Clara, et al., Occurrence of polycyclic musks in wastewater and receiving water bodies and fate during wastewater treatment, Chemosphere 82 (8) (2011) 1116–1123.
[142] I. González-Mariño, et al., Simultaneous determination of parabens, triclosan and triclocarban in water by liquid chromatography/electrospray ionisation tandem mass spectrometry, Rapid Commun. Mass Spectrom. 23 (12) (2009) 1756–1766.
[143] R. Karthikraj, et al., Occurrence and fate of parabens and their metabolites in five sewage treatment plants in India, Sci. Total Environ. 593–594 (2017) 592–598.
[144] W. Wang, K. Kannan, Fate of parabens and their metabolites in two wastewater treatment plants in New York state, United States, Environ. Sci. Technol. 50 (3) (2016) 1174–1181.
[145] R. Karthikraj, K. Kannan, Mass loading and removal of benzotriazoles, benzothiazoles, benzophenones, and bisphenols in Indian sewage treatment plants, Chemosphere 181 (2017) 216–223.
[146] S. Castiglioni, et al., Evaluation of uncertainties associated with the determination of community drug use through the measurement of sewage drug biomarkers, Environ. Sci. Technol. 47 (3) (2013) 1452–1460.
[147] J.W. O'Brien, et al., A model to estimate the population contributing to the wastewater using samples collected on census day, Environ. Sci. Technol. 48 (1) (2014) 517–525.
[148] M. Zessner, S. Lindtner, Estimations of municipal point source pollution in the context of river basin management, Water Sci. Technol. 52 (9) (2005) 175–182.
[149] G. Andreottola, et al., Methodology for the estimation of unit nutrient and organic loads from domestic and non-domestic sources, Eur. Water Manag. 4 (6) (1994) 13–19.
[150] C. Chen, et al., Towards finding a population biomarker for wastewater epidemiology studies, Sci. Total Environ. 487 (2013) 621–628.
[151] M. Rico, M.J. Andrés-Costa, Y. Picó, Estimating population size in wastewater-based epidemiology. Valencia metropolitan area as a case study, J. Hazard Mater. 323 (2017) 9.
[152] F. Been, et al., Population normalization with ammonium in wastewater-based epidemiology: application to illicit drug monitoring, Environ. Sci. Technol. 48 (14) (2014) 8162–8169.
[153] P.K. Thai, et al., Degradability of creatinine under sewer conditions affects its potential to be used as biomarker in sewage epidemiology, Water Res. 55 (2014) 272–279.
[154] M.F. Fitzsimons, S.T. Belt, Dynamic behaviour of 1-aminopropan-2-one in sewage: a preliminary synthetic and spectroscopic study, Environ. Chem. Lett. 3 (2) (2005) 70–73.
[155] T. Joy, et al., Increase of urinary 5-hydroxyindoleacetic acid excretion but not serum chromogranin a following over-the-counter 5-hydroxytryptophan intake, Can. J. Gastroenterol. (1) (2008) 22.
[156] Q.-D. Zheng, et al., Estimating nicotine consumption in eight cities using sewage epidemiology based on ammonia nitrogen equivalent population, Sci. Total Environ. 590 (2017) 226–232.
[157] Z. Yang, et al., A novel DNA biosensor using a ferrocenyl intercalator applied to the potential detection of human population biomarkers in wastewater, Environ. Sci. Technol. 49 (9) (2015) 5609–5617.
[158] J. Gao, et al., Measuring selected PPCPs in wastewater to estimate the population in different cities in China, Sci. Total Environ. 568 (2016) 164–170.
[159] P. Deville, et al., Dynamic population mapping using mobile phone data, Proc. Natl. Acad. Sci. U.S.A. 111 (45) (2014) 15888–15893.
[160] K.V. Thomas, et al., Use of mobile device data to better estimate dynamic population size for wastewater-based epidemiology, Environ. Sci. Technol. 51 (19) (2017) 11363–11370.
[161] E.E. Ntzani, et al., Literature review on epidemiological studies linking exposure to pesticides and health effects, EFSA Support. Publ. 10 (10) (2013).
[162] S. Mostafalou, M. Abdollahi, Pesticides and human chronic diseases: evidences, mechanisms, and perspectives, Toxicol. Appl. Pharmacol. 268 (2) (2013) 157–177.
[163] M.C. Aprea, Environmental and biological monitoring in the estimation of absorbed doses of pesticides, Toxicol. Lett. 210 (2) (2012) 110–118.
[164] D.B. Barr, Biomonitoring of exposure to pesticides, J. Chem. Health Saf. 15 (6) (2008) 20–29.
[165] R.P. Schwarzenbach, et al., The challenge of micropollutants in aquatic systems, Science 313 (5790) (2006) 1072–1077.
[166] N.I. Rousis, E. Zuccato, S. Castiglioni, Wastewater-based epidemiology to assess human exposure to pyrethroid pesticides, Environ. Int. 99 (2017) 213–220.
[167] N.I. Rousis, E. Zuccato, S. Castiglioni, Monitoring population exposure to pesticides based on liquid chromatography-tandem mass spectrometry measurement of their urinary metabolites in urban wastewater: a novel biomonitoring approach, Sci. Total Environ. 571 (2016) 1349–1357.
[168] T. Reemtsma, J. Lingott, S. Roegler, Determination of 14 monoalkyl phosphates, dialkyl phosphates and dialkyl thiophosphates by LC-MS/MS in human urinary samples, Sci. Total Environ. 409 (10) (2011) 1990–1993.
[169] D.B. Barr, et al., Assessing exposure to atrazine and its metabolites using biomonitoring, Environ. Health Perspect. 115 (10) (2007) 1474.
[170] N. Ratola, et al., Occurrence of organic microcontaminants in the wastewater treatment process. A mini review, J. Hazard Mater. 239–240 (2012) 1–18.
[171] F. Been, et al., Liquid chromatography–tandem mass spectrometry analysis of biomarkers of exposure to phosphorus flame retardants in wastewater to monitor community-wide exposure, Anal. Chem. 89 (18) (2017) 10045–10053.
[172] U.-J. Kim, J.K. Oh, K. Kannan, Occurrence, removal, and environmental emission of organophosphate flame retardants/plasticizers in a wastewater treatment plant in New York State, Environ. Sci. Technol. 51 (2017) 7872–7880.
[173] J.W. O'Brien, et al., Wastewater analysis of Census day samples to investigate per capita input of organophosphorus flame retardants and plasticizers into wastewater, Chemosphere 138 (2015) 328–334.
[174] J.D. Meeker, et al., Urinary metabolites of organophosphate flame retardants: temporal variability and correlations with house dust concentrations, Environ. Health Perspect. 121 (5) (2013) 580–585.
[175] L. Feng, et al., Levels of urinary metabolites of organophosphate flame retardants, TDCIPP, and TPHP, in pregnant women in Shanghai, J. Environ. Publ. Health 2016 (2016).
[176] N. Van den Eede, et al., Age as a determinant of phosphate flame retardant exposure of the Australian population and identification of novel urinary PFR metabolites, Environ. Int. 74 (2015) 1–8.
[177] D. Gao, Z. Wen, Phthalate esters in the environment: a critical review of their occurrence, biodegradation, and removal during wastewater treatment processes, Sci. Total Environ. 541 (2016) 986–1001.
[178] M. Zolfaghari, et al., Occurrence, fate and effects of Di (2-ethylhexyl) phthalate in wastewater treatment plants: a review, Environ. Pollut. 194 (2014) 281–293.
[179] I. González-Mariño, et al., Wastewater-based epidemiology as a new tool for estimating population exposure to phthalate plasticizers, Environ. Sci. Technol. 51 (7) (2017) 3902–3910.
[180] A.L. Heffernan, et al., Rapid, automated online SPE-LC-QTRAP-MS/MS method for the simultaneous analysis of 14 phthalate metabolites and 5 bisphenol analogues in human urine, Talanta 151 (2016) 224–233.
[181] H.M. Koch, J. Angerer, Di-iso-nonylphthalate (DINP) metabolites in human urine after a single oral dose of deuterium-labelled DINP, Int. J. Hyg Environ. Health 210 (2007) 9–19.
[182] C.A. Staples, et al., The environmental fate of phthalate esters: a literature review, Chemosphere 25 (4) (1997) 667–749.

[183] J.D. Blair, et al., Ultra-trace determination of phthalate ester metabolites in seawater, sediments, and biota from an urbanized marine Inlet by LC/ESI-MS/MS, Environ. Sci. Technol. 43 (16) (2009) 6262–6268.
[184] E.D. Tsochatzis, R. Tzimou-Tsitouridou, H.G. Gika, Analytical methodologies for the assessment of phthalate exposure in humans, Crit. Rev. Anal. Chem. 47 (4) (2017) 279–297.
[185] L.N. Vandenberg, et al., Hormones and endocrine-disrupting chemicals: low-dose effects and nonmonotonic dose responses, Endocr. Rev. 33 (3) (2012) 378–455.
[186] K. Prevedouros, et al., Sources, fate and transport of perfluorocarboxylates, Environ. Sci. Technol. 40 (1) (2006) 32–44.
[187] J.P. Giesy, K. Kannan, Perfluorochemical surfactants in the environment, Environ. Sci. Technol. 36 (7) (2002) 146A–152A.
[188] L. Ahrens, Polyfluoroalkyl compounds in the aquatic environment: a review of their occurrence and fate, J. Environ. Monit. 13 (2011) 20–31.
[189] M.M. Schultz, D.F. Barofsky, J.A. Field, Fluorinated alkyl surfactants, Environ. Eng. Sci. 20 (5) (2003) 487–501.
[190] A. Rotander, et al., Increasing levels of long-chain perfluorocarboxylic acids (PFCAs) in Arctic and North Atlantic marine mammals, Chemosphere 86 (2012) 1984–2009.
[191] D. Skutlarek, M. Exner, H. Farber, Perfluorinated surfactants in surface and drinking waters, Environ. Sci. Pollut. Res. 13 (5) (2006) 299–307.
[192] S. Convention, Stockholm Convention on Persistent Organic Pollutants (POPs), Stockholm Convention, Chatelaine, Geneva, Switzerland, 2017. COP-4, 2009 decision SC-4/27.
[193] USEPA, Drinking Water Health Advisories for PFOA and PFOS, 2017. https://www.epa.gov/ground-water-and-drinking-water/drinking-water-health-advisories-pfoa-and-pfos.
[194] EnvironmentCanada, Proposed Risk Management Measure for Perfluorooctanoic Acid (PFOA) its Salts, and its Precursors and Long-chain (C9-c20) Perfluorocarboxylic Acids (PFCAs), Their Salts, and Their Precursors, 2014.
[195] Australian Government Department of Health, Health Based Guidance Values for PFAS for Use in Site Investigations in Australia, April 2017. Available on-line: http://www.health.gov.au/internet/main/publishing.nsf/Content/2200FE086D480353CA2580C900817CDC/$File/fs-Health-Based-Guidance-Values.pdf.
[196] C. Gallen, et al., Occurrence and distribution of brominated flame retardants and perfluoroalkyl substances in Australian landfill leachate and biosolids, J. Hazard Mater. 312 (2016) 55–64.
[197] H. Hamid, L.Y. Li, Role of wastewater treatment plant in environmental cycling of poly- and perfluoroalkyl substances, Ecocycles 2 (2) (2016) 43–53.
[198] B.R. Shivakoti, et al., Occurrences and behavior of perfluorinated compounds (PFCs) in several wastewater treatment plants (WWTPs) in Japan and Thailand, J. Environ. Monit. 12 (2010) 1255–1264.
[199] E. Sinclair, K. Kannan, Mass loading and fate of perfluoroalkyl surfactants in wastewater treatment plants, Environ. Sci. Technol. 40 (5) (2006) 1408–1414.
[200] J. Heidler, R.U. Halden, Meta-analysis of mass balances examining chemical fate during wastewater treatment, Environ. Sci. Technol. 42 (17) (2008) 6324–6332.
[201] C.A. Huset, et al., Occurrence and mass flows of fluorochemicals in the Glatt Valley watershed, Switzerland, Environ. Sci. Technol. 42 (17) (2008) 6369–6377.
[202] B.G. Loganathan, et al., Perfluoroalkyl sulfonates and perfluorocarboxylates in two wastewater treatment facilities in Kentucky and Georgia, Water Res. 41 (2007) 4611–4620.
[203] Y.L. Mak, et al., Perfluorinated compounds in tap water from China and several other countries, Environ. Sci. Technol. 43 (13) (2009) 4824–4829.
[204] J. Thompson, G. Eaglesham, J.F. Mueller, Concentrations of PFOS, PFOA and other perfluorinated alkyl acids in Australian drinking water, Chemosphere 83 (2011) 1320–1325.
[205] E.A. Emmett, et al., Community exposure to perfluorooctanoate: relationships between serum concentrations and exposure sources, J. Occup. Environ. Med. 48 (8) (2006) 759–770.
[206] M. Wilhelm, S. Bergmann, H.H. Dieter, Occurrence of perfluorinated compounds (PFCs) in drinking water of North Rhine-Westphalia, Germany and new approach to assess drinking water contamination by shorter-chained C4–C7 PFCs, Int. J. Hyg Environ. Health 213 (2010) 224–232.
[207] H. Fromme, et al., Perfluorinated compounds – exposure assessment for the general population in western countries, Int. J. Hyg Environ. Health 212 (3) (2009) 239–270.
[208] Y. Zhang, et al., Biomonitoring of perfluoroalkyl acids in human urine and estimates of biological half-life, Environ. Sci. Technol. 47 (2013) 10619–10627.
[209] USEPA, Perfluoroalkyl and Polyfluoroalkyl Substances (PFAS), 2018. https://www.epa.gov/sites/production/files/2018-02/documents/pfas_methods_tech_brief_01feb17_508_compliant.pdf.
[210] Z. Wang, et al., A never-ending story of Per- and Polyfluoroalkyl Substances (PFASs)? Environ. Sci. Technol. 51 (5) (2017) 2508–2518.
[211] A. Rotander, et al., Elevated levels of PFOS and PFHxS in firefighters exposed to aqueous film forming foam (AFFF), Environ. Int. 82 (2015) 28–34.
[212] E.F. Houtz, et al., Poly- and perfluoroalkyl substances in wastewater: significance of unknown precursors, manufacturing shifts, and likely AFFF impacts, Water Res. 15 (95) (2016) 142–149.
[213] K.V. Thomas, M.J. Reid, What else can the analysis of sewage for urinary biomarkers reveal about communities? Environ. Sci. Technol. 45 (18) (2011) 7611–7612.
[214] C.G. Daughton, Using biomarkers in sewage to monitor community-wide human health: isoprostanes as conceptual prototype, Sci. Total Environ. 424 (Suppl. C) (2012) 16–38.
[215] C.G. Daughton, Monitoring wastewater for assessing community health: Sewage Chemical-Information Mining (SCIM), Sci. Total Environ. 619–620 (2018) 748–764.
[216] Y. Ryu, M.J. Reid, K.V. Thomas, Liquid chromatography–high resolution mass spectrometry with immunoaffinity clean-up for the determination of the oxidative stress biomarker 8-iso-prostaglandin F2alpha in wastewater, J. Chromatogr. A 1409 (2015) 146–151.
[217] A. Valavanidis, T. Vlachogianni, C. Fiotakis, 8-hydroxy-2′ -deoxyguanosine (8-OHdG): a critical biomarker of oxidative stress and carcinogenesis, J Environ. Sci. Health Part C 27 (2) (2009) 120–139.
[218] J. Li, et al., Exposure to polycyclic aromatic hydrocarbons could cause their oxidative DNA damage: a case study for college students in Guangzhou, China, Environ. Sci. Pollut. Control Ser. 22 (3) (2015) 1770–1777.
[219] G.A. Molnár, et al., Urinary ortho-tyrosine excretion in diabetes mellitus and renal failure: evidence for hydroxyl radical production, Kidney Int. 68 (5) (2005) 2281–2287.
[220] H. Mita, et al., Urinary 3-bromotyrosine and 3-chlorotyrosine concentrations in asthmatic patients: lack of increase in 3-bromotyrosine concentration in urine and plasma proteins in aspirin-induced asthma after intravenous aspirin challenge, Clin. Exp. Allergy 34 (6) (2004) 931–938.
[221] I. Garcia-Perez, et al., An analytical pipeline for quantitative characterization of dietary intake: application to assess grape intake, J. Agric. Food Chem. 64 (11) (2016) 2423–2431.
[222] A.B. Ross, Present status and perspectives on the use of alkylresorcinols as biomarkers of wholegrain wheat and rye intake, J. Nutr. Metab. 2012 (2012) 462967.
[223] M. Kristensen, et al., Urinary excretion of total isothiocyanates from cruciferous vegetables shows high dose-response relationship and may be a useful biomarker for isothiocyanate exposure, Eur. J. Nutr. 46 (7) (2007) 377–382.
[224] A.J. Lloyd, et al., Proline betaine and its biotransformation products in fasting urine samples are potential biomarkers of habitual citrus fruit consumption, Br. J. Nutr. 106 (06) (2011) 812–824.
[225] M.M. Ulaszewska, et al., Urinary metabolomic profiling to identify biomarkers of a flavonoid-rich and flavonoid-poor fruits and vegetables diet in adults: the FLAVURS trial, Metabolomics 12 (2) (2016) 32.
[226] T. Saenger, F. Hübner, H.-U. Humpf, Short-term biomarkers of apple consumption, Mol. Nutr. Food Res. 61 (3) (2017). 1600629-n/a.
[227] K.S. Krogholm, et al., Free fruit at workplace intervention increases total fruit intake: a validation study using 24[thinsp]h dietary recall and urinary flavonoid excretion, Eur. J. Clin. Nutr. 64 (10) (2010) 1222–1228.
[228] A.J. Cross, J.M. Major, R. Sinha, Urinary biomarkers of meat consumption, Cancer Epidemiol. Biomark. Prev. Publ. Am. Assoc. Cancer Res. Cosponsored Am. Soc. Prev. Oncol. 20 (6) (2011) 1107–1111.
[229] A.J. Cross, et al., Urinary 1-methylhistidine and 3-methylhistidine, meat intake, and colorectal adenoma risk, Eur. J. Cancer Prev. 23 (5) (2014) 385–390.
[230] M.S. Lundgren, P.J. Novak, Quantification of phytoestrogens in industrial waste streams, Environ. Toxicol. Chem. 28 (11) (2009) 2318–2323.
[231] S.S. Heinzmann, et al., Metabolic profiling strategy for discovery of nutritional biomarkers: proline betaine as a marker of citrus consumption, Am. J. Clin. Nutr. 92 (2) (2010) 436–443.
[232] S. Slow, et al., The betaine content of New Zealand foods and estimated intake in the New Zealand diet, J. Food Compos. Anal. 18 (6) (2005) 473–485.
[233] L.I. Mennen, et al., Urinary excretion of 13 dietary flavonoids and phenolic acids in free-living healthy subjects – variability and possible use as biomarkers of polyphenol intake, Eur. J. Clin. Nutr. 62 (4) (2007) 519–525.
[234] M.T. Kelly, et al., Associations between the portion sizes of food groups consumed and measures of adiposity in the British national diet and nutrition survey, Br. J. Nutr. 101 (9) (2009) 1413–1420.
[235] T. Tsuji, et al., Twenty-four-hour urinary water-soluble vitamin levels correlate with their intakes in free-living Japanese schoolchildren, Publ. Health Nutr. 14 (2) (2010) 327–333.
[236] T. Tsuji, et al., Twenty-four-hour urinary water-soluble vitamin levels correlate with their intakes in free-living Japanese university students, Eur. J. Clin. Nutr. 64 (8) (2010) 800–807.
[237] T. Tsuji, et al., Urinary excretion of vitamin B1, B2, B6, niacin, pantothenic acid, folate, and vitamin C correlates with dietary intakes of free-living elderly, female Japanese, Nutr. Res. 30 (3) (2010) 171–178.
[238] K. Shibata, J. Hirose, T. Fukuwatari, Relationship between urinary concentrations of nine water-soluble vitamins and their vitamin intakes in Japanese adult males, Nutr. Metab. Insights 7 (2014) 61–75.
[239] O.K. Chun, S.J. Chung, W.O. Song, Urinary isoflavones and their metabolites validate the dietary isoflavone intakes in US adults, J. Am. Diet Assoc. 109 (2) (2009) 245–254.
[240] M.S. Wolff, et al., Pilot study of urinary biomarkers of phytoestrogens, phthalates, and phenols in girls, Environ. Health Perspect. 115 (1) (2007) 116–121.

[241] M. Goni-Urriza, et al., Impact of an urban effluent on antibiotic resistance of riverine Enterobacteriaceae and Aeromonas spp, Appl. Environ. Microbiol. 66 (1) (2000) 125–132.
[242] A. Costan-Longares, et al., Occurrence and distribution of culturable enteroviruses in wastewater and surface waters of north-eastern Spain, J. Appl. Microbiol. 105 (6) (2008) 1945–1955.
[243] K.E. Shannon, et al., Application of real-time quantitative PCR for the detection of selected bacterial pathogens during municipal wastewater treatment, Sci. Total Environ. 382 (1) (2007) 121–129.
[244] M. Dolejska, et al., CTX-M-15-producing Escherichia coli clone B2-O25b-ST131 and Klebsiella spp. isolates in municipal wastewater treatment plant effluents, J. Antimicrob. Chemother. 66 (12) (2011) 2784–2790.
[245] S.L. McLellan, et al., Diversity and population structure of sewage-derived microorganisms in wastewater treatment plant influent, Environ. Microbiol. 12 (2) (2010) 378–392.
[246] AGWR, Australian Guidelines for Water REcycling: Managing Health and Environmental Risks (Phase 1), Natural Resource Ministerial Management Council, Environment Protection and Heritage Council and Australian Health Ministers, 2006.
[247] N. Montazeri, et al., Pathogenic enteric viruses and microbial indicators during secondary treatment of municipal wastewater, Appl. Environ. Microbiol. 81 (18) (2015) 6436–6445.
[248] J. Rodriguez-Manzano, et al., Analysis of the evolution in the circulation of HAV and HEV in Eastern Spain by testing urban sewage samples, J. Water Health 8 (2) (2010) 346–354.
[249] S.M. Bartsch, et al., Global economic burden of norovirus gastroenteritis, PLoS One 11 (4) (2016) e0151219.
[250] R.L. Atmar, M.K. Estes, Norovirus vaccine development: next steps, Expet Rev. Vaccine 11 (9) (2012) 1023–1025.
[251] J.H. Lun, et al., Emerging recombinant noroviruses identified by clinical and waste water screening, Emerg. Microb. Infect. 7 (1) (2018) 50.
[252] J.M. Fioretti, et al., Surveillance of noroviruses in Rio De Janeiro, Brazil: occurrence of new GIV genotype in clinical and wastewater samples, Food Environ. Virol. 10 (1) (2018) 1–6.
[253] R.J.D. Tebbens, et al., Insights from a systematic search for information on designs, costs, and effectiveness of poliovirus environmental surveillance systems, Food Environ. Virol. 9 (4) (2017) 361–382.
[254] S.C. Davies, Annual Report of the Chief Medical Officer, Volume Two, 2011, Infections and the Rise of Antimicrobial Resistance, 2013.
[255] PCAST, USA Report to the President on Combating Antibiotic Resistance, 2014.
[256] WHO, Global Action Plan on Antimicrobial Resistance, 2015.
[257] E. O'Neill, H. Humphreys, Surveillance of hospital water and primary prevention of nosocomial legionellosis: what is the evidence? J. Hosp. Infect. 59 (4) (2005) 273–279.
[258] J. O'Neill, Tackling Drug-resistant Infections Globally: Final Report and Recommendations in Review on Antimicrobial Resistance, 2016, 2016. UK.
[259] A.J. Alanis, Resistance to antibiotics: Are we in the post-antibiotic era? Arch. Med. Res. 36 (6) (2005) 697–705.
[260] J. Davies, Inactivation of antibiotics and the dissemination of resistance genes, Science 264 (5157) (1994) 375–382.
[261] P.M. Bennett, Integrons and gene cassettes: a genetic construction kit for bacteria, J. Antimicrob. Chemother. 43 (1) (1999) 1–4.
[262] H.K. Allen, et al., Call of the wild: antibiotic resistance genes in natural environments, Nat. Rev. Microbiol. 8 (4) (2010) 251–259.
[263] V.M. D'Costa, et al., Antibiotic resistance is ancient, Nature 477 (7365) (2011) 457–461.
[264] A. Moura, et al., Wastewater bacterial communities bring together broad-host range plasmids, integrons and a wide diversity of uncharacterized gene cassettes, Res. Microbiol. 161 (1) (2010) 58–66.
[265] R. Szczepanowski, et al., Detection of 140 clinically relevant antibiotic-resistance genes in the plasmid metagenome of wastewater treatment plant bacteria showing reduced susceptibility to selected antibiotics, Microbiology-Sgm 155 (2009) 2306–2319.
[266] A.R. Varela, C.M. Manaia, Human health implications of clinically relevant bacteria in wastewater habitats, Environ. Sci. Pollut. Control Ser. 20 (6) (2013) 3550–3569.
[267] T.U. Berendonk, et al., Tackling antibiotic resistance: the environmental framework, Nat. Rev. Microbiol. 13 (5) (2015) 310–317.
[268] L. Rizzo, et al., Urban wastewater treatment plants as hotspots for antibiotic resistant bacteria and genes spread into the environment: a review, Sci. Total Environ. 447 (2013) 345–360.
[269] J.L. Martinez, T.M. Coque, F. Baquero, What is a resistance gene? Ranking risk in resistomes, Nat. Rev. Microbiol. 13 (2) (2015) 116–123.
[270] M. Laht, et al., Abundances of tetracycline, sulphonamide and beta-lactam antibiotic resistance Genes in conventional Wastewater Treatment Plants (WWTPs) with different waste load, PLoS One 9 (8) (2014).
[271] M.C. Roberts, Update on acquired tetracycline resistance genes, FEMS Microbiol. Lett. 245 (2) (2005) 195–203.
[272] P. Antunes, et al., Dissemination of sulfonamide resistance genes (sul1, sul2, and sul3) in Portuguese Salmonella enterica strains and relation with integrons, Antimicrob. Agents Chemother. 49 (2) (2005) 836–839.
[273] S. Rodriguez-Mozaz, et al., Occurrence of antibiotics and antibiotic resistance genes in hospital and urban wastewaters and their impact on the receiving river, Water Res. 69 (2015) 234–242.
[274] Y.L. Zhang, et al., Wastewater treatment contributes to selective increase of antibiotic resistance among Acinetobacter spp, Sci. Total Environ. 407 (12) (2009) 3702–3706.
[275] M. Munir, K. Wong, I. Xagoraraki, Release of antibiotic resistant bacteria and genes in the effluent and biosolids of five wastewater utilities in Michigan, Water Res. 45 (2) (2011) 681–693.
[276] P. Gao, M. Munir, I. Xagoraraki, Correlation of tetracycline and sulfonamide antibiotics with corresponding resistance genes and resistant bacteria in a conventional municipal wastewater treatment plant, Sci. Total Environ. 421–422 (2012) 173–183.
[277] N. Al-Jassim, et al., Removal of bacterial contaminants and antibiotic resistance genes by conventional wastewater treatment processes in Saudi Arabia: is the treated wastewater safe to reuse for agricultural irrigation? Water Res. 73 (2015) 277–290.
[278] J.N. Li, et al., Occurrence and removal of antibiotics and the corresponding resistance genes in wastewater treatment plants: effluents' influence to downstream water environment, Environ. Sci. Pollut. Res. 23 (7) (2016) 6826–6835.
[279] S. Caucci, et al., Seasonality of antibiotic prescriptions for outpatients and resistance genes in sewers and wastewater treatment plant outflow, FEMS Microbiol. Ecol. 92 (5) (2016).
[280] N. Makowska, R. Koczura, J. Mokracka, Class 1 integrase, sulfonamide and tetracycline resistance genes in wastewater treatment plant and surface water, Chemosphere 144 (2016) 1665–1673.
[281] Q.X. Wen, et al., Monitoring and evaluation of antibiotic resistance genes in four municipal wastewater treatment plants in Harbin, Northeast China, Environ. Pollut. 212 (2016) 34–40.
[282] A.C. Cheng, et al., Control of fluoroquinolone resistance through successful regulation, Australia, Emerg. Infect. Dis. 18 (9) (2012) 1453–1460.
[283] S. Schwarz, A.P. Johnson, Transferable resistance to colistin: a new but old threat, J. Antimicrob. Chemother. 71 (8) (2016) 2066–2070.
[284] A. Karkman, et al., High-throughput quantification of antibiotic resistance genes from an urban wastewater treatment plant, FEMS Microbiol. Ecol. 92 (3) (2016).
[285] K.V. Thomas, et al., Comparing illicit drug use in 19 European cities through sewage analysis, Sci. Total Environ. 432 (2012) 432–439.
[286] H. Félix, et al., Mass spectrometric strategies for the investigation of biomarkers of illicit drug use in wastewater, Mass Spectrom. Rev. 37 (3) (2018) 258–280.
[287] A.L. van Nuijs, et al., Multi-year interlaboratory exercises for the analysis of illicit drugs and metabolites in wastewater: development of a quality control system, Trac. Trends Anal. Chem. 103 (2018) 34–43.