Mining Population Exposure and Community Health via Wastewater-Based Epidemiology

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Abstract

An individual's excreta contains chemical information that reflects the chemicals one has consumed or been exposed to. Wastewater-based epidemiology (WBE) is the discipline concerned with mining the chemical information from municipal wastewater. WBE, also known as wastewater epidemiology, sewer epidemiology, urban water fingerprinting or sewage chemical information monitoring (SCIM), has been applied in populations around the globe to mainly measure chemical consumption and exposure patterns. In particular, WBE studies have added to our knowledge of illicit and licit drug consumption patterns, and shows increasing potential as a tool for measuring aspects of public health and socioeconomics. This chapter presents readers with an overview of key methodologies, advances and perspectives in adapting WBE as a tool for better understanding relationships between biochemical consumption and exposure behaviour with public health and socioeconomics.

1 Wastewater-Based Epidemiology

1.1 Brief History and Overview

Anthropogenic chemicals, such as pharmaceuticals and drugs, have been quantified in aquatic environments for a few decades, and wastewater treatment plants (WWTPs) were identified as sources of discharge of anthropogenic chemicals into the environment. These wastewater analysis studies viewed anthropogenic chemicals as potential environmental contaminants that had to be managed by increasing their removal in water treatment processes and limit their release into the environment. However, the mid-2000s saw a number of wastewater analysis studies which (i) normalised chemical concentrations in wastewater influent by the size of the population served by the WWTP, and (ii) incorporated excretion factors to back-calculate the consumption of a parent chemical from its metabolite. This revealed the per capita load or consumption for said chemical within the catchment. This ushered in the era of wastewater-based epidemiology (WBE), where per capita use or excretion of a chemical could be deduced to inform population chemical consumption or exposure behaviour.

1.2 How Wastewater-Based Epidemiology Works

The modern WBE community has converged upon methodological consensuses to foster accurate and reproducible quantification of chemicals. Wastewater entering the WWTP is often sampled as high frequency flow- (preferred) or time-proportionally composited samples over a 24-hour period (Ort et al. 2010). The use of autosamplers allows automated collection and refrigeration of multiple consecutive composite samples, and allows different compositing periods depending on the research or monitoring aims. A temporal analysis can be achieved by analysing samples from different time points. This can be useful in measuring the effect of interventions, special events or the passing of time on biomarker loads. Similarly, normalised results from different communities can be compared to study spatial differences. Once sampled, wastewater is usually preserved with additives such as hydrochloric acid or sodium metabisulphite to minimise chemical transformation prior to freezing if there is a delay between sampling and analysis (Chen et al. 2012). Depending on the concentration of the analyte(s) of interest in the sample and the analytical detection limit, samples may be concentrated using techniques such as liquid–liquid or solid-phase extraction (SPE) (Kasprzyk-Hordern et al. 2008). WBE typically relies on liquid chromatography coupled to

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G. B. Jiang and X. D. Li (eds.), *A New Paradigm for Environmental Chemistry and Toxicology*, https://doi.org/10.1007/978-981-13-9447-8_8

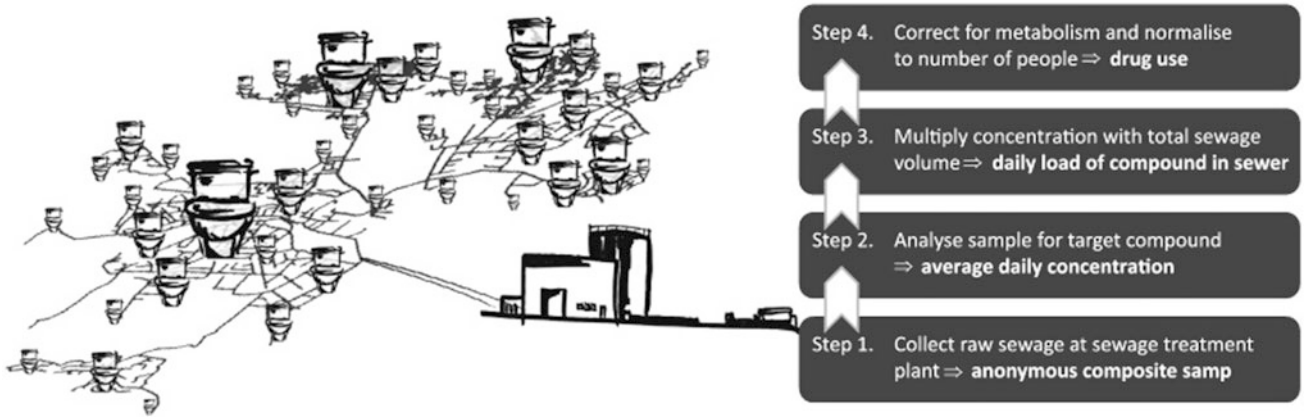

Fig. 1 Key steps in wastewater-based epidemiology. Reprinted from Science of the Total Environment, vol. 432, Thomas et al., Comparing illicit drug use in 19 European cities through sewage analysis, 432–439, Copyright (2012), with permission from Elsevier

tandem mass spectrometry (LC-MS/MS) for chemical analysis. While non-target high-resolution mass spectrometry (HRMS) is being increasingly embraced by WBE studies, triple quadrupole mass spectrometers remain popular as they provide sensitive targeted quantification. Once quantified, concentrations of a chemical of interest can be converted into daily load by multiplying by the flow of wastewater during the sampling period (Fig. 1). Dividing this by the number of people served by the WWTP results in load per capita per day. With information on the proportion of a drug excreted after ingestion, some biomarkers (e.g. drugs) can be expressed in terms of mass or number of doses ingested per capita per day (Rousis et al. 2017; O'Brien et al. 2015).

Chemical consumption or exposure may be determined by measuring the parent chemical compound or a metabolite. It is preferable to measure a human-specific metabolite as metabolism/transformation of chemicals within the sewer may occur, and in some cases agricultural wastewater is connected to the domestic wastewater network. In many cases, however, metabolites are often excreted in lower quantities than the parent compound, hindering quantification. Parent biomarkers can be considered indicators of consumption in scenarios where entry into the sewer by routes other than human excretion (e.g. dumping, trade waste input) can be ruled out or deemed negligible. The stability of a chemical is also an important consideration. Sewer systems contain a considerable diversity of microbial life which can transform a chemical between the time it enters the sewer system and the time it is sampled and preserved (McCall et al. 2016). There is a growing awareness of stability as a potential confounding factor. Stability studies using real sewers, experimental sewers and laboratory-scale sewer reactors have been used to document the extent of potential in sewer transformation, particularly for biomarkers frequently used in the WBE repertoire (Gao et al. 2019; Li et al. 2018).

1.3 Advantages and disadvantages

WBE has a number of characteristics that make it well suited as an alternative or complementary tool for measuring chemical consumption or exposure behaviour. High population coverage is one of the advantages of WBE. In developed countries, most households are connected to a sewer line, making it possible to obtain wastewater samples representing entire populations. This encourages spatial comparisons between different populations served by different WWTPs at a relatively small cost compared to traditional survey methods. Indeed, WBE studies comparing chemical consumption and exposure measures involving tens of different communities or countries are becoming increasingly common (Banta-Green 2009; Du et al. 2017; Gao et al. 2016; Ort et al. 2014; O'Brien 2017; SCORE 2018). Traditional survey methods may suffer from low response or compliance rates, particularly when addressing potentially sensitive information such as illicit behaviour, or involving lengthy study periods. This is aptly illustrated in a study in Lier, Belgium, where licit and illicit drug use was measured using an online survey and WBE methods in parallel (van Wel et al. 2016). While the response rate to the survey was 1% over the 12-week study period, the WBE study revealed detailed information on drug consumption patterns, such as differences in use between weekends and week-to-week variation. Indeed, many WBE studies have revealed the nature of yearly (van Nuijs et al. 2018; Lai et al. 2015, 2016a, b; Mackie et al. 2019; Bade et al. 2018), inter-week (e.g. weekdays vs weekends) (Lai et al. 2015, 2016; van Nuijs et al. 2009; Lai et al. 2013a; Salvatore et al. 2015; Kankaanpaa et al. 2014; Lai et al. 2016; Tscharke et al. 2016; van Nuijs et al. 2011; Boogaerts et al. 2016; Lai et al. 2017) and even within-day variations (Lai et al. 2013b) in drug consumption. In Australia, the National Wastewater Drug Monitoring Program publishes a quarterly WBE study, and the percentage of the Australian population covered in

each edition has ranged between 51 and 61%. Crucially, self-reporting bias is not a concern for WBE. Surveys and studies with self-reporting components are prone to bias from self-presentation concerns, and the magnitude of this bias increases with the sensitivity of the question asked (Krumpal 2013). This makes WBE a useful tool to measure consumption or exposure patterns which individuals may be oblivious to, such as consumption of artificial sweeteners (Subedi and Kannan 2014), exposure to flame retardants (O'Brien et al. 2015) or excretion of metabolites of illness (Ryu et al. 2015).

Naturally, there are a number of limitations and considerations for implementing or interpreting WBE studies. Wastewater is a form of pooled urine, and does not contain information about the distribution of a biomarker excreted by individuals. In other words, excretion of a biomarker in high quantities by a minority and in low quantities by a majority may give the same result in a WBE study. Consequently, strictly speaking, WBE studies report the 'load' or 'burden' of a biomarker in a population. These figures may be converted to prevalence, dose or frequency when assumptions for this conversion are acceptable, or for harmonisation with other measures of chemical consumption. Similarly, WBE is inherently unable to identify specific individuals in a catchment who contribute to loads of a biomarker. Consequently, WBE measures 'loads' of potentially sensitive biomarkers such as biomarkers of illicit drug use or disease. The positive side to this limitation is that anonymity is preserved (Hall et al. 2012).

A key distinguishing feature separating WBE from simple wastewater analysis lies in how WBE reports chemical loads or exposures on a per capita (i.e. population normalised) basis. Although this allows comparison with other sources of data (e.g. surveys) which report chemical consumption in units such as doses per person, it relies on accurate counts of the population served by a WWTP during the sampling period. There are a number of ways to obtain population data. Confident WWTP catchment population counts can be obtained by using census-derived population figures (O'Brien et al. 2019). Where this is unavailable, population may be ascertained using proxies of population, such as the number of mobile phones in a catchment (Thomas et al. 2017). Alternatively, chemicals excreted at approximately uniform rates by individuals can be measured as proxies of population size. Examples of such population biomarkers include ammonium, creatinine, 5-hydroxyoindole acetic acid or even mitochondrial DNA (Chen et al. 2014; Been et al. 2014; Yang et al. 2015). Loads of the former two can be affected by factors not related to population size such as industrial discharges, degradation of organic matter in the sewer or consumption of bodybuilding supplements. Hydrochemical parameters of wastewater, such as chemical oxygen demand or phosphorus, make poor population proxies for similar reasons (Rico et al. 2017). Chemicals consumed homogenously among different populations may also be used as population biomarkers. A notable example is acesulfame, whose loads correlated extremely well with population size ($R^2 = 0.995$) in Australian wastewater samples (O'Brien et al. 2014). Other biomarkers such as caffeine have been suggested for Valencia in Spain (Rico et al. 2017). However, anthropogenic population biomarkers should be calibrated regularly as they may be prone to changes over time, and consumption rates may differ between different cultures (Gao et al. 2016). Interpreting WBE results for their implications on population behaviour and health effects relies on pre-existing information regarding the absorption, distribution, metabolism and excretion (ADME) studies of a biomarker. This is paramount, particularly for quantitative interpretation where results hinge upon excretion factors. Ideally, excretion factors should be calculated using a large number of subjects whose demographics are reasonably representative of the wastewater catchment being studied.

To date, the focus of WBE has been largely limited to small molecule biomarkers which are water-soluble and are excreted primarily through urine. There are more ADME studies on (hydrophilic) chemicals excreted primarily through urine than hydrophobic, faecally excreted chemicals. Although some WBE studies feature methods for sampling biomarkers bound to solid particulate matter (SPM), such studies have been primarily used to determine the extent to which urine-excreted, mildly hydrophobic chemicals bind to SPM (Baker et al. 2012; Asimakopoulos et al. 2017).

Using techniques such as these, WBE has been successfully applied in measuring per capita consumption of illicit and licit recreational drugs, pharmaceuticals and personal care products, industrial chemicals and markers of stress. In the following sections, we briefly summarise the measurement of these different classes of chemicals from wastewater, with a focus on how WBE has contributed to public health and socioeconomic trend analysis.

2 Wastewater-Based Epidemiology for Public Health

2.1 Useful Wastewater-Based Epidemiology Biomarkers

2.1.1 Recreational Drugs

Illicit and licit recreational drug use is an important aspect of public health, as consumption of certain recreational drugs is associated with undesirable consequences at an individual and societal level. Since its inception, WBE has played an important role in monitoring recreational drug use in various communities and understanding temporal and spatial variations in their use. Cocaine was among the first WBE

biomarkers to be implemented. It can be measured as the parent metabolite or as its metabolite, benzoylecgonine, which is more stable than cocaine in wastewater (Chen et al. 2012). Co-consumption with alcohol can decrease urinary excretion of benzoylecgonine, but this can be accounted for by measuring cocaethylene, a biomarker of co-consumption of cocaine and ethanol (Harris et al. 2003). Alcohol consumption can be measured through ethyl sulphate and ethyl glucuronide, both metabolites of ethanol (Reid et al. 2011). In addition to cocaine, methamphetamine and MDMA (3,4-methylenedioxymethamphetamine) were the first biomarkers to be used in WBE (Zuccato et al. 2008). Methamphetamine metabolises to amphetamine, which is a drug in its own right. Consequently, methamphetamine is usually measured as the parent drug. Similarly, MDMA is generally measured as the parent drug as it also metabolises to another drug, MDA (3,4-methylenedioxyamphetamine) (Khan and Nicell 2011). Heroin consumption results in excretion of its major metabolite, morphine, as well as a minor metabolite, 6-monoacetylmorphine (MAM). As morphine and codeine consumption also results in morphine excretion, monitoring heroin consumption through morphine is problematic unless accurate morphine and codeine prescription or consumption data is available (Boleda et al. 2009). Although MAM excretion is unique to heroin consumption, care must be taken when interpreting its loads as it has low stability in sewer conditions (McCall et al. 2016).

Similar challenges are encountered when measuring cannabis consumption through its primary metabolites hydroxy-(THC-OH) or carboxy-tetrahydrocannabinol (THC-COOH). Owing to the hydrophobic nature of these metabolites, care must be taken to account for its sorption onto SPM in wastewater (Choi et al. 2018).

WBE has also been used to measure licit drugs which are abused illicitly, such as the synthetic opioid methadone (Baker and Kasprzyk-Hordern 2011) and ketamine (Castiglioni et al. 2015). New psychoactive substances (NPS), also known as 'designer drugs', have also been measured. Although such efforts rely on existing knowledge of emerging NPS though sources such as police raids (Castiglioni 2016), wastewater is a viable method for measuring population consumption of these difficult-to-regulate drugs (Gracia-Lor et al. 2017; Bade et al. 2017).

WBE has been used extensively to measure tobacco consumption. Nicotine, an alkaloid in tobacco, is metabolised to cotinine, and cotinine is metabolised to trans-hydroxycotinine. All the four chemicals, especially hydroxycotinine and cotinine, are used as WBE biomarkers for tobacco consumption. Alternatively, the tobacco alkaloids anabasine and anatabine can be measured. While these are present in wastewater at much lower concentrations, they have the advantage of not being excreted after application of nicotine replacement therapies (Zheng et al. 2019).

Many WBE studies have measured caffeine consumption by measuring caffeine or its metabolites, paraxanthine and 1,7-dimethyluric acid. Other metabolites of caffeine such as 1-methylxanthine and 7-methylxanthine have been proposed, but lack stability in wastewater (Senta et al. 2015).

2.1.2 Pharmaceuticals and Personal Care Products

A wide variety of pharmaceuticals and personal care products (PPCPs) have been measured through WBE, and per capita loads of a PPCP can be used as proxies of burden of specific illnesses, conditions or lifestyle characteristics in the population. Theoretically, prescription or sales volumes for pharmaceuticals and sales data for personal care products can be used to achieve accurate relationships between air pollution and pharmaceuticals. In the case of pharmaceuticals, this practise forms the basis of pharmacoepidemiology, a field in its own right. Unfortunately, prescription or sales data in most countries are not easily accessible, and may only cover small subsets of a population. Sales figures are even more difficult to arrive at for pharmaceuticals available over-the-counter. In this respect, WBE is a useful method of monitoring population PPCP use, as it can distinguish the day at which a product is used. Hundreds of different PPCPs have been measured in WBE to date (Choi et al. 2018). As with many other WBE biomarkers, properties that make PPCPs more amenable to WBE include: (i) excretion primarily through urine, (ii) a concentration in wastewater within the quantification range of the analytical method, (iii) well-established excretion factor and (iv) stability in sewer systems. It should be noted that availability of individual PPCPs may differ from jurisdiction to jurisdiction. Consequently, relationships found between a pharmaceutical measured with WBE and public health or socioeconomic phenomena may be affected by availability.

A subset of personal care products is considered endocrine-disrupting chemicals (EDCs) due to their potential to disrupt endocrine (hormonal) systems. Personal care products (e.g. cosmetics), food (e.g. bisphenols) and interaction with the anthropogenic environment (e.g. pesticides) are important routes of EDC ingestion. EDC exposure is typically measured using biomonitoring studies of urine or serum, which makes it costly to conduct studies on a large number of people due to the sheer number of individuals that must be sampled (Archer et al. 2017). Various WBE studies have measured EDCs, and WBE may be a useful alternative or complementary method for assessing exposure to EDCs (Lopardo et al. 2018).

2.1.3 Biomarkers of Industrial Chemicals

Exposure to industrial chemicals of concern has traditionally been measured using human biomonitoring samples on matrices such as serum or urine. As with the EDCs, WBE offers an effective alternative monitoring method. A number of pesticides, organophosphate esters, surfactants and similar chemicals have been measured using WBE (Rousis et al. 2017; O'Brien et al. 2015; Asimakopoulos et al. 2017; Gracia-Lor et al. 2012; Been et al. 2017). Many of these chemicals are poorly metabolised, and the ingested chemicals are often excreted unchanged. Therefore, it is difficult to distinguish between exposure, ingestion and input from sources other than human excreta. Although WBE cannot determine the magnitude of exposure to industrial chemicals in individuals, it nevertheless provides an effective method for monitoring, comparing and assessing population exposure.

2.1.4 Endogenous Markers of Stress

Prostaglandin F2α

8-iso prostaglandin F2α (8-iso-$PGF_{2\alpha}$) is a urinary marker of oxidative stress, and one of two endogenous (as opposed to exogenous) biomarkers of stress has been published in WBE studies. Formed from the oxidation of arachidonic acid, urinary 8-iso-$PGF_{2\alpha}$ is used as a measure of systemic lipid oxidative stress in clinical and metabolomics studies. In addition, the factors influencing its excretion, including various diseases, BMI, age, smoking behaviour, sex and even ethnicity have been thoroughly documented. A conceptual paper by Daughton (2012) discussed the theoretical challenges and potential possibilities in using 8-iso-$PGF_{2\alpha}$ (and other related biomarkers) in WBE. In 2015, Ryu and colleagues published a method for quantifying 8-iso-PGF2a for WBE. A major hurdle in measuring 8-iso-$PGF_{2\alpha}$ in wastewater was its relatively low excretion loads (500–5000 ng/person/day). Owing to the low concentration of 8-iso-$PGF_{2\alpha}$ in wastewater and interfering matrix effects in samples concentrated using traditional SPE methods, samples had to be concentrated 1000-fold using an immunoaffinity sorbent. While relatively time and resource-intensive, it is currently the only published method for measuring 8-iso-$PGF_{2\alpha}$ from wastewater. In 2016, Ryu and colleagues used this method to show that loads of 8-iso-$PGF_{2\alpha}$ in wastewater from 11 European cities correlated with the tobacco biomarker hydroxycotinine and not with the alcohol consumption biomarker ethyl sulphate. This study highlighted tobacco consumption as a major driver of lipid oxidative stress at a population level. The same study also observed that while there was no short-term temporal variation, baseline 8-iso-$PGF_{2\alpha}$ levels differed considerably from city to city. This implies that direct spatial comparison of 8-iso-$PGF_{2\alpha}$ levels in different cities may not be meaningful until more is understood about the causes of differences in loads between cities. Nevertheless, the ability to measure a population's oxidative stress immensely broadens avenues where WBE could be utilised to understand the aspects of public health. Long-term temporal analyses could be used to see how population oxidative stress burden changes over time in response to public health interventions such as changes to medical care or smoking policy/taxes.

Methyl Imidazole Acetic Acid

A WBE study of wastewater from seven different Australian catchments found that loads of the antihistamines, fexofenadine and cetirizine, were significantly correlated to loads of 1,4-methylimidazole acetic acid (MIAA), an endogenous marker of histamine turnover (Choi et al. 2018). This study was also unique in that it presented an endogenous marker of illness that could be quantified using standard analytical methods without any additional sample preparation steps as with 8-iso-$PGF_{2\alpha}$. The study also reinforces the notion that antihistamines, as well as MIAA, may be suitable indicators of allergic histamine burden.

2.1.5 Potential Future Biomarkers

Food and Diet Biomarkers

Information about an individuals' short-term food consumption and dietary status can be gained by urinalysis. Several biomarkers of food and diet have been proposed for application in WBE, including biomarkers of consumption of vitamins, wholegrains, fruits and meats (Choi et al. 2018; Thomas and Reid 2011), although no research has been published in this niche. Unlike traditional WBE markers, interpretation of food and diet markers in a WBE study will have several important considerations unique to food and diet biomarkers. Most of the proposed food and diet biomarkers are excreted in the urine unmetabolised. We can therefore expect the biomarkers to enter the sewer system by routes other than human excreta, such as through food processing, food scraps in the home or from industrial waste (e.g. restaurants, food processing factories). For example, wastewater generated by industrial sources contained levels of the soy consumption marker genistein at concentrations up to two orders of magnitude higher than concentrations in WWTP influent (Lundgren and Novak 2009). Quantitative comparisons of food markers will be difficult as levels of the parent food biomarker in food products will vary considerably depending on the cultivar of food components, seasonality, freshness of the food, preparation methods and other similar factors (Gibbons et al. 2017). In short, more research is required to assess the suitability of food and diet biomarkers in wastewater.

Proteins and Peptides

Urine contains a subset of proteins and peptides (henceforth proteins) found in serum, which reflects pathophysiological conditions experienced by an individual. Proteins measured in urine, many of which are validated for clinical settings, often reflect specific pathophysiological conditions such as glomerular diseases, prostate and bladder cancers, diabetes or coronary artery disease (Albalat et al. 2011; Hortin and Sviridov 2007). The nature of the conditions reported by urinary protein biomarkers therefore have little direct overlap with small molecules measured in urine, which are often byproducts of metabolism or metabolites reflecting food, drug or environmental contaminant intake (Bouatra et al. 2013). Accordingly, a number of proteins have been suggested as biomarkers for WBE, such as monocyte chemoattractant protein-1, a marker of renal disease, and gelsolin, a marker of cellular injury (Daughton 2018). While the prospect of measuring proteins in wastewater is an attractive one, a method capable of measuring specific proteins in influent or effluent wastewater is not available in the current literature. Methodological advances are required to access this as of yet untouched source of public health information from wastewater.

Biologicals

Wastewater contains an abundance of microbial life, which could be studied to learn more about the health of the population contributing to the wastewater. A small proportion of the taxonomic groups of the wastewater microbiome reflects the microbiome of the population contributing to wastewater (McLellan et al. 2010). In fact, in a study of wastewater influent from 71 US cities, 16S rRNA (ribosomal RNA) sequencing results were able to estimate the obesity of a population with 81–89% accuracy (Newton et al. 2015). The health surveillance potential of wastewater extends also to viruses. Viruses of modern public health concern such as norovirus and poliovirus can be surveilled in wastewater. By comparing clinical and wastewater strains of virus, new strains can be monitored and the epidemiology of a virus is assessed (Tebbens et al. 2017; Lun et al. 2018). Wastewater is also a medium for monitoring a multitude of antibiotic resistance genes, with some antibiotic resistance genes showing seasonal trends or changing in response to measures of antibiotic consumption (Laht et al. 2014; Caucci 2016). However, differences in selecting, measuring and reporting antibiotic resistance genes impede cross-comparisons between studies, and standardised approaches are required for (Laht et al. 2014) comparisons in future WBE efforts.

2.2 Comparison with Other Sources of Data

Comparing WBE studies with parallel data sources is an important step in establishing its relevance as a public health monitoring tool. Several WBE studies of drug consumption have reported their findings in conjunction with epidemiological or survey data (Andrés-Costa et al. 2016). Below, we briefly discuss such studies and the extent to which WBE agrees with the existing methods of epidemiological information.

Roadside drug tests (RDTs) allow law enforcement authorities to measure the prevalence of drug use in a population. Bade and colleagues measured methamphetamine, 3,4-methylenedioxymethamphetamine (MDMA) and cannabis (as tetrahydrocannabinol, THC) in wastewater from four wastewater treatment plants in Adelaide (Bade et al. 2018). Between 2011 and 2016, seven consecutive daily composite wastewater samples were collected every two months. RDTs were carried out as roadblocks on arterial roads within and in the vicinity of the WWTP catchments. Drivers of randomly selected cars were tested. In addition to a presumptive roadside test, oral swabs were collected from each driver for confirmatory laboratory analysis. The number of positive tests per drug per month was provided by the state police authority. The authors found significant Spearman correlations between the per capita load of drug and percentage of positive drugs tests for methamphetamine ($R = 0.772$) and MDMA ($R = 0.768$). Seasonal fluctuations in methamphetamine and MDMA levels matched well, and both data sources attested similar rates of increase and decrease in methamphetamine and MDMA consumption, respectively, over the study period. Agreement between WBE and RDT data for cannabis was robust during the first half of the sampling period, after which WBE and RDT trends diverged. A change in the time of day the RDT was conducted was suggested as an explanation, as THC levels in the oral cavity diminish relatively rapidly following cannabis use. Overall, however, the study shows that methamphetamine, MDMA, and, to a lesser extent, cannabis use measured by WBE reflects risk-taking behaviour (i.e. driving under the influence of drugs) among the population and reinforces the relevance of WBE to public health monitoring.

Opioid use is a problem in many communities. Intervention initiatives such as opioid substitution therapy or syringe distribution can provide quantitative proxies of opioid consumption with which to examine WBE data. In 2015, Been et al. published a WBE study focussing on methadone and heroin use in a catchment of Lausanne (population = 226,000), Switzerland (Been et al. 2015).

Methadone and its metabolite, 2-ethylidene 1,5-dimethyl-3, 3-diphenylpyrrolidine (EDDP), were measured as proxies of methadone consumption. Heroin consumption was determined through its exclusive metabolite 6-monoacetylmorphine (MAM) and non-specific metabolite morphine, which can also be excreted following consumption of morphine and other opioids. Twenty-eight composite wastewater samples collected between October 2013 and July 2014 were analysed by SPE-LC-MS/MS. External measures of methadone and heroin data were based on multiple estimates and sources. Methadone data was obtained in the form of mass of methadone supplied to pharmacies and the number of patients undergoing methadone therapy in Canton Vaud (Lausanne is the biggest city in Canton Vaud, and accounts for a third of its population). Heroin use was calculated using the number of syringes distributed by pharmacies and dedicated facilities within the catchment, and a number of assumptions regarding the dose and syringe use, which added uncertainty into calculations. Methadone consumption measured using WBE (19.1 ± 3.1 (SD) g/day) was similar to estimates from sales and prescription data (15.0 ± 0.5 (SD) g/day), especially considering the poor geographical specificity of the sales data. Interestingly, WBE was also able to illustrate the extent of variation in daily loads of methadone and EDDP, which was as high as one order of magnitude. In contrast, agreement was poor for comparisons with MAM. This was attributable to the low stability of MAM in wastewater samples, and the consumption of opioids other than heroin contributing to morphine loads in wastewater. Use of stable and specific biomarkers is an important consideration for a WBE study and can profoundly affect comparison with other sources of data.

Smoking is the highest risk factor for all-cause Disability Adjusted Life Years (DALYs) in many developed countries, and remains among the top 10 risk factors for developing countries (Reitsma et al. 2017). Accordingly, many governments are vested in reducing and monitoring tobacco consumption, and estimates for smoking behaviour may be freely available for many countries. In a temporal study spanning from 2010 to 2017 involving seven consecutive daily composite samples every two months, Mackie and colleagues measured the tobacco consumption markers nicotine, cotinine and hydroxycotinine using direct injection LC-MS/MS. When compared with national, state and consultancy-derived estimates, WBE estimates of numbers of cigarettes smoked per capita were on average 29% higher than survey and sales data (Mackie et al. 2019). Importantly, the long-term decreasing trend in number of cigarettes smoked as measured by WBE (3% per year) was consistent with survey and sales data (4–5% per year). It must be acknowledged that the results from this study may not have been replicable had the tobacco consumption levels in the specific catchment studied differed considerably to the national estimates of smoking. Comparisons with external sources of data are most meaningful when performed with catchment-specific data.

At least one study aimed to combine WBE with survey data from the WWTP catchment. In an aforementioned study, van Wel and colleagues aimed to address this issue in a WBE study of illicit drug, alcohol and tobacco consumption. During the 12-week study period, residents of the WWTP were asked to fill in an online survey through which they could report their drug use. Correlation between WBE and survey data for the illicit drugs, alcohol and tobacco use was poor, and this was attributed to the paltry (1%) survey response rate (Van Wel et al. 2016a, b).

WBE agreement with the external data sources largely depends on the scale of the external data used, which can range from national scale estimates in the case of the nicotine metabolites, or dispensing volumes in the case of methadone in Lausanne. Overall, however, WBE agrees well with parallel data from external sources. Comparative studies will be more meaningful if well coordinated with external data sources in terms of the population and timeframe for which wastewater was sampled. These same lessons should be carried over into WBE triangulation studies in order to find more accurate and relevant associations.

2.3 Data Sources for Triangulation

New relationships between WBE data and other phenomena can be established by triangulating with other data that is not directly related to the WBE biomarker in question. This approach differs from WBE studies that compare results with other data, whose aim is to show the congruence between consumption or exposure patterns measured by WBE and an equivalent method. The following is a discussion of WBE studies which feature triangulation with the external data sources with the primary purpose of understanding public health phenomena, as opposed to simply comparing wastewater measurements using external sources.

2.3.1 Events

Numerous WBE studies have sampled wastewater generated around and during the time of special events such as music festivals and bodybuilding events, typically for the purpose of identifying recreational drug use. One early example of such a study examined 13 illicit drugs in daily wastewater composite samples from a six-day music festival in 2010, the same music festival again in 2011 and from a nearby urban catchment in 2010. Conveniently, the music festival grounds had their own dedicated WWTP, allowing population size and demographics to be determined through ticket sales. Of the 13 drugs analysed, the study identified MDMA as the

only substance whose per capita consumption by festival participants exceeded consumption by the nearby urban catchment (Lai et al. 2013c). The authors attributed this to the relatively wide age demographic attending the festival compared with other music festivals.

In contrast, most of the other studies find higher illicit drug consumption during special events. This was almost consistently the case in a temporal study by Lai and co-workers, who analysed illicit drugs in daily composite wastewater samples from an urban WWTP catchment in Australia. Samples (n = 311) were mostly collected consecutively between June 2010 and June 2011. De facto catchment population was estimated with a Bayesian model that used the mass loads of eight PPCPs in wastewater influent. This allowed the authors to account for population flux during the sampling period on a per sample (i.e. per day) basis. Having consecutive daily sampling revealed a consistent, repeating weekly pattern in drug consumption, where per capita consumption of cocaine, MDMA and methamphetamine was significantly higher on weekends than weekdays (Lai et al. 2015). Importantly, per capita loads of cocaine more than doubled during New Year's Eve and New Year's Day compared with other equivalent days in each respective month (Fig. 2a). These same events also coincided with a several-fold increase in MDMA consumption (Fig. 2b). In contrast, methamphetamine loads increased only slightly relative to other days. For all three drugs, consumption was higher on weekends than weekdays ($p < 0.0001$). These results provide detailed illustration of the day-to-day variation in illicit drug consumption (i.e. public health burden from drug consumption). In other words, WBE can provide an objective insight into the public health burden that can be expected from different special events.

In a study of two WWTPs in Western Kentucky, USA, Foppe et al. sampled 24-hour, time-composited samples for seven consecutive days around Independence Day and for seven consecutive days during periods without special events (Foppe et al. 2018). For one of the WWTPs, samples

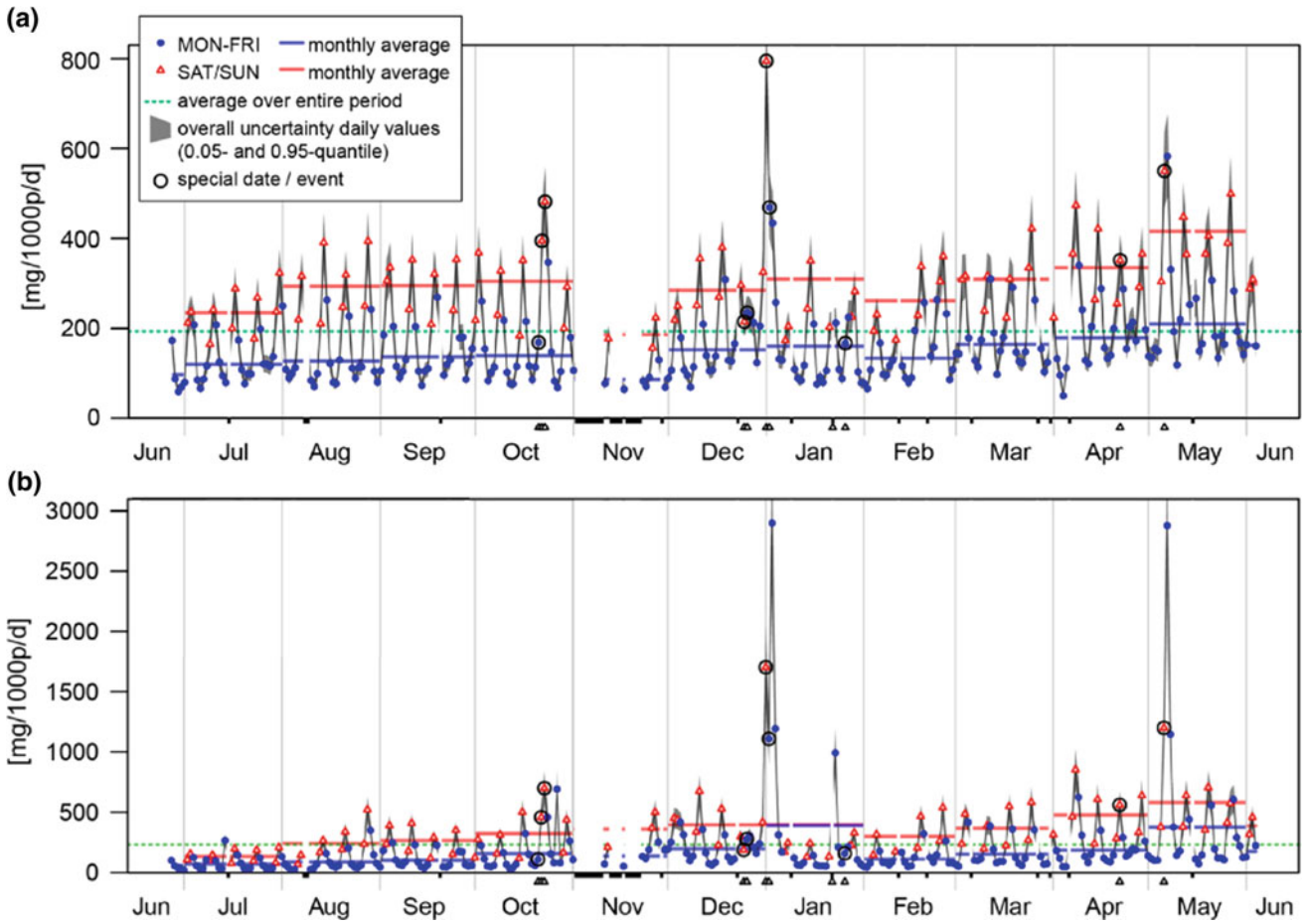

Fig. 2 Estimated population-normalized (mg/day/1000 capita) consumption of **a** cocaine and **b** MDMA using normalisation to de facto population. Black bars and triangles represent missing days and special event days, respectively. Reprinted (adapted) with permission from (Lai et al.). Copyright (2015) American Chemical Society

were also taken around the week of a solar eclipse. Ten recreational drugs were analysed from these samples using SPE followed by LC-MS/MS. For both WWTPs, per capita consumption of amphetamine, methamphetamine, cocaine, morphine and methadone was higher during Independence Day compared to the preceding and following days. Consumption of the same suite of drugs and also tetrahydrocannabinol (THC) was significantly higher in $WWTP_B$ during the time of the eclipse compared to the preceding and following days. Interestingly, when comparing the weekly sum of consumption rates of 'event' weeks to 'normal' weeks, differences ranged from negligible to minor. This suggests that the increased consumption during the special event day was offset by decreased consumption during the event week. WBE studies focussing on events can inform on drug consumption patterns during special events in an objective way that would be difficult to ascertain using traditional survey methods due to the illicit nature of the drugs consumed. Results from studies such as these can inform public health and law enforcement agencies on what to expect during special events, and provide a basis to help organise assistance or interventions.

Special events are ideal scenarios to measure doping substance use. Amateur athletes participating in sporting events are not always tested for performance enhancing drugs (PEDs) and doping substances to the same standard as professional athletes. In a study of three WWTP catchments, each holding a different sporting event, Causanilles and colleagues detected increases in the doping substances ephedrine, norephedrine, methylhexanamine and 2,4-dinitrophenol (a weight loss substance) in the days preceding but not during an amateur bodybuilding event (Causanilles et al. 2018). This was presumably due to athletes avoiding consuming the substances on the day of the event. Similarly, a spike in per capita loads of 2,4-dinitrophenol was registered during a 2-day bodybuilding event attended by over 100 amateur athletes and 8000 visitors. In addition to compromising integrity in sports, doping can lead to detrimental effects on the health of the affected individuals and is a matter of public health concern. The consumption of 2,4-dinitrophenol in both events is of particular concern due to its acute toxicity which has led to several confirmed deaths (Kamour 2015). Doping control was not used in either of the events in this study, which demonstrates WBE can be used to determine the extent of doping during sporting events.

In conclusion, WBE can be used to study chemical consumption and excretion behaviours associated with a special event, whether it may be a festival, cultural event or a public health intervention. Accurate knowledge of the population at the time of the event as well as appropriate sampling practise can strengthen the findings from these events.

2.3.2 Environmental

Temperature

Changes in ambient temperature contribute directly and indirectly to an individual's health, and changes in temperature even lead to mortality (Basu 2009; Gasparrini et al. 2015). Although most individuals can readily sense and respond to ambient temperature, the effects of changes in ambient temperature on a population scale are difficult to document using traditional survey-based methods. A temporal WBE study of a metropolitan Australian catchment spanning 475 days set out to find associations between temperature, humidity and rainfall with loads of a selection of eight PPCPs (Phung et al. 2017). A time-series regression analysis showed that a 1 °C increase in average temperature was associated with a 1.2% decrease (compared to mean) in loads of the antihypertensive atenolol, which reflected a greater incidence of cardiovascular irregularities in cooler times. A 1 °C increase was also associated with a 0.84% decrease in loads of caffeine and 1.9% increase in loads of acesulfame, and this likely reflects reduced consumption of popular caffeine-rich beverages (tea and coffee) and increased consumption of soft drinks in hotter weather. Loads of the non-steroidal anti-inflammatory drug (NSAID) naproxen increased 0.64% with a 1 °C rise, which was surmised to reflect increased incidence and exacerbation of arthritis in hotter, more humid climes. However, contrary results have been reported for other NSAIDs. A wastewater analysis study set in a French catchment measured 25 drugs from daily composite samples between 21 March and 11 June 2016. In this study, the highest weekly loads of the NSAIDs ketoprofen, ibuprofen and diclofenac coincided with the two coldest weeks (5–11 °C) during the sampling period (Thiebault et al. 2019). Higher loads of morphine and codeine also coincided, although to a lesser extent, with the cold snaps, suggesting that colder weather causes increased consumption of NSAIDs and analgesics. The cold snaps also coincided with lower loads of the stimulants (cocaine, benzoylecgonine, cocaethylene, ecstasy and amphetamine), suggesting reduced recreational use of these stimulants in colder weather. Together, these two studies are a good example of how freely and publically available weather measurements can be used to provide insights into how a population responds to changes in the environment. It also highlights the extent to which temperature influences the consumption of specific chemicals, which may be useful for public health management.

Relationship between temperature and chemical exposure has also been examined in a study of 36 WWTPs, which accounted for 48% of the Australian population during two

days in August of 2016. O'Malley and colleagues measured loads of seven organic UV filters as proxies of sunscreen use. Total per capita UV filter load correlated with maximum daily temperature (R^2 = 0.195), latitude (R^2 = 0.203) and daily global solar exposure (R^2 = 0.149) (O'Malley et al. 2019). The focus of this study was on establishing a national baseline for per capita UV filter use to inform environmental studies. Nevertheless, it identifies populations living closer to the equator as having greater exposure to UV filters, which may have endocrine disrupting properties. Future WBE studies triangulating with temperature could be used to determine and perhaps even quantify the role of temperature on public health and chemical exposure burdens.

Pollen

Individuals' response to environmental cues can be detected in wastewater. A WBE study set in Oslo measured PPCPs using passive samplers. The authors found that per capita loads of the antihistamine cetirizine peaked 2–3-fold above baseline levels during the pollen season (Harman et al. 2011). Although cetirizine appeared to be a suitable proxy of pollen in this study, differences in availability of different antihistamines may result in different antihistamines correlating with pollen in different countries. Antihistamines such as cetirizine are available over-the-counter in most jurisdictions, and monitoring sales or prescription data would be poorly suited to understanding population response to pollen. Measuring other biomarkers related to allergic burden, such as other antihistamines (fexofenadine, diphenhydramine) and MIAA and perhaps even pharmaceuticals used for chronic obstructive pulmonary disease (e.g. salbutamol, terbutaline) or anti-inflammatories (e.g. ibuprofen) may be useful in understanding the full extent of pollen on population pharmaceutical consumption behaviour.

Air Pollution

Air pollution exacerbates and predisposes to negative health outcomes such as asthma (Braman 2006). A study in Milan, Italy aimed to document the relationship between air pollution metrics and salbutamol, a short-acting beta2 agonist used to relieve bronchoconstriction, a hallmark symptom of asthma. The authors collected consecutive 24-hour composite wastewater samples from a WWTP serving the majority of Milan for 89 consecutive days. Wastewater samples were concentrated using an SPE method in order to measure salbutamol, which had minimum, median and maximum wastewater concentrations of 2.95, 5.85 and 9.97 ng/L, respectively. Despite this relatively narrow window of salbutamol concentrations, the authors found significant concentrations between daily per capita doses of salbutamol and levels of particulate matter. A log-linear Poisson model found that the correlations between PM2.5 and PM10 levels were slightly higher with 7 or 8 days of lag, suggesting a culminative effect of air pollution on salbutamol consumption by individuals. However, salbutamol is a prescription drug. The delay likely reflects in large part the time taken for the exposure to manifest in individuals and cause them to present to a doctor to obtain a prescription. Although WBE is limited by population-level resolution, it can be used to determine the time at which the population actually consumed the prescribed pharmaceutical. This may make it more useful for finding associations with short-lived events such as onsets of smog or thunderstorm asthma.

2.3.3 Socioeconomic

Urbanicity: Oregon

Previous epidemiological studies have shown that illicit drug use is influenced by the degree of urbanicity of a population. This was affirmed using WBE in a 2009 publication by Banta-Green et al. (Banta-Green 2009). The authors measured benzoylecgonine (cocaine biomarker), methamphetamine and MDMA in wastewater from 96 WWTPs collectively covering 65% of the population of the state of Oregon (United States). Each WWTP provides one 24-hour composite sample. Each WWTP was designated as 'urban', 'large rural city/town' or 'small rural town' based on rural–urban commuting area (RUCA) codes derived from census information. Benzoylecgonine and MDMA loads were highest in urban areas, whereas methamphetamine loads were not significantly different by urbanicity. This study showed that WBE could replicate established epidemiological findings regarding drug use in urban versus rural areas in the United States. This was despite basing drug loads on measurements from one convenience sample per WWTP. Although other WBE studies have also identified urbanicity as a factor for differences in drug consumption patterns (Du et al. 2017; Boogaerts et al. 2016; Castiglioni et al. 2015), linking WBE results to sociodemographic metrics makes findings more immediate and relevant to a transdisciplinary and public health focussed audience.

Additionally, it was the first study to link WBE results to a sociodemographic factor, asserting its relevance in sociodemographic aspects of public health.

Economic: Greece

Financial information may be triangulated with WBE to see how chemical consumption or exposure changes or differs with the financial wellbeing of a population. When drug and pharmaceutical incurs financial cost to the individual, it is inevitable that socioeconomic differences will influence

consumption behaviour. A WBE study in Athens, Greece was able to determine the extent to which the Greek economic downturn and subsequent austerity measures affected illicit drug and PPCP consumption between 2010 and 2014 (Thomaidis et al. 2016). During the study period, Greece saw economic hardships which manifested as a 20% drop in GDP, a doubling of its unemployment rate and cuts to public health spending including cuts to drug expenditure, and more. The WBE study was conducted as five-sampling campaigns between 2010 and 2014, with each campaign consisting of 7–11 consecutive daily samples. Wastewater samples were concentrated using SPE and 148 substances were quantified by LC-MS/MS. The study found a 14-fold increase in per capita doses of antidepressants and benzodiazepines, which are collectively used to treat depression, anxiety, insomnia and seizures. Doses of antipsychotics increased 35-fold. Pharmaceuticals used to treat stress-induced or stress-exacerbated conditions such as atenolol (hypertension) and gastric ulcer drugs also increased (Fig. 3). Per capita loads of certain illicit drugs, namely ecstasy, methamphetamine and methadone rose 2.2, 4.5 and 6.8-fold, respectively. This reflected the relatively low cost of street methamphetamine and the proliferation of methadone administration clinics, while no such trends could be established for cocaine, heroin, amphetamine and tetrahydrocannabinol (cannabis). There was a decrease in per capita doses of most antibiotics and most NSAIDs which could be explained by cuts in public health spending. Total number of doses of six benzodiazepines and antidepressants rose 10-fold over the sampling period. This study used broad, large-scale measures of socioeconomics and operated under a very unique scenario involving immense changes to the financial environment. Consequently, the degree to which methodological aspects or findings from this study can be replicated in other WBE studies, or the extent to which the findings can inform similar situations is unclear. Nevertheless, the study convincingly shows how dire socioeconomic changes led to increases in biomarkers of mental, physical and social distress.

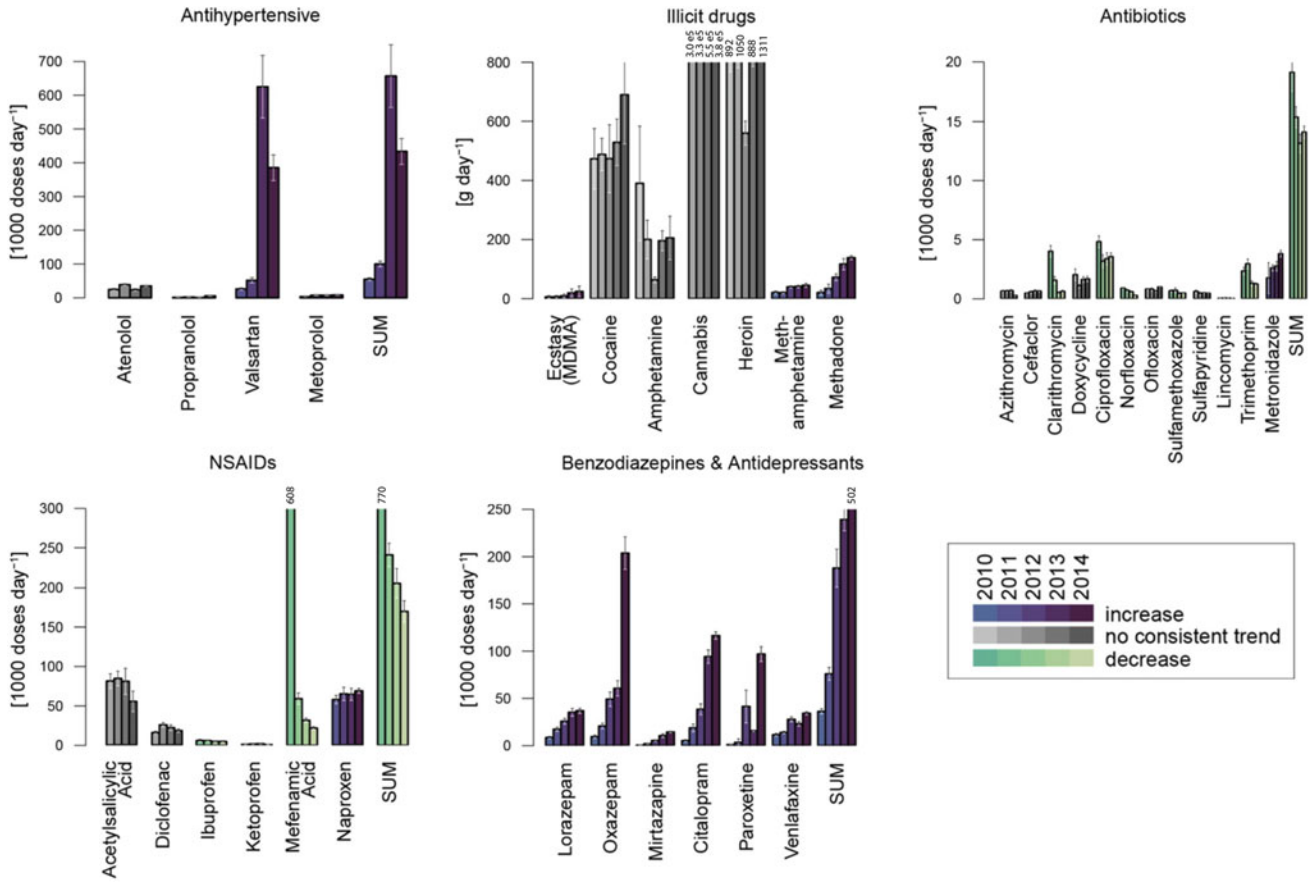

Fig. 3 Estimated use of selected pharmaceuticals (1000 doses per day) and illicit drugs (g per day) over 2010–2014. Error bars represent variability of daily values within each annual sampling campaign Reprinted (adapted) with permission from Thomaidis et al. Copyright (2016) American Chemical Society

Housing Price and Density: Beijing

WBE has also been used to examine the relationship between housing price and PPCP use. A study involving eight WWTP catchments covering most of the population of Beijing, China, was able to establish an association between housing price and per capita loads of PPCPs. In this study, Zhang et al. sampled one 24-hour time composite wastewater sample per WWTP between November 2016 and January 2017. A panel of 17 PPCPs (propranolol, carbamazepine, N,N-dimethyl-meta-toluamide, sulpiride, metoprolol, caffeine, diclofenac, indomethacin, ketoprofen, mefenamic acid, bezafibrate, clofibric acid, gemfibrozil, trimethoprim, nalidixic acid, chloramphenicol and acetaminophen) were measured using SPE-LC-MS/MS. Per capita loads of the panel of PPCPs correlated with catchment average housing price ($R = 0.92$) and population density ($R = 0.93$). Increased consumption of PPCPs among those living in more expensive areas was suggested as an explanation. However, this study had some limitations compared to other WBE triangulation studies. Only one sample was taken per catchment over a wide sampling window, which likely contributed towards unrepresentative sampling (Humphries et al. 2016). Population size was also determined using ammonia loads, a technique known to be better suited for measuring population fluctuations over time rather than between different catchments (Been et al. 2014). While this study revealed that variation in PPCP consumption behaviour can be attributed to socioeconomic measures, it must be noted that more synchronised sampling and more reliable population measurements would likely lead to more realistic findings.

2.3.4 Potential Future Sources of Data for Triangulation

Current best practise WBE methodologies have proven useful for accurately measuring chemicals excreted by individuals. Recent progress in WBE triangulation studies demonstrate that considerable insights into population health can be gained using existing WBE biomarkers. This is not to discount efforts in adopting other biomarkers for use in WBE. Rather, it is to stress that the success of future WBE triangulation studies which inform on public health will be proportional to the quality, novelty and public health relevance of the data used for triangulation. It is therefore imperative to look towards validated, well-curated, current and relevant sources of information for triangulation studies.

Where available, national census data is an attractive source of data for triangulation. Most nations conduct some form of census, which often record sociodemographic information about individuals. In countries such as Australia, census data are well curated, quality assured and validated by a census post enumeration survey (Harding et al. 2017). Following the census, sociodemographic information about residents of specific geographic locations are made available. The sociodemographic characteristics of a WWTP catchment can be determined by mapping geographically grouped census data onto WWTP catchment maps using georeferencing software as further explained elsewhere (O'Brien et al. 2019). In addition to providing accurate population of a WWTP, these approaches can be used to determine the extent to which sociodemographic factors (e.g. occupation, housing characteristics) associate with or influence chemical consumption or exposure patterns. The aforementioned 2009 study by Banta-Green et al. incorporating RUCA into WBE can be considered a pioneering step into this approach, since the RUCA was devised using census-derived data. However, censuses can provide more specific descriptors than urbanicity. Incorporating aspects of the census into WBE studies could prove fruitful for identifying sociodemographic disparities which can be actioned upon by public health or government interests.

In the burgeoning field known as digital epidemiology (Salathé 2018), the internet has proven to be fertile grounds from which to reap information about the health and wellbeing of individuals. Good examples abound. A digital epidemiology study by Bakker et al. found Google search instances of 'chicken pox' in several countries between 2010 and 2016 had good agreement with clinical chicken pox cases (Bakker et al. 2016). Search data could also be used to model future outbreaks and immunisation events manifested as declines in searches. Social media platforms have also been identified as platforms ripe with public health information. Twitter has been identified as a resource from which to monitor prescription medication abuse (Sarker et al. 2016; Chary et al. 2017) and illicit drug use (Kazemi et al. 2017), as well as health outcomes such as pregnancies, heart and birth defects (Klein et al. 2018). Although the software required to process the vast amounts of data often implicated in digital epidemiology studies is easily accessible, meaningful, high-quality datasets but generally not publicly accessible (Salathé 2018). For example, privacy restrictions commonly prevent researchers from dividing the dataset by demographics, which limits the extent to which results can be generalised to different demographic ranges (Kazemi et al. 2017). Any future triangulation with WBE will also need to consider the location (i.e. WWTP catchment) in which data points correspond to. Therefore the WBE community should be mindful of approaching digital WBE with good hypotheses and experimental plans in hand. Nevertheless, identifying similar keywords or activities which associate with chemical consumption or exposure as measurable by WBE could provide a simple way to assess chemical consumption or exposure in near real-time. This is quite appropriate, particularly given that social media platforms often shape or motivate substance use such as alcohol

consumption (Moreno et al. 2009). Alternatively, a combination of digital epidemiology and non-targeted WBE could be used to unveil chemical signatures corresponding to specific internet use patterns in a population scale metabolomics study.

2.4 Future Directions

Moving forward, future WBE studies that explore aspects of public health should employ suitable wastewater sampling practise, sufficient sample sizes for the research question being asked (including minimal gaps in triangulation data) and, wherever possible, robust statistics in order to reduce the uncertainty and ambiguity in the putative relationships found.

As the WBE field develops the main sources of uncertainty, such as flow and population, biomarker stability and biomarker excretion factors will need to be better understood and in some cases reduced if studies are to have sufficient power to reflect small changes in communities. Many of the studies performed to date have generally been pilots employing spatial analysis and we envisage that more longitudinal studies will be performed that will not only investigate changes in the weighted biomarker load over time but investigate those factors that influence biomarker change and use WBE, for example, as an intervention assessment tool. Understanding sampling power will be the key in such studies.

Humans are continually exposed to multiple chemicals from different environmental sources and pathways, both intentionally and unintentionally. The exposome paradigm acknowledges this complexity and seeks methods to measure all environmental exposures and the related biological responses over the life course. It is clear that integrating the exposome paradigm into WBE will improve exposure assessments. Non-target analysis employing HRMS has been established over recent years as one of the key approaches for tackling this complexity. When coupled to smart study design, time-trend screening and case–control type studies, it may not only allow for improved exposure assessments but also allow for novel wastewater biomarker discovery.

Metagenomic analysis in WBE has to date been rarely applied but offers clear opportunities in understanding the genetic material contained in wastewater. Current sampling procedures may need optimisation to achieve this goal and realise the benefits that such an approach may have in future public health assessments. Chemical and biological sensors may also have a role to play in terms of monitoring a multiplex of biomarkers in real time.

In many countries, a wealth of data that can be defined by a sewer catchment area is already available. This enables integration of biomarker data with available socioeconomic, pharmaceutical, biomonitoring, exposure, climatic and other data. One can envisage an approach that would focus on understanding the collective functioning of systems, in terms of their dynamic relationships, feedback loops, interactions and dependencies. In order to facilitate such an ambition, there is an important role for including wastewater in systematic sampling and archiving programs that allow retrospective analysis. In the age of ‘big data’, global collaboration and the sharing of data through open/social platforms may revolutionise the way WBE and associated data are processed in order to achieve significant outcomes. The Sewage analysis COre group Europe (SCORE) Network is already working towards a platform for storing its illicit drug biomarker data from extensive global surveys. Its further development may serve as a springboard to bring together data from other similar surveys, such as the Global Sewage Surveillance Project (focused on antimicrobial resistance) and the Human Health Repository in the USA.

Finally, and by far the most critical aspect to the success of the future direction of WBE is the need to establish national frameworks for WBE to encourage, support and formalise the crucial role that WWTP operators play in supporting this growing and exciting area of interdisciplinary science.

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