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Wastewater-based epidemiology as a surveillance tool to assess human consumption of psychotropic substances: Alcohol, nicotine and caffeine as case studies

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ABSTRACT

Wastewater-based epidemiology (WBE) is used as a surveillance tool to provide data on public health, drug use, exposure to chemicals and lifestyle habits. This approach can be used to monitor the achievement of the United Nations Sustainable Development Goals, such as reducing alcohol consumption and reducing the use of tobacco. In addition, it can be applied to monitor total caffeine consumption and assess levels according to the recommended daily safety limit as suggested by the European Food Safety Authority. The purpose of this review was to summarize and discuss analytical protocols for the determination of alcohol, nicotine, and caffeine WBE biomarkers in wastewater. The WBE requirements for these biomarkers were thoroughly investigated. Furthermore, back-calculated levels of alcohol, nicotine, and caffeine in the population were reviewed. Finally, challenges were identified, and future directions were proposed to fill knowledge gaps and improve the quality of information obtained from the WBE approach.

1. Introduction

Wastewater-based epidemiology (WBE) is a recognised approach to provide data at a community level related to public health, drug use, exposure to chemicals and lifestyle habits [1–5]. It was primarily used to deliver data on illicit drug consumption [6] and its applicability has been expanded to other classes, such as pesticides [7], phytoestrogens [8], new psychoactive substances [9], pharmaceuticals [10] and viruses [11]. WBE is also employed to assess the consumption of psychotropic substances, such as alcohol [12], nicotine [13], and caffeine [14].

Alcohol is one of the most popular psychotropic substances, with almost half of the global population consuming alcohol each year [15]. The percentage of consumers varies across regions from year to year, showing an increase or decrease attributable to several factors (e.g., religion, economic development and alcohol policies and interventions) [15–17]. Alcohol is considered a main contributor to disease burden and its harmful use has resulted in millions of deaths and hundreds of millions of disability-adjusted life years (DALYs) worldwide annually [15]. Therefore, it is important to explore tools that can monitor alcohol consumption and assess the efficacy of interventions (e.g., Non-Communicable Disease Framework and the Sustainable Development Goals for alcohol, goal 3.5).

Tobacco use is considered one of the greatest threats to public health, killing millions of people worldwide every year, including both direct users and non-smokers. In addition, tobacco use is a risk factor for six of the eight leading causes of death in the world and results in millions of DALYs as well. It is estimated that half of the users will die and considering that more than one billion people smoke globally, this highlights the need for preventive programs [18–20]. The World Health Organization (WHO) has put into force the WHO Framework Convention on Tobacco Control (FCTC), and introduced the MPOWER policy package, which is a practical, cost-effective way to help implement

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tobacco reduction provisions [20].

Caffeine is the most consumed psychotropic substance worldwide and has been used since ancient times. It is found in coffee beans, tea leaves, various plants, fruits, foods, pain medicines, beverages, and dietary supplements, among others. Although several positive effects of caffeine on human health have been reported, some side effects have also been stated, especially when caffeine is consumed at high levels [14,21]. Therefore, the European Food Safety Authority (EFSA) proposed a habitual daily intake from all sources that do not raise safety concerns of up to 400 mg per day for the general healthy population [22]. Information on caffeine consumption is commonly obtained from dietary surveys, but these studies are limited by several factors (e.g., consumer ignorance about products containing caffeine and caffeine amount is not always listed on products) and, thus, new approaches are needed to complement the existing data [14].

Monitoring the use of alcohol, nicotine and caffeine in the general population is considered necessary and of great importance. Therefore, this review aimed to investigate the WBE approach as a complementary surveillance tool that can provide spatiotemporal data in a cost-effective and timely manner. The main analytical methodologies, WBE biomarkers and levels of these substances estimated by WBE globally are reviewed and discussed. Finally, future research is suggested to fill current gaps and improve the quality of information obtained by the WBE approach.

2. Literature search

Manuscripts were searched in PubMed and Scopus databases. Search terms for all WBE biomarkers included: wastewater, influent wastewater, wastewater-based epidemiology, and metabolite. Additional terms for each biomarker included separately: alcohol and ethyl sulphate (EtS); nicotine, cotinine, and *trans*-3′-hydroxycotinine; and caffeine, paraxanthine, 1,7-dimethylxanthine, and 1,7-dimethyluric acid. The search started from 2005 (first application to small molecules in WBE) until May 2023. Only research papers were considered excluding reviews, editorials, letters, books, and conference proceedings. In addition, manuscripts in English were only evaluated. All publications were individually assessed, and irrelevant papers were excluded from further evaluation. Although parent compounds were used as search terms, papers studying only these substances were eliminated. Furthermore, studies applying grab sampling were also eliminated. Papers based on sampling small specific groups were excluded. For the section of analytical methods, including the Tables, papers dealing with validated analytical methods are mentioned and only the first publication is cited (papers using an already published method were not considered). For the section related to human consumption, papers that did not estimate consumption and were not easy to estimate from the data were excluded. In addition, when it was difficult to obtain the exact back-calculated consumption because it was not clearly reported (e.g., consumption shown only in the Figures), these papers were also not considered.

3. Wastewater-based epidemiology biomarkers

The selection of a WBE biomarker is considered a complex process as specific requirements must be met. A suitable biomarker must be excreted mainly via urine and be present in wastewater in measurable quantities [1]. Therefore, urinary biomarker concentrations should be at least at the μg/L level to ensure that they will be detectable in influent wastewater after dilution. The typical dilution factor ranges from 100 to 400 times, varying with population size and weather conditions (e.g., rain events) [23]. All selected biomarkers of alcohol, nicotine and caffeine are usually quantified in wastewater at high concentrations showing high detection frequencies in the various sampling campaigns.

Another important requirement is that a biomarker should be unique to human metabolism to ensure that it comes only from human excretion and not from exogenous sources [1]. The parent compounds alcohol, nicotine, and caffeine should be avoided as WBE biomarkers since their presence in wastewater may also originate from direct disposal/dumping into the sewer system. Alcohol could be detected in wastewater after its disposal from industry and alcoholic beverages. Nicotine levels are affected by external sources, such as flushing of cigarette ash and/or cigarettes in toilets [24]. The use of caffeine itself might lead to an overestimation of the total amounts due to the various additional sources (e.g., disposal of coffee that was not drunk or of coffee grounds through the sink drain) [14]. Therefore, these parent compounds are not discussed in the present review, which is focused on the specific metabolites, EtS for alcohol, cotinine and *trans*-3′-hydroxycotinine for nicotine and paraxanthine (1,7-dimethylxanthine) and 1,7-dimethyluric acid for caffeine to assess community-wide consumption. A few WBE studies on alcohol also investigated ethyl glucuronide as a potential biomarker and concluded that this compound was not suitable, since it was found to be unstable in wastewater [12,25,26]. Ethyl sulphate is considered the best biomarker due to its uniqueness and from the fact that unconsumed alcohol is not converted to EtS in wastewater [12]. The nicotine biomarkers cotinine and *trans*-3′-hydroxycotinine are mainly used in WBE and it should be emphasized that the degradation of nicotine in wastewater does not lead to the formation of these metabolites [27]. Several caffeine metabolites were tested (1-methylxanthine, 1-methyluric acid, 7-methylxanthine, etc.), but only paraxanthine and 1,7-dimethyluric acid are considered to be exclusive caffeine metabolites and were therefore used further [14].

A fraction of the metabolite may also be excreted conjugated (e.g., glucuronide conjugates) and should be considered when estimating the consumption. However, it is assumed that conjugated forms of the metabolites are transformed to the free form by the various enzymes found in many strains of faecal bacteria in wastewater [28]. A few studies performed a glucuronide deconjugation step by incubation with β-glucuronidase before the analysis of cotinine and *trans*-3′-hydroxycotinine. It was revealed that both metabolites are deconjugated in wastewater to a different extent, depending on the sewage system. In one study *trans*-3′-hydroxycotinine was completely deconjugated in the wastewater, but enzymatic deconjugation was necessary for cotinine prior the analysis [27]. In another study based on stability experiments in real sewers, it was found that both metabolites underwent de-glucuronidation within the sewer [26]. Therefore, enzymatic deconjugation may be necessary prior the analysis, especially for cotinine.

Pharmacokinetic data on human metabolism are essential to use a reliable correction factor (CF) for back-calculating the consumption [1]. CFs are obtained by considering the rate of urinary excretion of a metabolite and the molecular weight ratio of the parent compound and metabolite. For alcohol, most of the studies used a CF of 3047 considering an excretion of 0.012%. A range of 0.010–0.016% was also used resulting to similar CF (Table 1). However, one study suggested a CF of 4000 taking into account wastewater and sales data from Australia [29]. This CF may be useful for studies performed in Australia but needs to be carefully applied in other countries and sewage systems different from those investigated. In addition, this CF was corrected according to sales data and, thus, deviations due to several factors for alcohol, such as purchase but not consumption, transportation from other countries, use in cooking, illegal and home production, are expected. For nicotine back-calculation, a CF of 1.35 was used when the sum of cotinine and *trans*-3′-hydroxycotinine were considered, corresponding to an average excretion rate of the free and conjugated form of 74% (Table 2). When only one of the biomarkers was used for the back-calculation, the CF was 3.4 and 1.9, when considering an average excretion value of 27% and 44.5% for cotinine and *trans*-3′-hydroxycotinine respectively (Table 2). Several other CFs were used for cotinine, ranging from 0.45 to 2.85, that did not account the conjugated form. For the back-calculation of caffeine, two CFs were proposed, 23.4 for paraxanthine and 14.8 for 1,7-dimethyluric acid (Table 3).

Table 1
Studies investigating alcohol consumption by wastewater-based epidemiology.

Location	Number of WWTPs	Period	Population	CF	Excretion (%)	Consumption (mL alcohol/day/person)	Ref.
Oslo (NO)	1	September 2009 (twenty-five days)	500,000	n.a.	0.010–0.016	12.4–19.8	[12]
Galician city (ES)	1	April 2012 (one week)	100,000	n.a.	0.010–0.016	9.8–23.5	[30]
Barcelona (ES)	1	March 2013 (one week)	1,157,000	n.a.	0.011	18	[38] [a]
Santiago de Compostela (ES)	1	April 2012 (eight days)	136,500	3047	0.012	15.6 ± 5.0	[39] [c]
		March 2013 (eight days)				11.7 ± 6.7	
		March 2014 (one week)				12.9 ± 4.8	
Milan (IT)	1	November–December 2012 (eighteen days)	1,150,000			4.8 ± 1.4	
		March 2013 (two weeks)				6.3 ± 2.3	
		March 2014 (two weeks)				4.4 ± 1.4	
Mytilene (GR)	1	February–March 2015 (one week)	26,000	3047	0.012	2.2–11.2	[40]
Two small villages (island of Lesvos, GR)	1		1250			1.7–7.2	
Antwerp (centre) (BE)	1	March 2013 (one week)	1,600,000 (total)	3047	0.012	24.3	[41] [a, d]
		April 2014 (one week)				17.6	
		March 2015 (nine days)				21.8	
Ninove (BE)	1	March 2013 (one week)				16.7	
		March 2014 (one week)				14.6	
		March 2015 (one week)				12.5	
Antwerp (suburbs) (BE)	1	March 2013 (one week)				17.0	
		April 2014 (one week)				10.7	
		March 2015 (eight days)				13.3	
Lier (BE)	1	September 2014 (seventeen days)				12.4	
		October 2014 (seventeen days)				14.6	
		November 2014 (sixteen days)				14.8	
		December 2014 (eleven days)				14.6	
Geraardsbergen (BE)	1	March 2014 (one week)				11.0	
		March 2015 (eight days)				12.8	
Oostende (BE)	1	April 2015 (eight days)				14.8	
Brussels (BE)	1	March 2015 (eight days)				21.4	
Koksijde (BE)	1	March 2015 (one week)				9.2	
Lier (BE)	1	September–November 2014 (four two-week periods)	30,600	3047	0.012	12	[42] [a]
Oslo (NO)	1	March 2015 (one week)	n.a.	Not used	–	18.9	[43] [c]
Castellon (ES)	1					6.6	
Brussels (BE)	1					21.6	
Bristol (UK)	1					16.2	
Utrecht (NL)	1					10.8	
Milan (IT)	1					6.6	
Zurich (CH)	1					14.7	
Copenhagen (DK)	1					29.7	
Canberra (AU)	1	March 2014 (one week)	338,888	3047	0.012	9.3–22.3	[44] [a]
Toowoomba (AU)	1	March 2014 (one week)	125,000			6.9–14.5	
Montreal (CA)	1	March 2014 (one week)	1,958,257			29.2–44.3	
Granby (CA)	1	March 2014 (one week)	55,255			27.3–59.3	
Lugano (CH)	1	March 2014 (one week)	103,561			4.5–8.4	
Dortmund (DE)	1	March 2014 (one week)	371,788			18.1–34	
Dulmen (DE)	1	March 2014 (one week)	34,495			5.5–40.0	
Dresden (DE)	1	March 2014 (one week)	593,050			15.1–91.7	
Munich (DE)	1	March 2014 (one week)	1,000,000			0.5–47.4	
Berlin (DE)	4	March 2014 (one week)	3,840,000			13.8–22.3	
Copenhagen (DK)	1	March 2014 (one week)	531,000			24.6–74.0	
Barcelona (ES)	1	March 2014 (one week)	1,150,874			5.7–17.6	
Castellón (ES)	1	March 2014 (one week)	180,690			11.6–61.6	
London (UK)	1	March 2014 (one week)	3,400,000			10.9–36.0	
Milan (IT)	1	February 2015 (one week)	1,122,501			5.1–8.1	
Amsterdam (NL)	1	March 2014 (one week)	769,000			14.3–30.5	
Eindhoven (NL)	1	March 2014 (one week)	450,300			13.7–304	
Utrecht (NL)	1	March 2014 (one week)	300,000			7.7–20.7	
Oslo (NO)	1	March 2015 (eight days)	580,639			8.8–52.9	
Almada (PT)	1	March 2014 (one week)	138,685			8.4–24.1	
Valencia - Pinedo I (ES)	1	March 2014 (seventeen days)	335,825	n.a.	0.010–0.016	1.11–23.81	[45] [a]
Valencia - Quart Benager (ES)	1		154,421			3.31–56.11	
Valencia - Pinedo II (ES)	1		982,264			1.07–9.07	
Barcelona (ES)	1	March 2013 (one week)	998,846	3320.4	0.01	21.0	[46] [a]
		March 2014 (one week)	992,301			14.9	
		March 2015 (one week)	990,854			17.0	
Australian Capital Territory (urban, AU)	18	March–April 2014 at six WWTPs and March–May 2015 at 18 WWTPs (one week)	24,000,000 (total)	n.a.	0.012	7.40–16.0	[47] [a]
Queensland-A (urban, AU)						11.0–24.0	

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Table 1 (*continued*)

Location	Number of WWTPs	Period	Population	CF	Excretion (%)	Consumption (mL alcohol/day/person)	Ref.
Queensland-B (urban, AU)						9.1–24.0	
New South Wales-A (urban, AU)						7.3–36.0	
New South Wales-B (urban, AU)						9.5–21.0	
New South Wales-C (urban, AU)						6.0–12.0	
New South Wales-D (urban, AU)						3.7–15.0	
Northern Territory-B (urban, AU)						14.0–43.0	
Victoria-A (urban, AU)						9.3–18.0	
Victoria-B (urban, AU)						8.6–23.0	
Western Australia (urban, AU)						7.8–12.0	
Queensland-C (rural, AU)						7.7–15.0	
Queensland-D (rural, AU)						12.0–27.0	
Northern Territory-A (rural, AU)						16.0–37.0	
Community 1 (US)	1	A random weekday every month for 11 months (between March 2015 and 2016)	125,000	3047	0.012	24.7 ± 7.8 (total population) 30.9 ± 9.8 (>15 years)	[31][b]
Community 2 (US)	1		44,000			17.9 ± 11.6 (total population) 22.3 ± 14.5 (>15 years)	
Community 3 (US)	1		53,000			49.8 ± 23.5 (total population) 62.2 ± 19.4 (>15 years)	
Urban city (NZ)	1	October 2016 (one week) January 2017 (one week) May 2017 (one week) July 2017 (one week)	<100,000	3047	n.a.	7 7 7.8 7.3	[48] [c]
South Australia (Festival week)	1	November–December 2019 (three weeks)	<100,000	3043.82	0.012	17.7	[49] [e]
South Australia (Non-festival weeks)						12.7	
Adelaide (AU)	4	April 2016–December 2019 (one week every two months) February 2020 (one week) April 2020 (one week)	>150,000	3043.82	0.012	7.1–34.3 6.12–13.71 4.74–9.35	[50] [e]
South-East Queensland (AU)	1	2012 2013 2014 2015 2016 2017	100,000	3042	0.012	20.9–25.3 20.7–25.1 18.5–22.4 17.8–21.5 19.7–23.9 15.7–19.0	[51] [a]
Thirty-three cities (CN)	29 (17 cities) 19 (16 cities)	2014 2016	200,000–3,450,000 107,600–3,338,500	3042	n.a.	4.7 ± 3.0 8.1 ± 7.0	[52] [a]
P. Mallorca (ES)	17	Spring 2018 (one week)	454,453	3047	0.012	10–18	[53]
Bilbao (ES)			860,237			15–30	
Guadalajara (ES)			94,755			7.9–17	
Toledo (ES)			79,793			5.5–12	
Barcelona (ES)			1,163,154			7.8–21	
Lleida (ES)			143,612			6.4–13	
Reus (ES)			115,000			7.0–21	
Tarragona (ES)			142,635			7.5–46	
Madrid Centre (ES)			727,176			5.3–13	
Madrid North (ES)			227,869			6.6–21	
Móstoles (ES)			187,281			10–29	
Santiago de Compostela (ES)			136,500			4.5–12	
Castellón (ES)			171,669			6.3–16	
Valencia I (ES)			527,222			6.6–17	
Valencia II (ES)			788,242			8.4–13	
Valencia III (ES)			162,249			6.5–15	
Bratislava (SK)	1	June 2017 (one week) October–November 2017 (one week)	n.a.	3047	0.012	28.4 49.3	[54] [a]
Piestany (SK)	1	May 2017 (one week) October 2017 (one week)				25.1 17.2	

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Table 1 (*continued*)

Location	Number of WWTPs	Period	Population	CF	Excretion (%)	Consumption (mL alcohol/day/person)	Ref.
Adana (TR)	2	October 2016, January 2017, May–June 2017 and August 2017 (one week every period)	1.007.952 and 522.265	3047	n.a.	5.0 and 2.9	[55] [a]
Adana (TR)	3	March–April 2019, June–July 2019,	1,791,215	3320.4	n.a.	4.86 ± 1.65	[56] [a]
Ankara (TR)	1	September–October 2019, and December	4,735,531			3.74 ± 1.10	
Diyarbakır (TR)	1	2019 (one week every period)	1,069,893			2.42 ± 0.98	
Erzurum (TR)	1		371,645			2.88 ± 0.99	
Gaziantep (TR)	2		2,178,563			3.86 ± 1.83	
Kayseri (TR)	1		1,100,000			3.23 ± 0.81	
Konya (TR)	1		1,300,000			2.79 ± 1.18	
Mersin (TR)	2		1,379,464			5.66 ± 1.65	
Sanlıurfa (TR)	3		1,089,117			2.18 ± 0.97	
Trabzon (TR)	2		274,411			1.68 ± 0.95	
Van (TR)	1		400,000			2.38 ± 0.76	
Malé (MV)	Nine zones flowing into nine pumping stations	March 2015 (one day)	153,940	3047	0.012	1.32	[57]
Istanbul (TR)	14	March 2019 (one week) June 2019 (one week) September 2019 (one week) December 2019 (one week)	20,000,000 (total)	3320.4	n.a.	49.8 ± 29.5 54.9 ± 24.6 7.3 ± 2.5 6.7 ± 2.5	[58] [a]
Innsbruck (AT)	1	March 2020–April 2020 (COVID-19 lockdown, thirty-five days) March 2019–January 2020 (before COVID-19 lockdown)	174,000	3046	n.a.	12.5 ± 2.8 15.6 ± 6.1	[59] [d]
Milan, Turin, Verona, Bologna, Merano, Gorizia (North IT)	6	2013–2014	6,900,000 (total)	3047	0.012	7.4 ± 4.0	[60]
Florence, Perugia, Terni, Rome, Pescara (Centre IT)	5	2013–2015				4.2 ± 1.8	
Naples, Bari, Potenza, Palermo, Nuoro, Cagliari (South IT)	6	2013–2016				7.5 ± 2.6	
Milan Halloween (IT)	1	2013				9.2 ± 1.0	
Milan normal days (IT)	1	2013				7.6 ± 1.9	
Milan Fashion Week (IT)	1	2014				11.7 ± 4.1	
Milan normal days (IT)	1	2014				7.6 ± 1.9	
Milan Fashion Week (IT)	1	2015				12.6 ± 9.2	
Milan normal days (IT)	1	2015				8.6 ± 2.8	
Milan Summer Holidays (IT)	1	2017				19.1 ± 5.2	
Milan normal days (IT)	1	2017				10.8 ± 4.1	
Moscow region (RU)	1	March 2018 (seven ordinary days) December 2018 (thirty ordinary days) December 2018–January 2019 (ten days, New Year holidays)	7600	n.a.	0.011	47.9 48.7 118.8	[61] [a]
Antalya (TR)	5	March, June, September, and December	2,025,000	3320.4	n.a.	25.9	[62]
Aydin (TR)	1	in 2019 (one week every month)	246,153			18.2	
Bursa (TR)	7		1,683,729			53.4	
Denizli (TR)	1		475,218			29.7	
İzmir (TR)	2		3,043,489			32.7	
Samsun (TR)	1		3,000,000			18.3	
Auckland, Bay of Plenty, and Canterbury (NZ)	7	March 2018 (one week)	1,170,000	3047	0.012	14.6 ± 9.7	[63] [e]
Vilnius (LT)	1	April 2018 (eight days) and September 2019 (one week)	536,631	3049	n.a.	21.2–92.5	[64] [a, e]
Kaunas (LT)	1	April 2018 (eight days) and March–April 2019 (one week)	288,363				
Klaipeda (LT)	1	April 2018 (one week) and March–April 2019 (one week)	164,038				
Southern China	1	November 2017–October 2018 (270 days)	500,000	4000	n.a.	0.2–4.9	[65] [a]
American Indian reservation (US)	3	July 2018 (two days)	10s–100s	3047	0.012	90.1 (L1), 35.2 (L2) and 141.6 (L4)	[66] [d]
Bilbao (ES)	1	March–July 2020	860,237	3047	n.a.	21.0 ± 8.4	[67]
Castellón (ES)	1		179,661			26.5 ± 14.0	
Santiago de Compostela (ES)	1		136,500			21.0 ± 7.6	
Vitoria-Gasteiz (ES)	1		255,052			28.0 ± 17.5	
Porto (PT)	1		150,000			19.8 ± 7.0	
Vila do Conde (PT)	1		80,000			21.9 ± 9.1	
Riga (LV)	1	Winter 2020–2021 Spring 2021 Summer 2021	697,000	n.a.	n.a.	38 44 37	[68] [a]

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Table 1 (*continued*)

Location	Number of WWTPs	Period	Population	CF	Excretion (%)	Consumption (mL alcohol/day/person)	Ref.
		Autumn 2021				30	
Capital Territory (AU)	1	February 2020 (one week)	>150,000	3043	0.012	15.6–27.7	[69]
		April 2020 (one week)				11.4–20.6	
		June 2020 (one week)				13.7–27.8	
		2016–2019 (one week every two months)				12.4–16.0	
New South Wales (AU)	3	February 2020 (one week)	>150,000			11.8–27.4	
		April 2020 (one week)				9.2–16.8	
		June 2020 (one week)				13.3–37.7	
		2016–2019 (one week every two months)				18.6–21.4	
Northern Territory (AU)	1	February 2020 (one week)	30,000–150,000			28–42.1	
		April 2020 (one week)				21.6–38.2	
		June 2020 (one week)				25.6–40.9	
		2016–2019 (one week every two months)				31.3–52.3	
Queensland (AU)	3	February 2020 (one week)	>150,000			10.1–24.5	
		April 2020 (one week)				9.8–17.7	
		June 2020 (one week)				10.4–21.4	
		2016–2019 (one week every two months)				12.2–19.4	
South Australia	4	February 2020 (one week)	>150,000			6.1–13.8	
		April 2020 (one week)				4.8–9.4	
		June 2020 (one week)				7.8–14.6	
		2016–2019 (one week every two months)				12.9–20.5	
Tasmania (AU)	3	February 2020 (one week)	<30,000			21.6–35.1	
		April 2020 (one week)				17.9–26.5	
		June 2020 (one week)				18.4–24.0	
		2016–2019 (one week every two months)				22.7–38.0	
Victoria (AU)	2	February 2020 (one week)	>150,000			7.8–14.2	
		April 2020 (one week)				11.5–16.1	
		June 2020 (one week)				8.5–19.4	
		2016–2019 (one week every two months)				10.1–14.7	
Western Australia	3	February 2020 (one week)	>150,000			6.9–13.3	
		April 2020 (one week)				5.8–11.9	
		June 2020 (one week)				8.1–17.3	
		2016–2019 (one week every two months)				13.1–17.8	

*Abbreviations: Wastewater Treatment Plant, WWTP; Correction Factor, CF; Not available, n.a.

[a] Population only regarded those aged over 15.

[b] Included both total population and only those aged over 15 years. All other studies included the total catchment population.

[c] Consumption was calculated using a CF of 3047 for ethyl sulphate and the alcohol density ($p = 0.789$ g/mL).

[d] Consumption was calculated using the alcohol density ($p = 0.789$ g/mL).

[e] Consumption was calculated using the ethanol content in a standard drink of alcohol in that study and the alcohol density ($p = 0.789$ g/mL).

Finally, a key requirement for a biomarker is that it should be stable in wastewater during transport from the inlet (e.g., toilet) to the sampling point, and during sampling, storage, and analysis [1]. Stability tests have been performed in the laboratory (in-sample stability and laboratory-scale sewer reactors) and in real sewers. In-sample experiments showed little or no degradation of EtS for up to one week at room temperature (20°C) and refrigerated conditions (4°C) and for up to twelve months when frozen (−20°C) [12,30,31]. Experiments with a rising main sewer reactor, a gravity sewer reactor, and a control sewer reactor without biofilms at a 12 h monitoring period showed that the simulated sewer conditions increased the degradation of EtS [25]. Finally, real sewer stability experiments revealed that EtS can be used as a WBE biomarker to track alcohol consumption, but when the catchment has a long hydraulic retention time and a high area-to-volume ratio, correction needs to be made [26]. Cotinine and *trans*-3′-hydroxycotinine were stable during in-sample stability tests at room temperature (20°C and 25°C) and refrigerated conditions (4°C) for up to two weeks, for up to twelve months when frozen (−20°C) and for up to eight weeks when frozen at −80°C [24,31–36]. In addition, methanolic extracts of cotinine were stable at 4°C and −20°C for eight days [37]. Laboratory-scale sewer reactors showed that both metabolites were stable after an average sewer retention time of 6 h [25]. Paraxanthine was stable in wastewater during one day storage at 20°C and 4°C and at −20°C for up to four weeks [32]. Methanolic extracts of paraxanthine were also found to be stable at 4°C for two days and at −20°C for eight days [37]. Stability data for 1,7-dimethyluric acid were not available.

4. Analytical methods

4.1. Sampling procedure

Selecting a suitable sampling procedure and an analytical method is essential to ensure the quality of data obtained by WBE. Sampling should be performed at the inlet of a wastewater treatment plant and during dry periods to measure the entire catchment and avoid dilution due to rain (biomarker detection may be more difficult if diluted) respectively. Various sampling techniques are used, such as composite sampling, grab sampling and passive sampling. Composite sampling consists of pooling a number of discrete grab samples collected at a set frequency over a specified period of time (typically, 24 h). Composite samples are collected using automated devices, using a flow-proportional, time-proportional, or volume-proportional sampling mode. The choice of the specific mode of operation is inextricably linked to the type of device used in each wastewater treatment plant. When the infrastructure of a wastewater treatment plant does not consist of a sampling device, a portable automated sampler in time-proportional mode is usually used. Grab sampling is not considered an appropriate technique as it represents single moments in time and cannot capture concentration fluctuation over time. Therefore, studies performed with grab sampling were not considered in the current review. Passive sampling is an alternative approach that presents its own advantages and disadvantages compared to other techniques, and its application to the studied WBE biomarkers needs further investigation.

After sampling, which in most cases is done under refrigerated

Table 2
Studies investigating nicotine consumption by wastewater-based epidemiology.

Location	Number of WWTPs	Period	Population	Biomarker	CF	Excretion (%)	Consumption (mg nicotine/day/1000 person)	Ref.
Lisbon (PT)	1	October and November 2011 (twice a week, Tuesday and Thursday, for four weeks)	379,986	COT	n.a.	14.1	5900	[13]
Santiago de Compostela (ES)	1	April 2012 (one week) March 2013 (one week) March 2014 (one week)	130,000	COT and OH-COT (sum)	n.a.	27 (COT) 44.5 (OH-COT)	1600 1900 1800	[27]
Milan (IT)	1	October 2012 (one week)	960,300	COT and OH-COT (sum)	1.35	74	2200 ± 300	[24]
Turin (IT)	1		1,201,490				4000 ± 300	[a]
Como (IT)	1		79,926				3000 ± 600	
Bologna (IT)	1		470,640				2900 ± 300	
Rome (IT)	1		1,107,699				3400 ± 1600	
Naples (IT)	1		549,250				4500 ± 500	
Palermo (IT)	1		223,254				4300 ± 300	
Bari (IT)	1		296,922				3600 ± 100	
Albany (US)	2	July 2013 (one week)	15,000 and 100,000	COT	Not used	–	3150 and 4058	[70] [d]
Milan, Como, Bologna, Turin, Verona (North IT)	5	Spring and Autumn 2012	Each site either >50,000 or >500,000	COT OH-COT	Not used	–	1710 ± 300 2700 ± 600	[32] [d]
Pescara, Florence, Perugia, Rome (Centre IT)	4			COT OH-COT			1400 ± 300 2100 ± 800	
Bari, Palermo, Naples, Potenza (South IT)	4			COT OH-COT			2400 ± 700 3000 ± 800	
Bratislava (SK)	1	March 2014 (three days), August 2014 (one day) and November 2014 (one day)	n.a.	COT	n.a.	14.1	4000	[71] [b]
Bratislava International Gypsy Fest (SK)		August 2014 (two days)					4000	
Piestany (SK)	1	June 2014 (three days) and August 2014 (two days)					4000	
Piestany Festival Topfest (SK)		June 2014 (three days)					8000	
Piestany Festival Grape (SK)		August 2014 (two days)					6000	
Skalica (SK)	1	September 2014 (two days)					9000	
Skalica Festival Skalické dni (SK)		September 2014 (two days)					5000	
Zubri (CZ)	1	August 2014 (six days)					10,000	
Zubri Festival Vandaalfest (SK)		August 2014 (three days)					11,000	
Valaské Medzirici (CZ)	1	July 2014 (six days)					5000	
Velaké Festival Gulas Fest (CZ)		July 2014 (three days)					6000	
Bratislava (SK)	5	March 2014 (one week)	450,000	COT	n.a.	14.1	4200	[72]
Petržalka (SK)			125,000				5100	
Košice (SK)			215,000				2400	
Nitra (SK)			81,000				8000	
Piešťany (SK)			35,000				2200	
Prague (CZ)			1,300,000				4300	
České Budějovice (CZ)			120,000				3600	
Oslo (NO)	1	March 2015 (one week)	n.a.	COT and OH-COT (sum)	Not used	–	9045	[43] [d]
Castellón (ES)	1						7020	
Brussels (BE)	1						7155	
Bristol (UK)	1						4590	
Utrecht (NL)	1						2835	
Milan (IT)	1						3510	
Zurich (CH)	1						8100	
Copenhagen (DK)	1						2835	
Dalian (CN)	11	June 2015 (two weekdays)	2,200,000 (total)	COT and OH-COT (sum)	n.a.	32.3 (COT) 43.4 (OH-COT)	1920	[73] [b]
Geraardsbergen (BE)	1	March 2015 (eight days)	29,000	OH-COT COT	Not used	–	2820 2540	[35] [d]
Ninove	1	March 2014 (eight days)	36,000	OH-COT			2460	

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Table 2 (*continued*)

Location	Number of WWTPs	Period	Population	Biomarker	CF	Excretion (%)	Consumption (mg nicotine/day/1000 person)	Ref.
				COT			2280	
Jilin province, eight cities (CN)	10	August 2016 (two days)	66,300–1,500,000	COT	2.85	32.3	2390	[74] [b]
Australian Capital Territory (urban, AU)	18	March–April 2014 at six WWTPs and March–May 2015 at 18 WWTPs (one week)	24,000,000 (total)	COT and OH-COT (sum)	n.a.	28.05 (COT) 45.23 (OH-COT)	1210–1280	[47] [b]
Queensland-A (urban, AU)							1950–2540	
Queensland-B (urban, AU)							130–1690	
New South Wales-A (urban, AU)							1150–3900	
New South Wales-B (urban, AU)							750–1340	
New South Wales-C (urban, AU)							350–1370	
New South Wales-D (urban, AU)							520–820	
Northern Territory-B (urban, AU)							1610–2990	
South Australia (urban, AU)							1830–1960	
Victoria-A (urban, AU)							1740–2010	
Victoria-B (urban, AU)							2010–2300	
Western Australia (urban, AU)							1590–1760	
Queensland-C (rural, AU)							1160–1720	
Queensland-D (rural, AU)							2460–3150	
Northern Territory-A (rural, AU)							2930–3500	
Athens (GR)	1	January 2017 (one week)	3,700,000	COT	Not used	–	3950	[75] [d]
				OH-COT			3720	
Geneva (CH)	1	February 2017 (one week)	600,000	COT			1770	
				OH-COT			1920	
Community 1 (US)	1	A random weekday every month for 11 months (between March 2015 and 2016)	125,000	COT and OH-COT (sum)	1.35	74	2700 (total population) 3400 (>15 years)	[31] [c]
Community 2 (US)	1		44,000				2400 (total population) 3000 (>15 years)	
Community 3 (US)	1		53,000				2600 (total population) 3300 (>15 years)	
South-East Queensland (AU)	1	2010	110,000	COT	3125	32	3489	[76] [e]
		2011					3084	
		2012					2849	
		2013					3095	
		2014					2896	
		2015					2763	
		2016					2733	
		2017					2545	
Urban city (NZ)	1	October 2016 (one week)	<100,000	COT	Not used	–	1432 ± 89	[48] [d]
				OH-COT			2122 ± 27	
		January 2017 (one week)		COT			1463 ± 126	
				OH-COT			2105 ± 70	
		May 2017 (one week)		COT			1389 ± 48	
				OH-COT			2147 ± 27	
		July 2017 (one week)		COT			1333 ± 72	
				OH-COT			2179 ± 38	
Merano (IT)	16	November 2013 (one week)	75,000	COT and OH-COT (sum)	1.35	74	4200 ± 500	[77] [a]
Milan (IT)			1,040,000				3200 ± 500	
Turin (IT)			1,370,000				4100 ± 400	
Verona (IT)			287,000				2900 ± 300	
Gorizia (IT)			33,000				2400 ± 900	
Bologna (IT)			530,000				2900 ± 800	
Terni (IT)			100,000				2700 ± 400	

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Table 2 (*continued*)

Location	Number of WWTPs	Period	Population	Biomarker	CF	Excretion (%)	Consumption (mg nicotine/day/1000 person)	Ref.
Perugia (IT)			68,880				2000 ± 600	
Rome (IT)			1,279,000				1400 ± 600	
Cagliari (IT)			270,000				1900 ± 500	
Nuoro (IT)			21,000				5000 ± 600	
Pescara (IT)			154,000				1400 ± 600	
Naples (IT)			650,000				4100 ± 1900	
Palermo (IT)			265,000				3700 ± 900	
Bari (IT)			341,290				5100 ± 600	
Potenza (IT)			80,000				1900 ± 600	
Palma de Mallorca (ES)	17	Spring 2018 (one week)	454,453	COT and OH-COT independently (the average value is presented)	n.a.	27 (COT) 44.5 (OH-COT)	2300 ± 300	[78]
Bilbao (ES)			860,237				2800 ± 400	
Guadalajara (ES)			94,755				2700 ± 300	
Toledo (ES)			79,793				1800 ± 100	
Barcelona (ES)			1,163,154				2600 ± 200	
Lleida (ES)			143,612				2000 ± 200	
Reus (ES)			115,000				2800 ± 200	
Tarragona (ES)			142,635				2600 ± 500	
Madrid I (ES)			727,176				1800 ± 300	
Madrid II (ES)			227,869				1400 ± 300	
Móstoles (ES)			187,281				3700 ± 800	
Santiago di Compostela (ES)			136,500				1400 ± 300	
Castellón (ES)			171,669				2700 ± 300	
Valencia I (ES)			527,222				1800 ± 300	
Valencia II (ES)			788,242				2300 ± 500	
Valencia III (ES)			162,249				2000 ± 300	
Adana (TR)	3	March–April 2019, June–July 2019, September–October 2019, and December 2019 (one week every period)	1,791,215	COT	0.45	n.a.	2911 ± 1437	[56] [b]
Ankara (TR)	1		4,735,531				2740 ± 652	
Diyarbakır (TR)	1		1,069,893				2756 ± 1529	
Erzurum (TR)	1		371,645				2764 ± 1211	
Gaziantep (TR)	2		2,178,563				3647 ± 1600	
Kayseri (TR)	1		1,100,000				4282 ± 1332	
Konya (TR)	1		1,300,000				3276 ± 1219	
Mersin (TR)	2		1,379,464				3884 ± 1083	
Sanlıurfa (TR)	3		1,089,117				2116 ± 1287	
Trabzon (TR)	2		274,411				987 ± 394	
Van (TR)	1		400,000				1877 ± 1314	
Malé (MV)	Nine zones flowing into nine pumping stations	March 2015 (one day)	153,940	COT and OH-COT (sum)	1.35	74	2600	[57]
South-East Queensland (AU)	1	February, April, June, August, October, and December in 2015 (one week every month)	100,000	COT OH-COT	Not used	–	680–2210 720–2450	[79] [d]
Thirty-three cities (CN)	29 (17 cities)	2014	200,000–3,450,000	COT	1.94	32.3	1600 ± 900	[52] [b]
	19 (16 cities)	2016	107,600–3,338,500				1900 ± 1100	
Yingkou working days (CN)	1	September 2018 (six days), October 2018 (two days), December 2018 (two days), January 2019 (two days), February 2019 (four days), April 2019 (two days) and May 2019 (two days)	510,000	COT	2.85	19.4	980 ± 230	[80]
Yingkou The Mid-Autumn Festival (CN)		September 2018 (three days)					950 ± 120	
Yingkou National Day (CN)		October 2018 (one week)					1270 ± 210	
Yingkou New Year's Day (CN)		January 2019 (three days)					730 ± 80	
Yingkou The Spring Festival (CN)		February 2019 (one week)					860 ± 170	
Yingkou The Tomb - Sweeping Day (CN)		April 2019 (three days)					1870 ± 290	
Yingkou Labor Day (CN)		May 2019 (four days)					1320 ± 120	
Istanbul (TR)	14	March 2019 (one week) June 2019 (one week)	20,000,000 (total)	COT	3.33	n.a.	4294 ± 2110 4366 ± 4114	[58] [b]

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Table 2 (*continued*)

Location	Number of WWTPs	Period	Population	Biomarker	CF	Excretion (%)	Consumption (mg nicotine/day/1000 person)	Ref.
		September 2019 (one week)					7055 ± 4692	
		December 2019 (one week)					6313 ± 2553	
Innsbruck (AT)	1	March 2020–April 2020 (COVID-19 lockdown, thirty-five days)	174,000	COT	7.08	n.a.	4300 ± 600	[59]
		March 2019–January 2020 (before COVID-19 lockdown)					4300 ± 800	
Moscow region (RU)	1	March 2018 (seven days) and December 2018 to January 2019 (forty days)	7600	COT	n.a.	14.1	7830	[61] [b]
Auckland, Bay of Plenty, and Canterbury (NZ)	7	March 2018 (one week)	1,170,000	COT and OH-COT (sum)	1.35	n.a.	1910 ± 515	[63] [e]
Athens (GR)	1	March 2019 (one week)	3,995,020	COT	3.4 (COT)	n.a.	1300 ± 100	[81]
				OH-COT			2100 ± 200	
		March–April 2020 (COVID-19 lockdown, fifteen days)	4,009,346	COT	1.9 (OH-COT)		800 ± 70	
				OH-COT			1700 ± 100	
Vilnius (LT)	1	April 2018 (eight days) and September 2019 (one week)	536,631	COT and OH-COT (sum)	3.13 (COT)	n.a.	3200–9540	[64] [b, e]
Kaunas (LT)	1	April 2018 (eight days) and March–April 2019 (one week)	288,363		2.31 (OH-COT)			
Klaipeda (LT)	1	April 2018 (one week) and March–April 2019 (one week)	164,038					
Antalya (TR)	5	March, June, September, and December in 2019 (one week every month)	2,025,000	COT	3.33	n.a.	5375 ± 2272	[62] [b]
Aydın (TR)	1		246,153				8791 ± 8362	
Bursa (TR)	7		1,683,729				5144 ± 2322	
Denizli (TR)	1		475,218				1855 ± 786	
İzmir (TR)	2		3,043,489				4355 ± 1859	
Samsun (TR)	1		3,000,000				4762 ± 4436	
Chongqing I (CN)	27	2018–2019	n.a.	COT	2.85	n.a.	680 ± 150	[82] [b]
Chongqing II (CN)							570 ± 130	
Chengdu (CN)							880 ± 210	
Guiyang I (CN)							960 ± 50	
Guiyang II (CN)							1880 ± 300	
Changdu (CN)							610 ± 150	
Kunming (CN)							530 ± 40	
Dalian (CN)							1220 ± 270	
Haerbin (CN)							900 ± 140	
Changzhou (CN)							800 ± 10	
Hefei (CN)							670 ± 190	
Qingdao (CN)							910 ± 50	
Jinan (CN)							450 ± 160	
Weihai (CN)							580 ± 130	
Beijing (CN)							350 ± 100	
Hohhot (CN)							370 ± 20	
Baoding I (CN)							720 ± 40	
Baoding II (CN)							170 ± 10	
Baoding III (CN)							1680 ± 10	
Zhengzhou (CN)							1020 ± 90	
Xiangtan I (CN)							920 ± 130	
Xiangtan II (CN)							630	
Guangzhou (CN)							880 ± 150	
Xian (CN)							210 ± 10	
Lanzhou (CN)							480 ± 110	
Dingxi (CN)							370 ± 80	
Yinchuan (CN)							340 ± 50	
American Indian reservation (US)	3	July 2018 (two days)	10s–1000s	COT and OH-COT (sum)	1.35	74	4630 (L1), 870 (L2) and 4230 (L4)	[66]
Bilbao (ES)	1	March–July 2020	860,237	COT and OH-COT independently (the average value is presented)	3.41 (COT)	n.a.	1674 ± 672	[67]
Castellón (ES)	1		179,661				1497 ± 594	
Santiago de Compostela (ES)	1		136,500		1.90 (OH-COT)		1659 ± 663	
Vitoria-Gasteiz (ES)	1		255,052				2654 ± 1421	
Porto (PT)	1		150,000				1501 ± 204	
Vila do Conde (PT)	1		80,000				1391 ± 514	
Novo Hamburgo (BR)	1	March 2020–March 2021 (24 sets of 3 POCIS samplers)	778–1679	COT	3.1	30	163 ± 65	[83]
Riga (LV)	1	Winter 2020–2021	697,000	COT	n.a.	27	5100	[68] [b]
		Spring 2021					5700	
		Summer 2021					6800	
		Autumn 2021					6100	

(*continued on next page*)

Table 2 (*continued*)

Location	Number of WWTPs	Period	Population	Biomarker	CF	Excretion (%)	Consumption (mg nicotine/day/1000 person)	Ref.
Beijing (CN)	1	December 2018 (one week), December 2018–January 2019 (one week) and April–May 2019 (nine days)	120,000	COT	2.88	32	300–5400	[84]

*Abbreviations: Wastewater Treatment Plant, WWTP; Correction Factor, CF; Not available, n.a.; Cotinine: COT; *trans*-3′-hydroxycotinine: OH-COT; Polar organic chemical integrative samplers, POCIS.

[a] Population only regarded those aged over 14.

[b] Population only regarded those aged over 15.

[c] Included both total population and only those aged over 15 years. All other studies included the total catchment population.

[d] Consumption was calculated using a CF of 3.4 for cotinine and 1.9 for *trans*-3′-hydroxycotinine.

[e] Consumption was calculated using the nicotine content of a cigarette reported in that study.

Table 3
Studies investigating caffeine consumption by wastewater-based epidemiology.

Location	Number of WWTPs	Period	Population	Biomarker	CF	Excretion (%)	Consumption (mg caffeine/day/person)	Ref.
Albany (US)	2	July 2013 (one week)	15,000 and 100,000	Paraxanthine	Not used	–	161 and 188	[70] [a]
Milan, Como, Bologna, Turin, Verona (North IT)	5	Spring and Autumn 2012	Each site either >50,000 or >500,000	Paraxanthine	Not used	–	354 ± 89	[32] [a]
Pescara, Florence, Perugia, Rome (Centre IT)	4						262 ± 110	
Bari, Palermo, Naples, Potenza (South IT)	4						356 ± 35	
Bristol (UK)	1	March 2015 and April 2015 (Porto) (one week)	886,650	1,7-dimethyluric acid	14.8	6.7	190 ± 37	[14]
Brussels (BE)	1		954,000				162 ± 15	
Castellón (ES)	1		180,000				122 ± 28	
Copenhagen (DK)	1		530,000				229 ± 19	
Lugano (CH)	1		103,560				97 ± 16	
Milan (IT)	1		1,100,000				86 ± 18	
Oslo (NO)	1		580,000				211 ± 21	
Porto (PT)	1		150,000				121 ± 27	
Utrecht (NL)	1		300,000				107 ± 28	
Zurich (CH)	1		410,000				263 ± 23	
Merano, Milan, Turin, Verona, Gorizia, Bologna (North IT)	6	November 2013 (one week)	33,000–1,370,000	1,7-dimethyluric acid	14.8	6.7	134.6 ± 43.6	[77]
Terni, Perugia, Rome Cagliari, Nuoro, Pescara (Centre IT)	6		21,000–1,279,000				110.5 ± 69.5	
Naples, Palermo, Bari, Potenza (South IT)	4		80,000–650,000				101.7 ± 47.7	
Milan (IT)	1	November 2013 (one week) March 2014 (one week) May 2014 (one week) March 2015 (one week)	1,040,000				115.9 ± 15.6 136.7 ± 26.1 130.1 ± 28.8 83.0 ± 18.1	
Malé (MV)	Nine zones flowing into nine pumping stations	March 2015 (one day)	153,940	1,7-dimethyluric acid	14.8	6.7	60	[57]
American Indian reservation (US)	3	July 2018 (two days)	10s–1000s	Paraxanthine	23.4	4.6	276–693	[66]
Beijing (CN)	1	December 2018 (one week), December 2018–January 2019 (one week) and April–May 2019 (nine days)	120,000	Paraxanthine	23.42	4.6	16–121	[84]

*Abbreviations: Wastewater Treatment Plant, WWTP; Correction Factor, CF.

[a] Consumption was calculated using a CF of 23.4.

conditions (4°C), another critical step is storage to avoid degradation of biomarkers. Following collection, samples are usually frozen at −20°C until analysis.

4.2. Analytical methods for the determination of ethyl sulphate in influent wastewater

Assessment of alcohol consumption is mainly performed by measurements of EtS in influent wastewater (Table 4). Wastewater is centrifuged and/or filtered prior to analysis and no losses of EtS are expected in this first step, as EtS is not retained on the filter [60]. In addition, adsorption to suspended matter is not likely to occur, as EtS should remain in the aqueous phase due to its physico-chemical properties (e.g., high mobility in soils and high polarity and thus strong affinity for water [85]). For the analysis, liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) in negative electrospray ionization mode is used (Table 4). The first studies used ion-pair chromatography due to the ionic nature of EtS employing hydrophobic stationary phases. Several ion-pair reagents were used, such as dihexyl ammonium acetate, dibutyl ammonium acetate or tetrabutylammonium bromide to increase the retention time of the analyte and improve the peak shape [12,30,38,45]. These reagents were added to the mobile phase or the sample itself; addition of the ion-pair reagent to the sample allows the use of stronger non-volatile amines (e.g., tetrabutylammonium bromide), keeps the MS cleaner and does not adversely affect ionization efficiency (analyte signal suppression) [30,38,45]. EtS is poorly retained on conventional (C8 or C18) reverse phase chromatographic columns due to its high polarity and, thus, hydrophilic interaction chromatography (HILIC) could be an alternative option. However, experiments showed that retention time was poor and peak shape was not improved [30]. In addition, HILIC needs a further dilution of the sample (e.g., 1:10, v/v) with an organic solvent as it is not compatible with high water percentage. Other studies used polar columns based on silica and conventional solvents for the mobile phases, such as water, methanol and/or acetonitrile containing formic or acetic acid showing good chromatographic characteristics (Table 4). The EtS detection is performed using mainly QqQ instruments and a few studies used a quadrupole ion trap (QTrap), however, as a QqQ without using the potential of the trap. The confirmation of EtS should be done using both characteristic product ions (96.960, HSO_4^- and 79.957, SO_3^-) and quantification with the assistance of the corresponding labelled internal standard (EtS-d5) to minimize uncertainties related to matrix effect and instrumental drift.

4.3. Analytical methods for the determination of nicotine biomarkers in influent wastewater

Nicotine consumption is evaluated by measurements of its urinary metabolites, cotinine and/or *trans*-3′-hydroxycotinine in influent wastewater (Table 5). Both biomarkers are expected in the aqueous phase of wastewater rather than adsorbed to suspended matter due to their physico-chemical properties, such as high mobility in soils,

Table 4
Main analytical methods for the determination of ethyl sulphate in influent wastewater.

Pre-treatment	IS, EtS-d5 (μg/L)	Injection volume (μL)	Liquid chromatography		Mass spectrometry	LOD/LOQ (μg/L)	Ref.
			Column	Mobile phase			
•Centrifugation (20,000 g, 10 min)	50	n.a.	Acquity UPLC Bridged-Ethyl Hybrid C8 (1.7 μm, 50 × 2.0 mm) at 50 °C	H_2O and MeOH both containing 7 mM dihexyl ammonium acetate at 0.6 mL/min	MS/MS, ESI (−), SRM (125 > 97)	-/8 (nM)	[12]
•Centrifugation (10,000 rpm, 10 min, 4°C)	25	20	Purospher STAR RP-18 end-capped (5 μm, 125 × 2.0 mm)	H_2O and MeOH both containing 5 mM of dibutyl ammonium acetate at 0.3 mL/min	QqLIT-MS/MS, ESI (−), SRM (125 > 97, 80)	0.02/0.07	[38]
•Filtration (glass fiber prefilters and 0.22 μm nitrocellulose filters) •Addition of tetrabutylammonium bromide (50 mM)	10	100	Synergi Fusion-RP (4 μm, 100 × 2.0 mm) at 45°C	H_2O and MeOH both containing 0.1% formic acid at 0.4 mL/min	QqQ-MS/MS, ESI (−), MRM (125 > 97, 80) QTOF, ESI (−), (124.991 > 96.960, 79.957)	0.1/0.3 (QqQ) 0.2/0.6 (QTOF)	[30]
•Addition of 0.5 M of tributylamine and 0.1% of formic acid	25	5	Kinetex C18 (1.7 μm, 50 × 2.1 mm) at 30°C	H_2O and MeOH both containing 0.1% formic acid at 0.2 mL/min (isocratic)	QqQ-MS/MS, ESI (−), SRM (125 > 97, 80)	0.1/0.3	[45]
•Centrifugation (8000 rpm, 5 min) •Filtration (0.2 μm) •Centrifugation (8000 rpm, 5 min)	50	4	Atlantis T3 (3 μm, 150 × 2.1 mm) at 30°C	H_2O (0.1% formic acid) and ACN at 0.18 mL/min	QqQ-MS/MS, ESI (−), MRM (125 > 97, 80)	-/1.5	[41]
•Filtration	5	10	EVO C18 (1.7 μm, 50 × 2.0 mm) at 45°C	H_2O and MeOH both containing 5 mM of dihexyl ammonium acetate at 0.27 mL/min	Qtrap, ESI (−), MRM (125 > 97, 80)	0.2/0.5	[26]
•Centrifugation (4000 g, 10 min)	1	10	Symmetry C18 (3.5 μm, 150 × 4.6 mm)	H_2O (0.2% formic acid) and MeOH at 0.4 mL/min	QqQ-MS/MS, ESI (−), MRM (125 > 97, 80)	0.073/0.245	[31]
•Filtration (glass microfiber filters GF/A 1.6 mm)	46.7	5	Synergi Hydro-RP (4 μm, 150 × 4.6 mm)	H_2O (5 mM ammonium formate, 0.1% formic acid) (A) and MeOH with ACN (50:50, v/v, B) at 0.5 mL/min	Qtrap, ESI (−), MRM (125 > 97, 80)	0.17/0.5	[49]
•Centrifugation (3500 rpm, 10 min) •Filtration (0.22 μm)	5	10	Restek Raptor (2.7 μm, 100 × 2.1 mm) at 30°C	H_2O (0.1% acetic acid) and ACN at 0.3 mL/min	MS/MS, ESI (−), MRM (125 > 97, 80)	0.03/0.1	[55]
•Centrifugation (17,000 g, 5 min) •Addition of triethylammonium acetate (40 mM)	n.a.	10	Kinetex Biphenyl (2.6 μm, 100 × 2.1 mm) at 50°C	Linear gradient of 2–10% MeOH in H_2O with 0.5% acetic acid at 0.2 mL/min	Qtrap MS/MS, ESI (−), MRM (125 > 97)	-/1.0	[59]
•Centrifugation (12,000 rpm, 5 min)	20	n.a.	Synergy Polar RP (4 μm, 250 mm × 2.0 mm)	H_2O and ACN both containing 0.1% formic acid	QqQ-MS/MS, ESI (−), MRM (125 > 97, 80)	0.10/0.32	[61]
•Filtration (glass microfiber filter 1.6 μm GF/A and 0.45 μm nitrocellulose filter) •Centrifugation (2500 rpm, 5 min)	50	4	Atlantis T3 (3 μm, 150 × 2.1 mm)	H_2O (0.1% acetic acid) and ACN at 0.18 mL/min	QqQ-MS/MS, ESI (−), MRM (125 > 97, 80)	0.05/0.18	[60]

*Abbreviations: Internal standard, IS; Limit of detection, LOD; Limit of quantification, LOQ; Not available, n.a.; Water, H_2O; Methanol, MeOH; Acetonitrile, ACN.

Table 5
Main analytical methods for the determination of cotinine and *trans*-3′-hydroxycotinine in influent wastewater.

Biomarkers	Pre-treatment	IS, μg/L	Injection volume (μL)	Liquid chromatography		Mass spectrometry	LOD/ LOQ (ng/ L)	Ref.
				Column	Mobile phase			
COT	•Filtration (glass microfiber GF/A filters) •SPE (HLB)	COT-d3, 1.5	5	BEH C18 (1.7 μm, 100 × 2.1 mm)	H_2O (30 mM formic acid/ ammonium formate, pH 3.5) and ACN (0.1% formic acid) at 0.5 mL/min	QqQ-MS/MS, ESI (+), SRM (177 > 80, 98)	-/500	[37]
COT	•pH = 6 •Centrifugation (4°C, 3800 rpm, 10 min) •Filtration (0.45 μm glass microfiber filter) •SPE (HLB)	COT-d3, 50	5	Luna HILIC (5 μm, 150 × 3.00 mm) at 35°C	H_2O (ammonium acetate 5 mM) and ACN at 0.30 mL/ min	QqQ-MS/MS, ESI (+), SRM (177 > 80, 98)	-/14,900	[13]
COT, OH-COT	•Filtration (glass fiber filters and 0.22 μm nitrocellulose filters) •Glucuronides deconjugation •pH = 5	COT-d3 and OH-COT-d3, 20	100	Synergi Fusion-RP (4 μm, 100 × 2.0 mm) at 45°C	H_2O and MeOH, both containing 5 mM ammonium acetate (pH 8.5) at 0.20 mL/ min	QqQ-MS/MS, ESI (+), MRM (COT: 177 > 80, 98; OH-COT: 193 > 80, 134)	COT: 50/ 200 OH-COT: 100/400	[27]
COT	•Centrifugation (5000 rpm, 10 min) •Filtration (glass fiber filter) •SPE (HLB)	COT-d3, 50	10	Ultra-Biphenyl (5 μm, 100 × 2.1 mm)	H_2O (0.1% formic acid) and MeOH	QqQ-MS/MS, ESI (+), MRM (177 > 80, 98)	n.a.	[70]
COT, OH-COT	•Filtration (1.6 μm GF/ A glass microfiber filters and 0.45 μm mixed cellulose membrane filters) •pH = 6.0–7.5 •SPE (HLB)	COT-d3, 20	2	X-Terra C18 (3.5 μm, 100 × 1 mm)	H_2O (10 mM ammonium acetate) and ACN at 0.07 mL/min	QqQ-MS/MS, ESI (+), SRM (COT: 177 > 80, 98; OH-COT: 193 > 80, 134)	COT: -/0.43 OH-COT: -/1.9	[32]
COT	•Filtration •pH = 4.5–5 •SPE (UCT-XRADH506)	COT-d3, 1.6	5	HS F5 (5 μm, 150 × 4 mm) at 40°C	5% ACN in H_2O and 5% H_2O in ACN and both containing 10 mM ammonium formate, 0.1% formic acid and 0.1% tetrahydrofuran at 1 mL/min	Triple TOF, (+) mode (177 > 98)	-/400	[33]
COT, OH-COT	•Filtration (0.45 μm glass filter) •SPE (Cleanert PEP-2)	COT-d3 and OH-COT-d3, 20	10	Symmetry Shield C18 (3.5 μm, 150 × 2.1 mm)	H_2O and MeOH both containing 5 mmol/L ammonium acetate at 0.4 mL/min	MS/MS, ESI (+), SRM (COT: 177 > 80; OH-COT: 193 > 80)	COT: 12/- OH-COT: 14/-	[73]
COT, OH-COT	•Filtration (1.6 μm glass microfiber filters)	COT-d3, 10	2	Atlantis T3 (3 μm, 150 × 2 mm) at 40°C	H_2O and MeOH both containing 5 mM ammonium formate with 0.1% formic acid at 0.3 mL/min	QqQ-MS/MS, ESI (+), MRM (COT: 177 > 80, 146; OH-COT: 193 > 80, 134)	COT: 100/120 OH-COT: 23/37	[35]
COT, OH-COT	•Dilution with H_2O •Centrifugation (4000 rpm, 3 min)	morphine-d3 and cocaethylene-d3, 50	5	Kinetex C18 (1.7 μm, 50 × 2.1 mm) at 30°C	H_2O and MeOH both containing 0.1% formic acid at 0.2 mL/min	QqQ-MS/MS, ESI (+), MRM (COT: 177 > 80, 98; OH-COT: 193 > 80, 134, 53)	COT: 500/ 2000 OH-COT: 500/ 2000	[34]
COT, OH-COT	•Direct injection	COT-d3	n.a.	Kinetex Biphenyl (2.6 μm, 50 × 2 mm) at 45°C	H_2O and MeOH both containing 0.1% formic acid at 0.3 mL/min	QTrap, ESI (+), MRM (COT: 177 > 80, 98; OH-COT: 193 > 80, 134)	COT: 30/ 100 OH-COT: 20/50	[26]
COT	•Filtration (glass microfiber filters) •pH adjustment •SPE (Strata-X-C)	COT-d4	20	Kinetex C18 (1.7 μm, 100 × 2.1 mm) at 30°C	H_2O and ACN both containing 0.1% formic acid at 0.3 mL/min	QqQ-MS/MS, ESI (+), MRM (177 > 80, 53)	n.a.	[87]
COT, OH-COT	•SPE (HLB)	COT-d3, 250	10	XBridge C8 (3.5 μm, 150 × 4.6 mm)	H_2O and MeOH at 0.5 mL/ min	QqQ-MS/MS, ESI (+), MRM (COT: 177 > 80, 98; OH-COT: 193 > 80, 134)	COT: 2/8 OH-COT: 6/29	[31]
COT	•Filtration (0.7 μm glass microfiber filter) •pH = 7.5–8.5 •SPE (HLB)	COT-d3, 100	15	RP C18 (1.7 μm, 150 × 1.0 mm) at 25°C	H_2O:MeOH (80:20, v/v; 5 mM ammonium acetate and 3 mM acetic acid, pH 4.7) and MeOH at 0.04 mL/min	QqQ-MS/MS, ESI (+), MRM (177 > 80, 98)	0.27/ 1.34	[86]
COT	•Filtration (0.7 μm glass fiber filter) •SPE (HLB)	COT-d3, 4	10	Allure pentafluorophenyl propyl (5 μm, 50 × 2.1 mm) at 40°C	H_2O (10 mM ammonium formate) and MeOH at 0.4 mL/min	QqQ-MS/MS, ESI (+), MRM (177 > 80, 53)	40/100	[56]

(*continued on next page*)

Table 5 (*continued*)

Biomarkers	Pre-treatment	IS, μg/L	Injection volume (μL)	Liquid chromatography		Mass spectrometry	LOD/ LOQ (ng/ L)	Ref.
				Column	Mobile phase			
COT	•Filtration (GF/F filters) •SPE (HLB)	COT-d3, 100	20	Chiral CBH (1.7 μm, 100 × 1.0 mm) at 25°C	H_2O (1 mM ammonium acetate)/MeOH (85:15, v/v) at 0.1 mL/min (isocratic)	QqQ-MS/MS, ESI (+), MRM	n.a.	[88]
COT, OH-COT	•Filtration (0.7 μm glass fiber filter) •pH = 6.5 •SPE (mix of Strata-X, Strata-X-CW, Strata-X-AW, isolute ENV+)	Various	5	Acclaim RSLC C18 (2.2 μm, 100 × 2.1 mm) at 30°C	H_2O:MeOH (90:10, v/v) and MeOH both containing 5 mM ammonium formate and 0.01% formic acid	QToF-MS/MS, ESI (+)	n.a.	[81]
COT	•Centrifugation (17,000 g, 5 min)	COT-d3	10	Kinetex Biphenyl (2.6 μm, 100 × 2.1 mm) at 50°C	H_2O (0.5% acetic acid) and MeOH at 0.2 mL/min	QTrap MS/MS, ESI (+), MRM (177 > 80)	-/100	[59]
COT	•pH = 2 •Centrifugation (12,000 rpm, 5 min)	COT-d3, 20	n.a.	Synergy Polar RP (4 μm, 250 × 2.0 mm)	H_2O and MeOH both containing 0.1% formic acid	QqQ-MS/MS, ESI (+), MRM (177 > 80, 98)	100/320	[61]
COT	•Centrifugation (10,000 g, 4°C, 10 min) •pH adjustment •SPE (Cleanert PEP-2)	Codeine-d3	10	Phenomenex C18 (2.6 mm, 50 × 3.0 mm) at 35°C	H_2O (0.1% formic acid) and ACN at 0.4 mL/min	QqQ-MS/MS, ESI (+)	0.08/ 0.27	[84]

*Abbreviations: Internal standard, IS; Limit of detection, LOD; Limit of quantification, LOQ; Not available, n.a.; Cotinine, COT; *trans*-3′-Hydroxycotinine, OH-COT; Water, H_2O; Methanol, MeOH; Acetonitrile, ACN.

hydrophilicity and high water solubility [85]. As pre-treatment procedures, centrifugation, filtration and SPE have been used (Table 5). Various SPE procedures were applied and most of them were based on universal sorbents, such as hydrophilic-lipophilic-balanced (HLB) (e.g. Refs. [13,37]), Cleanert PEP-2 [73,84], and mix of Strata-X, Strata-X-CW, Strata-X-AW, isolute ENV+ [81]. The main reason was probably that most studies determined other classes of compounds as well, such as illicit drugs [37,70], pharmaceuticals [84], personal care products, pesticides and industrial chemicals [81,86] and, thus, a generic extraction procedure was required. The relative recoveries for cotinine were 72%–106% and 77%–112% for *trans*-3′-hydroxycotinine when their deuterated internal standards were used [13,27,31,32,37,73,86,87]. Therefore, the aforementioned SPE types are considered suitable for the extraction of these compounds. In addition, several studies proposed a non-SPE analysis due to the relatively high concentrations of these compounds in wastewater, considering direct injection after centrifugation and/or filtration of the sample [26,34,61]. Chromatographic separation was done using a great variety of columns as most studies were based on multi-residue methods. However, it should be mentioned that separation can also be achieved by conventional reverse phase chromatographic columns (e.g., C18 and C8) (Table 5). The detection is performed using mainly QqQ instruments in positive mode. Two product

Table 6
Main analytical methods for the determination of paraxanthine in influent wastewater.

Pre-treatment	IS, μg/L	Injection volume (μL)	Liquid chromatography		Mass spectrometry	LOD/ LOQ (ng/L)	Ref.
			Column	Mobile phase			
•Filtration (glass microfiber GF/A filters) •SPE (HLB)	CAF-^{13}C3, 15	5	BEH C18 (1.7 μm, 100 × 2.1 mm)	H_2O (30 mM formic acid/ ammonium formate, pH 3.5) and ACN (0.1% formic acid) at 0.5 mL/min	QqQ-MS/MS, ESI (+), SRM (181 > 124, 96)	-/850	[37]
•Centrifugation (5000 rpm, 10 min) •Filtration (glass fiber filter) •SPE (HLB)	PXT-d6, 50	10	Ultra-Biphenyl (5 μm, 100 × 2.1 mm)	H_2O (0.1% formic acid) and MeOH	QqQ-MS/MS, ESI (+), MRM (181 > 124, 96)	n.a.	[70]
•Filtration (1.6 μm GF/A glass microfiber filters and 0.45 μm mixed cellulose membrane filters) •pH = 6.0–7.5 •SPE (HLB)	CAF-^{13}C3, 200	2	X-Terra C18 (3.5 μm, 100 × 1 mm)	H_2O (10 mM ammonium acetate) and ACN at 0.07 mL/ min	QqQ-MS/MS, ESI (+), SRM (181 > 124, 96)	-/6.6	[32]
•Filtration (0.7 μm glass microfiber filter) •pH = 7.5–8.5 •SPE (HLB)	COT-d3, 100	15	RP C18 (1.7 μm, 150 × 1.0 mm) at 25°C	H_2O:MeOH (80:20, v/v; 5 mM ammonium acetate and 3 mM acetic acid, pH 4.7) and MeOH at 0.04 mL/min	QqQ-MS/MS, ESI (+), MRM (181 > 124)	560/ 2165	[86]
•Filtration (GF/F filters) •SPE (HLB)	COT-d3, 100	20	Chiral CBH (1.7 μm, 100 × 1.0 mm) at 25°C	H_2O (1 mM ammonium acetate)/MeOH (85:15, v/v) at 0.1 mL/min (isocratic)	QqQ-MS/MS, ESI (+), MRM	n.a.	[88]
•Centrifugation (10,000 g, 4°C, 10 min) •pH adjustment •SPE (Cleanert PEP-2)	Acetaminophen-^{13}C2–^{15}N	10	Phenomenex C18 (2.6 mm, 50 × 3.0 mm) at 35°C	H_2O (0.1% formic acid) and ACN at 0.4 mL/min	QqQ-MS/MS, ESI (+)	0.68/ 2.26	[84]

*Abbreviations: Internal standard, IS; Limit of detection, LOD; Limit of quantification, LOQ; Not available, n.a.; Caffeine, CAF; Paraxanthine, PXT; Cotinine, COT; Water, H_2O; Methanol, MeOH; Acetonitrile, ACN.

ions should be monitored for the confirmation of these compounds (e.g., 80 and 98 for cotinine and 80 and 134 for *trans*-3′-hydroxycotinine) and the quantification should be done using the corresponding labelled compounds as internal standards.

4.4. Analytical methods for the determination of caffeine biomarkers in influent wastewater

Caffeine consumption is evaluated by measurements of its exclusive metabolites, paraxanthine (1,7-dimethylxanthine) and/or 1,7-dimethyluric acid (1,7-DUA) in influent wastewater. Most analytical methods have focused on the determination of paraxanthine (Table 6) and one on 1,7-dimethyluric acid [14]. The pre-treatment procedure is based on centrifugation and/or filtration followed by SPE using an HLB cartridge. The use of a universal polymeric reversed phase sorbent for the extraction is explained by the fact that most methods determined a wide range of chemical classes simultaneously and, thus, a generic protocol was needed [37,70,84,86,88]. It must be stated that generic SPE cartridges extract many compounds and cannot clean-up the matrix effectively leading to high matrix effect, especially in complex samples, such as influent wastewater. Therefore, for accurate quantification, the corresponding labelled compounds (internal standards) should be used. The analytical methods (Table 6) have used for paraxanthine, isotopic labelled caffeine [32,37], deuterated paraxanthine [70] or surrogate internal standards [84,86,88]. Caffeine is structurally related to paraxanthine, as it differs by one methyl group, and might be a good alternative when the isotopic labelled standard of paraxanthine is not available. Indeed, recovery experiments have shown pretty good results ranging from 71% [37] to 76% [32]. The quantification of 1,7-DUA with caffeine-^{13}C3 was not considered acceptable and, thus, this compound was excluded from the method [32]. When its deuterated analogue was used (1,7-DUA-d3), the performance improved leading to 87% recovery [14]. Chromatographic separation is performed using universal columns and conventional solvents as multi-residue methods were developed (Table 6). The LC-MS/MS by QqQ is the preferred technique because of its high sensitivity and for the quantification the two main transitions were monitored; one work monitored only one transition for paraxanthine and, thus, provided semi-quantitative data [86].

5. Wastewater-based epidemiology studies for the estimation of human consumption of alcohol, nicotine, and caffeine

5.1. Alcohol consumption

Alcohol has been measured in many locations around the world and spatial patterns can be observed (Table 1). The highest levels of alcohol consumption have been seen in locations in Northern Europe – particularly in sites in Denmark, Latvia, Russia, Lithuania as well as Turkey, with measured consumed loads up to 118 mL/day/person [44,58,61,62,64,68]. High levels were also seen in sites in Canada and remote areas in the United States [31,44,66]. The lowest consumption of alcohol, according to these WBE studies were in Asia, especially sites in China, with calculated consumption of 0.2–8.1 mL/day/person. Differences between countries could be explained by the habits of the population, the profile of beverages consumed (e.g., spirits, wine, or beer) and the level of stringency of any government control. Finally, it should be noted that some studies used the total population of the catchment when performing the calculations rather than the population >15 years.

Apart from spatial trends, temporal trends can also be investigated. Temporal variations are detected for a specific location on short-term and long-term analysis. Information for a short period of time is usually obtained when performing analysis in consecutive days and is proved useful as it can detect variations that ascribed for instance to specific habits. Perhaps expectedly, alcohol consumption during the week has shown an increase on weekends. One study compared alcohol consumption across 20 cities in 11 countries showed that there were some spatial patterns in weekly consumption. For example, sites in Italy and Canada showed little variation between weekday and weekend consumption, while sites in Denmark, Germany and Norway showed a large difference [44]. Sampling campaigns for a long-term showed differences among the years or months of the same year in many cases due to several reasons, such as measures taken by the authorities, change on habits, holding events and availability of products [31,41,46,49,51,55,58,60,64]. One study investigated the impact of increasing the minimum unit price (MUP) of alcohol to curb alcohol consumption in one State in Australia. Analysing four years of WBE data found that there was a significant decrease in alcohol consumption immediately following the introduction of the MUP but after 15 months the alcohol consumption was back to levels similar to before its introduction [89].

The impact of specific events on the consumption of alcohol have also been examined. For example, alcohol consumption was shown to greatly increase during a large festival in Valencia, Spain [45] and during a school-leaver festival in Australia [49]. The impact of the COVID-19 pandemic on alcohol consumption has also been explored in various locations. Decreases in alcohol consumption were particularly evident over the weekends due to the implementation of COVID-19 social restrictions in cities in Austria and Australia [50,59]. However, when studies examined multiple sites, spatial differences were observed. For example, from six cities in Portugal and Spain, only two showed a significant difference in alcohol consumption before and after the lockdowns [67]. Similar findings were evident in Australia, where samples were analysed before, during and after the implementation of the lockdowns from capital cities of all States and Territories. Significant decreases during the lockdowns were only observed in three States [69].

It has been noted that alcohol consumption sometimes occurs in conjunction with other recreational substances and, therefore, correlations between alcohol consumption and drugs of abuse have also been investigated. Positive correlations were found in Barcelona, Spain in a specific period between alcohol and recreational drugs of abuse, such as cocaine, MDMA and amphetamine. In contrast, no correlation was found for ephedrine, methamphetamine, diazepam, morphine, and cannabis [38]. Another study found significant positive correlations between alcohol and cocaine, MDMA and amphetamine consumption for some cities and a poor correlation for others in Australia, Switzerland, Germany, Denmark, Portugal, Spain, Italy, England, Norway, Canada, and the Netherlands. Methamphetamine consumption was only significantly positively correlated with alcohol in Canberra, Australia [44]. However, it should be emphasized that the correlation does not indicate co-consumption but rather common habits of the population in specific periods. Finally, the metabolite cocaethylene was detected in wastewater in Spain and Italy indicating co-consumption of alcohol and cocaine; this metabolite is only formed after consumption of both substances [38,39].

5.2. Nicotine consumption

Nicotine consumption showed similar spatial trends to alcohol, with highest levels observed in Austria, Russia, Latvia, and Turkey [56,58,61,62,68]. Lowest consumption was seen in China [82,84] and Brazil [83]. Care must be taken when comparing the consumed mass loads in Table 2, as the biomarkers analysed and correction factors and percentage excretion values were not consistent across all studies. For example, either cotinine or *trans*-3′-hydroxycotinine (or a combination of the two) were predominantly analysed as the biomarker. Furthermore, some studies used the total population for the back-calculation and others the population >15 years.

Many studies estimated the number of cigarettes smoked considering the content of nicotine in an average cigarette and the average yield of nicotine uptake during smoking. Although this calculation can be used to compare WBE data to epidemiological data, it must be mentioned that different amounts of nicotine content in a cigarette (e.g., 0.8 [13,27,67,72], 0.9 [47,52,64,73,74], 1.0 [76], or 1.25 [24,31,57,59,63,81]) and

different absorption rates (e.g., 85% [13,71,72], 66% [52,73,74], or none [24,27,31,47,57,59,63,64,67,76,81]) have been used leading to inconsistencies in the final data and increasing the uncertainty of the methodologies.

Temporal trends were also investigated for nicotine consumption. No weekly pattern was observed for nicotine consumption, such as the characteristic peak observed for alcohol at weekends, possibly because tobacco is not specifically consumed during recreational activities but on a daily basis [27,32,35,47,55,68,72]. Long-term sampling campaigns were also carried out with mixed results depending on the country. For example, a three-year study in Spain found the same level of nicotine throughout [27]. Other studies in Turkey and Latvia detected elevated levels during some months but these periods were different for the countries [58,68]. A study in Lithuania assessed population response to stricter regulations and government efforts to reduce tobacco use and found that in two cities the nicotine consumption remained stable and in one city it decreased [64]. A study in Australia found that nicotine use declined with increased tobacco taxation over the same period [47]. There have been some studies examining the use of anatabine and anabasine as tobacco-specific markers. Methods including these have been applied in Australia [33,90,91], and evidence a decline in tobacco use [90], unable to be explained by analysing cotinine alone.

Nicotine use has also been examined during special events. Unlike alcohol, there is generally no significant difference before or after festivals (e.g. Ref. [49]). The impact of the COVID-19 pandemic and associated restrictions were also investigated. In six sites in Spain and Portugal, there was only one location where a decrease in consumption was observed, with no change in the other five [67]. In a similar study conducted in Athens, Greece a significant decrease was found for nicotine consumption during the COVID-19 pandemic [81]. These discrepancies indicate that nicotine was not as affected as other compounds during the pandemic.

5.3. Caffeine consumption

Caffeine has not been as thoroughly examined as nicotine and alcohol using WBE (Table 3), with the majority of studies coming from the United States and Europe. Either paraxanthine (1,7-dimethylxanthine) or 1,7-dimethyluric acid have been used as the biomarkers. Nevertheless, consumed mass loads of caffeine were around 100–200 mg/day/person in these studies. The highest mass loads were seen in sites in Italy [32] and the United States [66], with levels of 260–360 mg/day/person and one site in the United States up to 650 mg/day/-person. The lowest levels were found in China [84] and the Maldives [57] of less than 60 mg/day/person. In all but one location in the United States, the consumed mass loads were below the daily intake, which does not raise safety concerns recommended by EFSA (Table 3).

Caffeine consumption showed spatial trends between countries and cities within the same country and was attributed to the habits of each population rather than geographical location [14,32]. For instance, caffeine consumption was related to coffee type and caffeine content (e.g., Arabica or Robusta), preparation method (e.g., brewed, or filtered coffee), and consumption of other products containing high amounts of caffeine, such as tea, chocolate, and dietary supplements [14]. Finally, positive correlations among caffeine consumption and nicotine and other stimulants consumption were revealed through WBE analysis [70,77]. However, increased caffeine intake was not attributed to increased nicotine consumption and vice versa, but in locations where more caffeine was consumed, more cigarettes were smoked [77].

5.4. Comparison of wastewater-based epidemiology with other data sources

There are many large studies looking at the global consumption of alcohol, tobacco, and caffeine. Alcohol consumption has been monitored by the WHO, with their most recent data from 2019 showing that the highest per capita consumption of alcohol is in Europe, followed by Australia, New Zealand, and the United States. The lowest levels were in Asia and Africa [92]. This compares well with the wastewater data. The highest levels found in literature were also from Europe, while the lowest levels were in China.

In 2019 [18], it was estimated that there were 1.14 billion current smokers, with age-standardized prevalence of current use in those aged over 15 being approximately 33% for males and 7% for females. The countries with the highest prevalence of smoking tobacco were in Central Europe, Eastern Europe, and South-East Asia. The lowest prevalence was in Latin America, Africa, North America, and Oceania. This is somewhat reflected in the studies in this review, with highest levels measured in wastewater in Eastern Europe and lowest levels in Brazil.

A study investigating the global use of caffeine found that countries in North America and Europe consumed the most caffeine. This included coffee, tea, and carbonated beverages. Asia and Africa consumed the least [93]. Although there have only been few papers investigating the wastewater-based consumption of caffeine, the studies that have been published show that highest levels of caffeine were also in the United States and Italy, with lowest levels in China.

Admittedly, the studies reviewed here cannot reflect global consumption of alcohol, nicotine, and caffeine, with only small numbers of countries and sites therein monitored. Despite this, it is clear that the estimated consumption metrics calculated in these studies are comparable to that of other data sources. A comprehensive understanding of the consumption of the investigated psychotropic substances requires the collection of data from different sources, such as questionnaires, ecological momentary assessments studies, sales data, human biomonitoring studies, dietary surveys, data from handheld devices (e.g., breathalyzers) and WBE. Each technique has its own strengths and weaknesses, and no one can answer all research questions. Therefore, collaboration and data triangulation among all interested parties is required to investigate the prevalence of these substances.

6. Future perspectives and conclusions

The WBE approach is widely used as a surveillance tool to assess alcohol, nicotine, and caffeine consumption in the general population. Analytical methods are based on filtration and/or centrifugation followed by direct injection (e.g., ethyl sulphate, cotinine, and *trans*-3′-hydroxycotinine) or SPE (e.g., cotinine, *trans*-3′-hydroxycotinine, paraxanthine, and 1,7-dimethyluric acid). The main technique is LC-MS/MS using mostly QqQ mass analysers. The selected biomarkers are considered suitable as they meet all WBE requirements (detectable, excreted via human urine, unique to human metabolism and stable). This approach has been applied worldwide (e.g., the United States, Brazil, many European countries, Turkey, Russia, China, Australia, and New Zealand) under normal and special periods, such as festivals and the COVID-19 pandemic.

WBE is considered an essential, robust, and well-established surveillance tool that can provide public health data on alcohol, nicotine, and caffeine consumption in a cost-effective and timely manner without ethical issues. The great potential of WBE is seen when it delivers temporal and spatial information that can be used as an early warning system for authorities and help them make decisions and evaluate any taken actions. This approach can be used to track the achievement of the United Nations Sustainable Development Goals, such as limiting per capita alcohol consumption (goal 3.5) and limiting the use of tobacco and tobacco-related products (goal 3.a). One of the main advantages of this tool is that it can track unrecorded alcohol and nicotine, both illegal and legal, and provide overall indicators of consumption. For instance, unrecorded alcohol was estimated at 9%–44% of total consumption with a global average of 25% [94]. In addition, monitoring tobacco and interpretating the data obtained has created many difficulties between countries as different models and methods are used, producing non-comparable results, and leading to different conclusions [19].

Therefore, the use of WBE on a global scale would be an important tool that can resolve this issue and provide homogeneous comparable data for all countries and monitor tobacco use through nicotine. Caffeine consumption is mainly monitored by surveys, but this technique cannot measure total intake as in many cases the amount is not listed on the products and people do not know which products contain caffeine, leading to high uncertainties of the final estimate. To assess the total caffeine consumption in the population and compare it to the recommended by EFSA [22] usual daily intake that does not raise safety concerns (up to 400 mg per day), the WBE approach proves to be the most appropriate tool. Considering the available studies in the per-reviewed scientific literature, a few suggestions can be made to fill current gaps and improve the quality of data obtained by WBE.

- Composite samples are highly recommended to minimize uncertainties due to sampling process and obtain accurate data. When not possible (e.g., inadequate infrastructure in low-income countries), other techniques such as passive sampling should be considered, and grab sampling must be avoided. However, passive sampling needs to be investigated for these substances.
- Filtration and/or centrifugation of the samples as the first pretreatment step is highly recommended to remove solid particles, reduce matrix effects, and keep the chromatographic system in good conditions. A direct injection or SPE approach can be further applied. The direct injection can be used when the analytes are detected at high concentration levels in wastewater and the SPE when a further clean-up and/or preconcentration step is considered necessary.
- The LC-MS/MS technique is considered suitable for the analysis of the biomarkers in wastewater and two product ions should be monitored for each compound. In addition, to obtain quantitative data, the corresponding labelled compounds should be used as internal standards.
- The specific metabolites (ethyl sulphate for alcohol, cotinine with *trans*-3′-hydroxycotinine for nicotine and paraxanthine (1,7-dimethylxanthine) with 1,7-dimethyluric acid for caffeine) should be used as WBE biomarkers and not the parent compounds.
- A wide range of CF, specifically for alcohol and nicotine, have been used in different studies. This could lead to some biases when comparing the results. In addition, new pharmacokinetic studies are needed, as most of them were carried out many years ago and were relatively limited in scope. From the reviewed literature, the following CF could be applied: 3047 for ethyl sulphate, 3.4 for cotinine, 1.9 for *trans*-3′-hydroxycotinine, 23.4 for paraxanthine and 14.8 for 1,7-dimethyluric acid. When the sum of cotinine and *trans*-3′-hydroxycotinine is used, CF 1.35 can be applied.
- New studies should be performed to investigate if the conjugated forms of the metabolites are fully reverted to the free compound in raw wastewater.
- More stability studies (in-sample and in-sewage) are required, especially for data-poor compounds, such as 1,7-dimethyluric acid and paraxanthine. It would be useful to obtain real sewer experiments from different catchments to assess stability in different systems.
- The presence of nicotine metabolites in wastewater might not be due to tobacco smoking, but due to smoking cessation products or e-cigarettes, therefore, other tobacco related metabolites, such as anabasine and anatabine should be further explored.
- The studies investigated demonstrated spatial trends in alcohol and nicotine consumption within a country and temporal trends within a year in the same location. Therefore, to monitor the achievement of the United Nations Sustainable Development Goals, sampling should be done in different locations of a country and in many months of the year.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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