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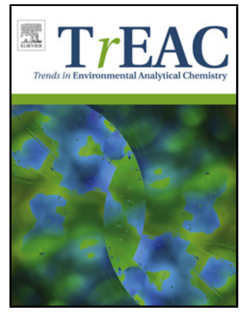

Site- and event-specific wastewater-based epidemiology: current status and future perspectives

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PII: S2214-1588(20)30033-7

DOI: https://doi.org/10.1016/j.teac.2020.e00105

Reference: TEAC 105

To appear in: *Trends in Environmental Analytical Chemistry*

Received Date: 26 July 2020

Revised Date: 15 September 2020

Accepted Date: 17 September 2020

Site- and event-specific wastewater-based epidemiology: current status and future perspectives

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Highlights

- Obtaining representative samples of wastewater from specific sites is challenging
- Cannabis use is prevalent among adolescents
- Prisoners use substitutional drugs daily
- Monitoring of drug use at special events acts as an early warning system
- WBE requires ethical consideration when applied to specific communities

Abstract

Wastewater-based epidemiology (WBE) can provide objective and reliable data to monitor spatio-temporal patterns of licit and illicit drug use. Numerous studies have been published relating to sampling, sample stability, validation of analytical protocols and the back-calculation of drug consumption. The majority of these studies focus on sampling from municipal sewage treatment plants, but an increasing number of studies have used WBE to monitor community-specific substance use and use during special events. This review presents a systematic review of published WBE studies of drug use trends in educational institutions and prisons, as well as during music festivals, sporting events, and holidays. A discussion on the application and benefits of using wastewater-based epidemiology in these specific cases is presented together with an examination of current challenges and future perspectives.

Abbreviations

2CB, 2,5-dimethoxy-4-bromophenethylamine; 4-FA, 4-fluoroamphetamine; 4-MEC, 4-methylethcathinone; 6-AM, 6-acetylmorphine; ADHD, attention deficit hyperactivity disorder; AE, anhydroecgonine; AEME, anhydroecgonine methyl ester; AMP, amphetamine; BE, benzoylecgonine; BUP, buprenorphine; COC, cocaine; COD, codeine; COE, cocaethylene; COST, the European Cooperation in Science and Technology; COT, cotinine; DHNK, dehydronorketamine; ECG, ecgonine; EDDP, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine; EDR, the European Drug Report; EMCDDA, the European Monitoring Centre for Drugs and Drug Addiction; EME, ecgonine methyl ester; EPH, ephedrine; ESPAD, European School Survey Project on Alcohol and Other Drugs; EtS, ethyl sulfate; GHB, gamma-hydroxybutyrate; HBSC, Health Behavior in School-aged Children survey; HER, heroin; KET, ketamine; LC, liquid chromatography; LOQ, the limit of quantification; LSD, lysergic acid diethylamide; LSD-OH, 2-oxo-3-hydroxy-lysergic acid diethylamide; M3G, morphine-3-glucuronide; MAMP, methamphetamine; MBDB, 3,4-methylenedioxy-N-methyl-butanphenamine; mCPP, meta-chlorophenylpiperazine; MDA, 3,4-methylenedioxyamphetamine; MDEA, 3,4-methylenedioxy-N-ethylamphetamine; MDMA, 3,4-methylenedioxymethamphetamine; MDPV, methylenedioxypyrovalerone; MDT, mandatory drug testing; MEPH, mephedrone; MOR, morphine; MPA, methiopropamine; MS/MS, tandem mass spectrometry; MTHD, methadone; MXE, methoxetamine; ND, not detected; NIC, nicotine; nor-BE, nor-benzoylecgonine; nor-COC, nor-cocaine; nor-KET, nor-ketamine; nor-LSD, N-demethyl-lysergic acid diethylamide; NPS, new psychoactive substances; PCP, phencyclidine; PEPH, pseudoephedrine; PMA, 4-methoxyamphetamine; PMMA, 4-methoxymethamphetamine; SCORE, Sewage analysis CORe group Europe; SPE, solid-phase extraction; THC, tetrahydrocannabinol; THC-COOH, 11-nor-Δ9-carboxy-Δ9-tetrahydrocannabinol; THC-OH – 11-Hydroxy-Δ9-tetrahydrocannabinol; US, the United States; USA, the United States of America; WBE, Wastewater-based epidemiology; WHO, World Health Organization; WWTP, wastewater treatment plant;

Keywords: Wastewater-based epidemiology, illicit, licit, drug, alcohol, tobacco, special event, consumption, school, prison

1. Introduction

Licit and illicit drug abuse is a global issue and represents a social, economic and health burden for the abuser and on society [1]. According to World Health Organization (WHO) data, 2.3 billion people drink alcohol worldwide, and about 1.1 billion people smoke tobacco (15 years and older), making them the most popular licit drugs [2]. The European Drug Report (EDR) states that 29% of adults (15–64 years) and 16% of young adults (15–34 years) have tried illicit drugs [3]. Over the last decade, a large variety of new psychoactive substances (NPSs) have emerged that can be easily bought either on the drug market or in physical and online shops and on the darknet [3]. In this review, the consumption of the most common licit drugs (alcohol and nicotine/tobacco), and illicit drugs (cocaine, amphetamine, methamphetamine, ecstasy, lysergic acid diethylamide – LSD, heroin and cannabis), together with NPSs and opioids, such as morphine, codeine, methadone, buprenorphine and fentanyl, are discussed.

Drug consumption in the population is usually assessed by traditional epidemiological methods (e.g., surveys), while other information, such as crime statistics (e.g., seizure data) and medical records (e.g., overdoses) are also a valuable source of information [3, 4, 5]. The problem with surveys is that they are subject to errors arising from reporting biases, have low response rates, raise ethical issues and are slow to detect the use of NPSs [4,5]. This review is focused on studies utilizing an alternative approach referred to as wastewater-based epidemiology (WBE). WBE is becoming an increasingly popular approach for providing additional information on substance use and misuse due to its objectivity, cost-effectiveness and its ability to provide data in near-real-time. It is based on the accurate determination of human metabolic excretion products (biomarkers) of licit and illicit drugs in wastewater. From the measured levels of biomarkers, consumption in a target population can be back-calculated by taking in account additional information such as wastewater flow and by applying a correction factor that takes into account human metabolism [4,5]. Since its first application in 2005 [4], WBE has undergone continuous development and improvement [6,7]. For example, in 2012, a group of scientists supported by the European Cooperation in Science and Technology (COST) established the Sewage analysis CORe group Europe (SCORE) to gather experts to discuss, develop and standardize the WBE approach [8]. The European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) has also recognized and supported the SCORE group and promoted WBE as an additional tool for estimating drug use [9]. To date, numerous studies relating to sample collection [10], sample stability [11], validation of analytical protocol [7,12] and back-calculating drug consumption [5,6] have been published, and are evidence of WBE's potential for studying spatio-temporal consumption patterns in the general population [13–16].

However, obtaining drug use trends is not only of interest concerning the general population but also specific populations that are highly susceptible to drug use (e.g., adolescents, prisoners). For example, in comparison to the general population, illicit drug use is more frequently reported in nightlife settings (clubs and bars) and during music festivals, where they are often co-consumed with other psychoactive substances, including alcohol and tobacco [3]. Moreover, according to the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) *"prisoners report higher lifetime rates of drug use and more harmful patterns of use, including injecting, than the general population"* [3]. Several WBE studies exploring the impact and extent of substance abuse in specific catchments, e.g. educational institutions, prisons, fitness centers and at an airport (site-specific WBE), and during special events such as music festivals, sporting events and holidays (event-specific WBE), have emerged and are summarized and discussed in this review.

This review presents a discussion on the application and benefits of using WBE for assessing licit and illicit drug consumption in specific catchments. The results of published studies are presented together with an examination of current challenges and future perspectives. Referenced studies were found by searching the following keywords: wastewater analysis, wastewater-based epidemiology, illicit drug, alcohol, tobacco, school, prison, small population, event, festival, holiday.

2. Methodological challenges relating to sampling

Sample collection, filtration, sample preparation and instrumental analysis are the first steps in the WBE approach. Sample preparation mainly involves extraction of analytes by solid-phase extraction (SPE) followed by liquid chromatographic (LC) separation coupled to tandem mass spectrometry (MS/MS) or high-resolution mass spectrometry (HRMS). When determining alcohol and tobacco biomarkers, direct sample injection without extraction is possible because of their high concentrations in the samples. Analytical protocols are well established and regulated by "best practice protocols" developed in the frame of COST SCORE actions [6,7] and summarized elsewhere [12]. In site- and event-specific studies, similar analytical protocols are applied. In this section, the focus is on sampling strategies since obtaining a representative wastewater sample represents a significant challenge in WBE when applied to specific catchments.

In the case of site-specific studies (studies conducted in small sub-catchments, e.g. educational institutions, prisons, fitness centers, airport), raw wastewater samples are typically collected directly from the sewage outlet or at the inlet of a wastewater treatment plant (WWTP) installed at the studied site (Table 1). At the same time, sampling during special events has been mostly performed at municipal WWTPs (Table 2). Site-specific studies face unique challenges, notably when sampling upstream from the WWTP in small sub-catchments or at specific sites [17,18]. Here, good knowledge of the sewer system is essential, since the physical boundary of the sewer system (e.g. depth of the sewer) and the availability of a power source can disable a sampling campaign (e.g., when an autosampler is used over an extended period) [19,20]. Also, obtaining an adequate composite wastewater sample using an autosampler becomes an issue, since a small and inconsistent wastewater flow may prevent subdivisions of the composite samples from being obtained, such as during times of no flow [21].

Moreover, low and inconstant wastewater flow makes it difficult to measure wastewater flow, which is needed to calculate drug consumption estimates. In such cases, the flow rate can be estimated from the monthly water bill obtained for a specific site. If no leakage is assumed, the water used that month is drained as wastewater from the sampling site. The average daily wastewater flow can be calculated by dividing used water (read from water bill) and the number of days in the month. However, calculating the flow this way introduces uncertainty into the consumption estimates, since wastewater flow can vary from day to day. When sampling at source in a site-specific situation, a higher content of solids, namely sanitary tissue, feces, and solid waste creates additional problems such as clogging of the autosampler [22]. Solutions to this problem include installing a solids separator and adapting the autosampler to operate under compressed air [23].

Typically, 24-hour composite samples are collected in studies assessing drug consumption in the general population [13,14]. However, when sampling wastewater at specific sites, sampling intervals have to be frequently adjusted (Table 1). For instance, Zuccato *et al.* [24] collected samples during an 8-h school day, while others collected 2-h composite samples [23,25], hourly samples to study time-dependent use patterns [26], and samples collected over an extended period (72-h composite samples) at sites with limited access [22,27,28]. For estimating drug consumption during special events, 24-h composite samples are commonly collected (Table 2) while grab sampling, which is not recommended because of its inability to capture high intraday variability of drug excretion, has rarely been applied [29–31]. Tables 1 and 2, list the three most common autosampling modes: flow-, volume- and time-proportional, which were used to obtain composite wastewater samples in specific catchments and during special events. Flow-proportional sampling is used to overcome flow rate variability [27] and thus is recommended as the most suitable sampling mode for collection of wastewater samples in small catchments. Several site- and event-specific studies have used volume-proportional sampling, even though it does not provide accurate averages of analyte concentrations since individual samples are not corrected for wastewater flow [10,19]. In this respect, volume-proportional sampling is similar to time-proportional sampling (constraint frequency and sampling volume), which is frequently used in site-specific studies [19].

A critical factor, when time-proportional sampling is used, is sampling frequency [19,20], and the main parameter dictating sampling frequency is the variability in the number of pulses, e.g., toilet flushes [10,19]. At specific sites, such as prisons and schools, a high sampling frequency is essential because of the low number of pulses. In such cases, the optimal sampling frequency should be determined using a preliminary dye tracer test [22,37] or by analyzing flow dynamics [24].

Table 1. Overview of WBE application in site-specific studies

Population	Country	Sampling period	Type of raw wastewater sample	Sampling location and type of sampling	Target drugs	Reference
EDUCATIONAL INSTITUTIONS						
Educational institution *(less than 1 000 students)*	USA	Different days during regular class sessions, final exams and summer break *(sampling between 10 a.m.–12 a.m. and 12 a.m.–2 p.m.)*	2-hour composite samples	Sewer pipe output from the building Manual collection *(up to 500 mL every 20 min)*	Illicit drugs (cannabis, MDMA, AMP, COC, HER) NPSs (MDA) Opioids (COD, MOR)	[25]
Main university campus and dorms *(9 456 people in the university campus and 560 people in dorms)*	USA	a) Dorms *(2012–2013)*: - Beginning of the semester *(12 days at the beginning of September)* - End of the semester *(9 days during the second and third week in December)* - Middle of the semester *(17 days between February and March)* b) Main university campus *(sampling over 3 weeks in April 2013)*	24-hours composite samples	Pump station Time-proportional sampling *(200 mL every 4 hours)*	Illicit drugs (cannabis, AMP, MAMP, MDMA, COC, HER, LSD) NPSs (MDA, MDEA) Opioids (COD, MOR, MTHD)	[32]
Private college of art *(4 dorms, 476 students)*	USA	a) First semester (4 samples): the first week, midterms, post-midterms, finals week	72-hours composite samples	Sanitary sewer line Time-proportional samples *(125 mL every*	Illicit drugs (AMP)	[22]

		(August–December 2011) b) <u>Second semester (5 samples):</u> the first week, midterms, post-midterms, last week, finals week *(January–July 2012)*		*hour*) integrated into a total volume over 72-hour period regarding the measured flow		
College campus (4 residences halls, 476 undergraduate students)	USA	The first week of school, midterms and shortly before final exams *Additionally: Web-based surveys (400–627 respondents) was conducted in the same time frames*	72-hours composite samples	On-campus sampling location Time-proportional samples *(125 mL every hour*) integrated into the total volume over 72-hour period regarding the measured flow	Illicit drugs (AMP)	[28]
University campus *(in total 15 000–60 000 persons)*	USA	7 consecutive samples, collected once per month over 5 months *(August–December 2017)*	24-hour composite samples	Two sampling locations at the campus sewer system Flow-proportional sampling	Illicit drugs (AMP, MDMA, COC, HER) Opioids (MOR, COD, MTHD, BUP, fentanyl)	[33]
8 secondary schools *(3 classic, scientific or artistic education and 5 professional or vocational schools*), located in different cities *(Bologna,*	Italy	a) 5 or 6 consecutive daily samples *(May 2010)* b) Repeated sampling in schools from Rome, Turin, Verona *(October 2011, March and October 2012, November 2013)*	8-hours composite samples *(samples collected during lessons period)*	Main sewage pipe Time-proportional sampling *(1L every hour)*	Illicit drugs (cannabis, AMP, MAMP, MDMA, COC, HER) NPSs (KET, MEPH) Opioids (MOR)	[24]

Florence, Milan, Naples, Palermo, Rome, Turin, Verona) *(in total: >6 000 students)*						
University campus without dormitories *(1 600 ± 130 people)* *Additional sampling sites: Mytilene (26 000 people), the island capital, and two small villages (1 250 people)*	Greece	a) University 5 consecutive days over the week b) Additional sampling sites 7 consecutive days *All samples were collected between 10th February to 10th March 2015.*	24-hours composite samples	3 WWTP, each serves a particular population (university, Mytilene or villages) Time-proportional sampling *(6 mL per minute)*	Illicit drugs (cannabis, AMP, MAMP, MDMA, COC, HER) NPSs (KET, butylone, ethylone, methylone, MPA, PMMA, PMA, MEPH, MXE, MDPV) Opioids (MTHD) Alcohol	[34]
PRISONS						
Two prisons (*covering three buildings)*	France	Not specified	24-hour composite samples	Sewer pipe output of the participated buildings Flow-proportional sampling	Illicit drugs (cannabis, MDMA, COC, HER) NPSs (MEPH,4-MEC) Opioids (MOR, MTHD, BUP)	[35]

Prison *(around 3 500 people in total)*	Spain	a) 10 or 11 consecutive days in the middle of each month *(June 2008–January 2009)* b) 1 sample every Monday *(during the rest of the month)*	24-hour composite samples	WWTP serving exclusively the penalty complex Type of sampling was not reported	Illicit drugs (cannabis, AMP, MAMP, MDMA, COC, HER, LSD) NPSs (EPH) Opioids (MOR, MTHD)	[36]
Prison *(no data on target population)*	USA	a) Sampling over 28 days *(30 July–2 August 2011)* b) Hourly samples collected over 3 days *(13–15 August 2011)* *Additionally: During the sampling period, 243 urinalysis tests were carried out.*	a) 24-hour composite samples b) Hourly samples	Details on sampling spot were not reported Constraint collection with a peristaltic pump *(flow rate of 8 mL/min)*	Illicit drugs (MAMP, COC)	[26]
Prison *(467–523 people in total)*	Australia	7 consecutive days *(January 2013)*	24-hour composite samples	Sewer pipe output outside the prison grounds Volume-proportional sampling *(median sampling interval: 2 min or 20 mL every 250 L of flow)*	Illicit drugs (cannabis, AMP, MAMP) NPSs (KET) Opioids (COD, MOR, MTHD)	[37]
Prison *(437 people in total)*	Australia	Two periods of 12 consecutive days *(May–July 2013)*	24-hour composite samples	Sewer pipe output of the building	Illicit drugs (cannabis, COC, MDMA, MAMP)	[38]

		Additionally: During sampling periods, 40 urinalysis tests were carried out.		Volume-proportional sampling *(median sampling interval: 2 min or 20 mL every 250 L of flow)*	NPSs (MDA, MDEA, KET, MEPH, methylone) Opioids (COD, MTHD, BUP)	
OTHER SPECIFIC SITES						
3 fitness centres *(no data on targeted population)*	Germany	2 days *(12 samples per day)*	2-hours composite samples	Sewer pipe output from the building Time-proportional sampling	Illicit drugs (AMP, MAMP, MDMA) NPSs (EPH)	[23]
National airport of Amsterdam - Schiphol *(40 000 people)* *Additional sampling sites: Utrecht (529 000 people), Eindhoven (544 030 people), Apeldoorn (351 500 people), Amsterdam (913 435 people)*	Netherlands	a) Airport Sampling 3 days of the week and all weekend b) Additional sampling sites Sampling over one week. *All samples were collected between the third and fourth week of February 2010.*	a) Airport Weekdays: 24-hours composite samples Weekend: 72-hours composite sample b) Additional sampling sites: 24-hours composite samples	a) WWTP serving the airport b) WWTP serving each city All samples were collected in flow-proportional mode.	Illicit drugs (cannabis, AMP, MAMP, MDMA, COC, HER) NPSs (KET) Opioids (COD, MOR, MTHD)	[27]

4-MEC – 4-methylethcathinone, AMP – amphetamine, BUP – buprenorphine, COC – cocaine, COD – codeine, EPH – ephedrine, HER – heroin, KET – ketamine, LSD – lysergic acid diethylamide, MAMP – methamphetamine, MDA – 3,4-methylenedioxyamphetamine, MDEA – 3,4-methylenedioxy-N-ethylamphetamine, MDMA – 3,4-methylenedioxymethamphetamine, MDPV – methylenedioxypyrovalerone, MEPH – mephedrone, MOR – morphine, MPA – methiopropamine, MTHD – methadone, MXE – methoxetamine, PMA – 4-methoxyamphetamine, PMMA – 4-methoxymethamphetamine

Table 2. Overview of WBE applications for monitoring drug consumption trends during special events

Special event	Country	Population	Sampling period	Type of raw wastewater sample	Sampling location and type of sampling	Target drugs	Reference
MUSIC FESTIVALS							
Largest music festivals in Slovakia	Slovakia	a) Pohoda Festival - multicultural festival (near Trenčín) 30 000 attendants (47 000 inhabitants inTrenčín) b) Lodenica Festival – folk and country festival (near Piešťany) 10 000 attendants (35 000 inhabitants Piešťany)	a) 12–13 July 2013 b) 30–31 August 2013 *(plus control samples one week later)*	24-h composite sample	a) Trenčín WWTP including contents of special reservoirs Time proportional sampling b) Piešťany WWTP Time proportional sampling *(15-min intervals)*	Illicit drugs (cannabis, AMP, MAMP, MDMA, COC)	[39]

Annual music festival *(includes a wide range of arts and attendants of different ages 6–60+)*	Australia	*a) festival 2010* In total 100 000 attendants (on average 16 700 attendants/day) *b) festival 2011* In total 88 600 attendants (on average 14 700 attendants/day)	Music festival 2010 and 2011 One week in summer *Additionally: In 2010, samples were collected in a nearby urban area (~350 000 inhabitants)*	24-h composite sample	One-site WWTP *(received wastewater only from the festival)* a) festival 2010: Continuous sampling side stream b) festival 2011: Flow-proportional sampling	Illicit drugs (cannabis, COC, AMP, MAMP, MDMA) NPSs (MEPH, methylone, benzylpiperazine)	[40]
7 music festivals with different music genre preferences	The Czech Republic and Slovakia	a) Guláš Fest (Valašské Meziříči) – country/folk music b) VanDaal fest (Zubří) – metal music c) Grape Festival (Piešťany) – dance music d) Topfest (Piešťany) – pop/rock music e) Gypsy Fest (Bratislava) f) Skalické dni (Skalica) – multi-genre music	several days before, during and after festivals	24-h composite sample	Wastewater from WWTPs of cities where festivals took place (including portable toilet Contents) Time-proportional sampling *(15-min intervals)*	Illicit drugs (cannabis, AMP, MAMP, MDMA, COC, HER, LSD) NPSs (MDEA, MBDB, KET, cathinone, mephedrone) Opioids (COD, MTHD, BUP)	[41]

		g) Pohoda Festival (Trenčin) – pop/rock music 8 000–20 000 festival attendants 14 000-45 000 inhabitants					
Music day event	France	2 WWTPs about 500 000 inhabitants	9 consecutive days a) 15–23 June 2017 (including music event on 21 June) b)17–22 May 2018	24-h composite sample	2 WWTPs in Bordeaux Flow-weighted sampling *(6 times per hour)*	Illicit drugs (COC, MDMA, cannabis)	[42]
Two Amsterdam street festivals	Netherlands	300 000 attendants 769 000 inhabitants (Amsterdam)	Samples collected before and during the festival *(Thursday to Sunday in Summer 2012 and 2014)*	24-h composite sample	Amsterdam WWTP Flow-proportional sampling	NPSs (560 different NPSs)	[43]
The youth festival - Spring Scream	Taiwan	600 000 attendants	During the week of the youth festival *(1–7 April 2011)*	Grab sample	Nanwan and Kenting WWTPs	Illicit drugs (cannabis, AMP, MAMP, MDMA, COC, HER) NPSs (KET, PEPH, GHB)	[30]

Music festivals with different music genre preferences	The Czech Republic and Slovakia	a) Guláš Fest (Valašské Meziříči) – country/folk music b) VanDaal fest (Zubří) – metal music c) Grape Festival (Piešťany) – dance music d) Topfest (Piešťany) – rock/metal music e) Gypsy Fest (Bratislava) f) Skalické dni (Skalica) – multi-genre music *In total: 10 000–20 000 festival attendants and 13 500–45 000 inhabitants*	a) 18–20 July 2014 b) 16–18 August 2014 c) 15–16 August 2014 d) 26–28 June 2014 e) 8–9 August 2014 f) 20–21 September 2014	24-h composite sample	WWTPSs of cities where the festival took place Time-proportional sampling	Tobacco	[44]
Music festival *(Fallas festivity)*	Spain	Total of 1 500 000 inhabitants (Valencia city)	4–20 March 2014 (Fallas festivity: 15–19 March 2014)	24-h composite sample	3 WWTPs in Valencia city No data about sampling type	Alcohol	[45]
Largest Spanish and European	Spain	a) Music event Approximately 40 000 attendants	a) Music event *One week in July 2008 (the event*	24-h composite sample	Benicasim WWTP	Illicit drugs	[46]

annual pop, rock and electronic festivals		b) Benicasim (city) 15 564 inhabitants	*took place on 17–20 July)* b) Periods without a music festival *(one week in June and January 2008)*		including contents of portable toilets Time-proportional sampling *(1 L every hour)*	(cannabis, AMP, MAMP, MDMA, COC) NPSs (MDA, MDEA)	
The graduates' celebration – Russ	Norway	50 000 high school graduates 500 000 inhabitants (Oslo)	Year-long sampling in 2010 *(sampling period during the festival: 12)*	Passive sampling (Polar organic chemical integrative samplers, POCIS)	From the large gravity tunnel tube system before WWTP of Oslo POCIS were replaced every two weeks	Illicit drugs (AMP, MAMP, MDMA, COC)	[47]
Street parade	Switzerland	600 000 attendants	a) Street parade *(4 September 2009)* b) Reference week *(Wednesday)* c) The day after the event *(Sunday)*	24-h composite sample	Central WWTP Zurich Flow-proportional sampling	Illicit drugs (cannabis, AMP, MAMP, MDMA, COC, HER) Opioids (COD, MOR, MTHD)	[48]
HOLIDAYS							
Christmas holiday season	Croatia	Zagreb *(688 163 inhabitants)*	a) holidays 2012–2013 *(21 December 2012–4 January 2013)*	24-h composite sample	Central WWTP Zagreb Time-proportional sampling	Illicit drugs (AMP, MDMA, COC, HER) Opioids (MOR, MTHD)	[16]

			b) holidays 2013–2014 *(20 December 2013–3 January 2014)*		*(15-min intervals)*		
Christmas holiday season	Australia	a) urban area *(350 000 inhabitants)* b) semi-rural area *(120 000 inhabitants)* c) vacation area *(1 100–2 400 inhabitants)*	holiday *(23 December 2010–3 January 2011)* normal day *(26 February–3 March 2011)*	24-h composite sample	Urban area and vacation area: Flow-proportional sampling Semi-rural area: Volume-proportional sampling	Illicit drugs (Cannabis, MAMP, MDMA, COC)	[49]
Independence Day 2017 The 2017 solar eclipse The first week of an academic semester, 2017	USA	2 communities A: 20 000 inhabitants B: 25 000 inhabitants *(40% of the B community are professors and students)*	a) Independence Day celebration *(30 June–6 July)* b) Total solar eclipse observation day *(19–22 August)* c) the first week of an academic year *(11–17 August)* d) typical week	24-h composite sample	2 WWTPs in Western Kentucky Time-proportional sampling *(15-min intervals)*	Illicit drugs (cannabis, AMP, MAMP, MDMA, COC) NPSs (MDA, MDEA) Opioids (MOR, MTHD)	[50]

			(26 July–1 August 2017)				
a) Memorial Day b) 4th of July c) Labor Day d) New Year	USA – New York	a) North River WWTP: 588 772 inhabitants b) Newton Creek WWTP: 1 068 012 inhabitants c) Hunts Point WWTP: 684 569 inhabitants d) Tallman Island WWTP: 410 812 inhabitants e) Jamaica WWTP: 728 123 inhabitants	Sampling on days before and after major holidays in 2016: Memorial Day *(27 and 31 May 2016)* 4th of July *(1 and 5 July 2016)* Labor Day *(2 and 6 September 2016)* New Year *(30 December, 3 January 2017)*	One-time grab samples between 8:00 am and 11:00 am	5 WWTPs No data about sampling type	Illicit drugs (AMP, MAMP, MDMA, COC) NPSs (MDA) Opioids (COD, MOR, MTHD, fentanyl) Tobacco	[31]
a) Chinese Spring Festival b) National Day	China	WWTP in Guangzhou No data about the population	8 weeks across 3 months 2017: 1–10 January 23 January–5 February (including Chinese Spring Festival)	24-h composite sample	WWTP in Guangzhou No data about sampling type	Illicit drugs (AMP, MAMP, MDMA, COC) NPSs (KET) Opioids (COD, MTHD)	[51]

			9–22 May 27 September–12 October (including National Day)				
Carnival	Brazil	A North-Wing WWTP: 145 000 inhabitants A South-Wing WWTP: 525 000 inhabitants	8 consecutive days *(30 May 2017–6 June 2017)* Carnival day *(13 February 2017)*	24-h composite sample	2 WWTPs Flow-proportional a sampling	Illicit drugs (COC)	[52]
Easter holiday (including the examination period at university and end of university year)	France	70 000 inhabitants *(students presented ~20% of the total population)*	84 consecutive days *(21 March–11 June 2016; except on 31 March)*	24-h composite sample	WWTP Flow-proportional sampling *(50 mL every 30 m^3 of Influent)*	Illicit drugs (AMP, HER, COC, cannabis, MDMA) Opioids (COD, MOR, MTHD, BUP)	[53]
Summer tourist Season	Croatia	a) Zadar *(64 324 inhabitants + 16 % increase of population during summer season)* b) Zagreb *(688 163 inhabitants)*	*a) Zadar* - *off-season (18–27 March 2013)* - *tourist season (21 July–1 August 2013)* c) Zagreb	24-h composite sample	WWTP Zadar Time-proportional sampling *(15-min intervals)*	Illicit drugs (AMP, MDMA, COC, HER) Opioids (MOR, MTHD)	[54]

			(24–31 July 2013)				
Summer holiday season	Korea	a) WWTP 1: semi-rural area b) WWTP2: residential area c) WWTP 3: vacation area No data about the population	8–10 July 2013 (before the main holiday period) 1–3 August 2013 (in the peak Holiday period) 12–14 September 2013 (after the main holiday period)	24-h composite sample	3 WWTPs Time-proportional sampling	Illicit drugs (MAMP, MDMA, HER) NPSs (MDA, MDEA, meperidine, KET) Opioids (COD, MOR, MTHD, BUP, fentanyl)	[55]
Summer holiday season (coinciding with school holidays)	Italy	a) WWTP 1 Fondo Verde: 45 000 inhabitants b) WWTP 2 Acqua dei Corsari 340 000 inhabitants	March–November 2015 *(with the exception of May and August)*	24-h composite sample	2 WWTPs in Palermo No data about sampling type	Illicit drugs (cannabis, MAMP, MDMA COC) NPSs (MDA, MDEA)	[56]
Christmas holiday season	Australia	a) WWTP 1: 728 759 inhabitants b) WWTP 2: 75 225 inhabitants c) WWTP 3: 155 604 inhabitants	19 December 2018–1 January 2019	24-h composite sample	4 WWTPs Flow-proportional sampling	NPSs (21 NPSs in total)	[57]

		d) WWTP 4: 212 309 inhabitants					
a) Christmas holiday season b) Australian Day	Australia	South-East Queensland 2015: 105 532–106 788 inhabitants 2016: 107 037–108 292 inhabitants 2017: 108 542–109 294 inhabitants	7 consecutive days in every second month *(February, April, June, August, October, December) 2012–2017* a) Christmas–New Year holiday *(21 December–31 January) 2015/2016 2016/2017* b) Australian Day *(26 January)*	24-h composite sample	WWTP located in South-East Queensland Flow-proportional sampling	Alcohol	[58]
SPORTING EVENTS							
FIFA Soccer World Cup	Brazil	68 000 people per game only inside the stadium 700 000 inhabitants *(served by 2 WWTPs from Brazilian Federal District)*	a) Argentina vs Belgium *(5–6 July 2014)* b) 3rd place playoff *(12–13 July 2014)* c) reference week *(21–22 April 2012)*	24-h composite sample	2 central WWTPs Flow-proportional	Illicit drugs (AMP, MAMP, MDMA, COC) NPSs (MDA, MDEA, MBDB)	[59]

National Football League's - Super Bowl	USA	Not reported	a) Super Bowl *(7–8 February 2010)* b) Baseline week *(7–8 March 2010)*	12-h composite sample	WWTP Time-proportional	Illicit drugs (cannabis, AMP, MAMP, MDMA, COC, HER) NPSs (MDA) Opioids (MOR)	[60]
Football game at the university	USA	a) University - *Fall semester: 18 800 students* - *During football games:>60 000 attendants (depends on the game held)* b) City of Oxford (20 800 inhabitants)	During weekends on which football home games were held: - Friday (before the game) - Saturday (during the game) - Sunday (after the game) Non-game days *(Two Wednesdays, before the games)*	Grab sample	a) University WWTP Samples manually collected from the influent reservoir b) Oxford WWTP Samples collected using a pump	Illicit drugs (AMP, MAMP, MDMA, COC, HER) NPSs (MDA, MDEA, PCP) Opioids (COD, MOR, MTHD, fentanyl)	[29]

AMP – amphetamine, BUP – buprenorphine, COC – cocaine, COD – codeine, GHB – gamma-hydroxybutyrate, HER – heroin, KET – ketamine, LSD – lysergic acid diethylamide, MAMP – methamphetamine, MBDB – 3,4-methylenedioxy-N-methyl-butanphenamine, MDA – 3,4-methylenedioxyamphetamine, MDEA – 3,4-methylenedioxy-N-ethylamphetamine, MDMA – 3,4 methylendioximethamphetamine, MEPH – mephedrone, MOR – morphine, MTHD – methadone, PCP – phencyclidine, PEPH – pseudoephedrine

The primary source of uncertainty in sampling, which can exceed 100%, depends on catchment characteristics, mode of sampling, and sampling frequency [10,29]. Burgard *et al.* [22] are the only group so far to determine sampling uncertainty connected with at source sampling (e.g. dorms) by measuring mass loads of creatinine and a human urine marker in wastewater. The authors surmise that since excretion, and consequently the mass load, of creatinine, is assumed constant, the calculated deviation in measured loads is attributable to sampling uncertainty, which was 31%. Others have also determined creatinine levels [22,28,31] but in order to account for population variations and dilutions.

Passive sampling can be used to overcome autosampling issues (e.g., varying wastewater flow, missing pulses – toilet flushes, power requirements) [19]. Passive samplers, such as polar organic chemical integrative sampler (POCIS), have been applied in WBE studies of the general population [47]. Their application is, however, limited due to practical challenges, e.g., clogging and difficulties in determining uptake rates [19]. To the author s' knowledge, passive samplers have not been used in site-specific WBE studies, although POCIS was used to monitor illicit drugs and their metabolites in wastewater during the Norwegian "russefeiring" or "the russ" celebrations for high school graduates [47].

To summarize, in site-specific studies flow-proportional sampling mode should be used to overcome variations in flow, while for time-proportional sampling, the sampling frequency should be optimized (using dye trace test or similar) to minimize the chance of missing wastewater pulses (e.g., toilet flushes). As an alternative to active sampling, passive sampling can be used, but its possible application in specific sites still needs to be explored. In the case of event-specific studies, wastewater is commonly collected at WWTP using standardized sampling procedures performed by the WWTPs' staff. As such, the sampling procedure is appropriate to address drug consumption patterns during special events, such as music festivals, holidays and sporting events and needs no adjustment.

3. Applicability of WBE for studying drug consumption patterns in site-specific studies

WBE has been utilized to gain insight into licit and illicit drug consumption patterns in susceptible populations, namely adolescents, prisoners, travelers and fitness users. Details of the sampling strategies used and the data obtained are presented in Tables 1 and 3, respectively. The data are hard to compare since the data are presented differently in different studies (e.g., as concentration, normalized concentrations, mass loads, consumption estimates), while consumption estimates are made based on different biomarkers (e.g. heroin consumption estimated from morphine or 6-acetylmorphine).

3.1. Educational institutions

Monitoring drug use is especially important for preventing the spread of drugs among young people, including schoolchildren and students. In Europe, data on drug use among teenagers derive mainly from the European School Survey Project on Alcohol and Other Drugs – ESPAD (15- to 16-year olds) [61] and the Health Behavior in School-aged Children – HBSC survey (11-, 13-, 15- and 17-year olds) [62]. In the US, several public secondary schools have considered mandatory drug testing (MDT) of their students, but such demands have so far been met with resistance by the public because of the personal invasiveness of the tests [25].

To date, several WBE studies in educational institutions, including universities [22,25,28,32–34] and secondary schools [24], have been completed. The focus was on opioid abuse and consumption of NPSs, while only one study investigated ethyl sulfate, a biomarker of alcohol consumption (Table 1). Biomarkers of conventional drugs (cannabis, amphetamines, cocaine, and heroin) were present in wastewater from universities, as well as in wastewater from secondary schools (Table 3). The data show that cannabis was the most commonly used drug being used almost exclusively in secondary schools (cannabis consumption: up to 2 893 mg/day/1 000 people *vs* cocaine consumption: up to 187 mg/day/1 000 people). In contrast, biomarkers of opioids, such as codeine, morphine, methadone, and fentanyl, were detected sporadically although, in a US university, opioid biomarkers, such as codeine [32] and morphine-3-glucuronide [32] were present in 100% and 98% of the wastewater samples, respectively. Also, Gushgari *et al.* [33] observed a high average consumption of cocaine (551

± 49 mg/day/1 000 people) and heroin (474 ± 32 mg/day/1 000 people, based on 6-acetylmorphine) at a US college campus (cannabis consumption was not examined) compared to other studies conducted in educational institutions, where up to 280 mg of cocaine/day/1 000 people and up to 69 mg of heroin/day/1 000 people (based on morphine) were estimated to be consumed. Among the NPSs, two US studies [25,32] report the presence of 3,4-methylenedioxyamphetamine (MDA). Ethyl sulfate (alcohol biomarker), although detected, was below the limit of quantification (LOQ) in wastewater from a university campus in Greece [34].

Several groups also used WBE to correlate drug use patterns with varying levels of stress during different school periods [22,25,28,32]. Panawennage *et al.* [25], found that drug use was higher during the final exam period compared to regular class time, with cannabis consumption doubling during this period. Heuett *et al.* [32] also observed an increase in cannabis consumption at the end of the semester, while amphetamine consumption remained relatively constant. In contrast, Burgard *et al.* [22], who explored the non-prescribed use of attention deficit hyperactivity disorder (ADHD) medications such as Adderall (mixed amphetamine salts, excreted in the urine as 30–40% intact amphetamine) during periods of low and high stress, found an increase in amphetamine concentration during stressful times. Although sampling uncertainty was high because of the low sampling frequency, the results revealed an eight-fold increase in amphetamine concentration, normalized for creatinine, during times of high academic stress (final exams). Moore *et al.* [28] also reported that the use of Adderall increased during high-stress periods. In their study, up to a 3-times higher concentration of amphetamine (normalized for creatinine) was found in wastewater samples during high-stress periods compared to the first week of class. The results were in good agreement with results obtained by a self-reporting survey, conducted in parallel, where the number of people reporting the non-prescriptive use of Adderall increased by up to 4-times during high-stress periods.

Weekly consumption trends at a university campus were explored by Gushgari *et al.* [33] but found no statistically significant variation in the mass loads of opioids and 3,4-methylendioximethamphetamine (MDMA). In contrast, higher loads of amphetamine and benzoylecgonine (the main cocaine metabolite) were detected during weekdays and the weekend, respectively. Also, Heuett *et al.* [32] observed a higher consumption of cannabis and amphetamine during weekdays on the main campus, which is probably a likely consequence of the higher number of students present during weekdays. Annual variations in drug consumption in Italian secondary schools were explored by Zuccato *et al.* [24]. They found up to a 20-fold increase in cannabis consumption over a four-year monitoring period (2010–2013), although morphine and cocaine use varied such that no time consumption trends were observable.

Differences in drug consumption between institutions offering different educational programs, such as classic, scientific, artistic, vocational, and professional secondary schools, was also explored by Zuccato *et al.* [24]. The data show that illicit drug consumption is higher in secondary schools that offer classic, scientific or artistic education. For example, 11- and 75-times higher consumption of cannabis and cocaine was observed in the former compared to vocational or professional schools.

A good agreement between a national survey and WBE was reported by Panawennage *et al.* [25], both studies found that cannabis was the drug of choice among adolescents in the USA. In comparison to the general population, cannabis (schools in Milan, Turin, Verona and Naples) and morphine use (a school in Verona) in Italian secondary schools was in the same order of magnitude, while the consumption of morphine (except in a school in Verona) and cocaine was 40% and 20% lower [24]. Lower consumption of cannabis (100 mg/day/people) was observed on the university campus on the island of Lesvos, Greece compared to two small nearby villages (1 200 mg/day/1 000 inhabitants) and the capital, Mytilene (2 800 mg/day/1 000 inhabitants) [34].

Despite the usefulness of WBE, its application to educational institutions is not without its limitations, i.e., students spend only limited time in school and may excrete drug biomarkers elsewhere [24,33]. Also, an individual's excretion profile may have a significant effect on drug consumption trends and, therefore, sampling for an extended period at institutions with a larger number of students is desirable. In order to avoid bias, students should be unaware of the research being undertaken, and even if all of the purposed measures are taken into account, the data may not necessarily reflect drug use only among the students, since it is not possible to distinguish between students and staff.

3.2. Prisons

Another site-specific application, where WBE can be of benefit, is in evaluating drug use in prisons, where consumption has a significant impact on inmate detoxification, treatment, reintegration and prison security [37]. In Europe, the prevalence of drug consumption among prisoners is traditionally assessed by surveys and MDT, with urinalysis as the method of choice [63]. Accurate data is difficult to obtain from surveys, while MDT is expensive and may force prisoners to switch from less harmful drugs that persist longer in the body, such as cannabis, to "harder" drugs with shorter half-lives like heroin, methamphetamine, cocaine, or opioids like morphine [37,63]. In contrast to the methods commonly used to estimate drug consumption in prisons, WBE approach provides the possibility of obtaining more objective data [25]. Also, it can be performed without the prisoners' knowledge and does not require extra security measures [35]. Similar to an educational institution, an individual's excretion profile may affect drug consumption estimates, while the contribution of employees and visitors to overall drug consumption cannot be accurately determined [25].

So far, most WBE studies of prisons focus on illicit drugs (e.g., cannabis, amphetamines, cocaine) and opioids (e.g. substitutional drugs, such as morphine, methadone, buprenorphine), while fewer studies have looked at NPSs (MDA, 3,4-methylenedioxy-N-ethylamphetamine – MDEA, ketamine – KET, mephedrone – MEPH, ephedrine – EPH, methylone and 4-methylethcathinone – 4-MEC). The results suggest that

WBE is suitable for detecting the use of new substances in such settings (Tables 1 and 3). Substitutional drugs were daily found in all of the prisons studied, while illicit drugs and NPSs were detected only sporadically or not at all (Table 3). The occurrence of illicit drugs in prisons (e.g. cannabis, cocaine, and methamphetamine), reflected that of a city or countrywide demand. For example, there was widespread use of cannabis in French prisons and the general population [35]. The consumption of cocaine and cannabis was also regularly detected in a Spanish prison and the city of Barcelona [36], and as a result of its widespread usage in the general population, methamphetamine was frequently detected in Australian and US prison facilities [26,37,38]. Despite society perceptions, Postigo *et al.* [36] found that illicit drugs (e.g. cocaine, heroin, and amphetamines), except for cannabis, were consumed less in prison in comparison to the nearest major population (Barcelona).

Monitoring prescription drug use to prevent abuse is also of concern, and several authors [35,37,38] compared methadone loads in wastewater with the amounts administered to the inmates. In all three studies, there was an agreement between the estimated and the administrated amounts. The results suggested little if any additional non-prescribed usage of methadone over the monitoring periods. One reason could be the difficulties in dividing liquid methadone since its distribution in the prisons is strictly supervised or difficulties in smuggling a large number of methadone tablets into the prison [37]. Also, caution is needed when making such observations, since methadone consumption was estimated based on levels of 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine – EDDP (methadone metabolite), the excretion rates of which can vary considerably [37,38,64]. Thus, consumption can be underestimated (using higher excretion rate = 55%) or overestimated (using lower excretion rate = 3%). In contrast, up to 5-times higher consumption of buprenorphine was observed in comparison to administrated dose in the study by van Dyken *et al.* [38], suggesting greater buprenorphine misuse in a small regional prison in Australia. Buprenorphine is consumed mostly in tablet form or as a sublingual film, which makes its concealment easier. Other reasons include the availability of buprenorphine on the prison drug market, its increased use in the wider population, and the exclusion of buprenorphine from traditional prison screening processes. The levels of buprenorphine detected in French prisons is consistent with the proportions dispensed by the prison's pharmacy healthcare unit [35].

Weekly trends in drug use in prison were explored by Postigo *et al.* [36], who observed an increase in ephedrine (900 to 1 425 mg/day/1 000 people from Tuesday to Sunday) and cocaine (average working days: 280 mg/day/1 000 people, the weekend average: 350 mg/day/1 000 people) consumption through the week. In contrast, methadone consumption decreased over the week (Monday, Tuesday: 4 350 mg/day/1 000 people, Sunday: 3 025 mg/day/1 000 people), possibly due to weekend permits of the inmates.

A WBE approach is also a cost-effective alternative to MDT, providing a more comprehensive picture of substance use in prisons [26,38]. For example, in the study of van Dyken *et al.* [38], only a few of the drugs (cannabis, buprenorphine) were detected by both, WBE and MDTs, while drugs, such as methamphetamine, ketamine and methylone, were detected only by wastewater analysis. Alternatively, Brewer *et al.* [26] found good agreement between MDT and WBE data for methamphetamine and cocaine use. Methamphetamine consumption was confirmed, and cocaine consumption was disproven by both methods.

3.3. Other specific sites

The WBE approach has also been used to monitor drug consumption in public places, such as fitness centers and an airport (Tables 1 and 3). In the study of Schröder *et al.* [23] stimulants, e.g. ephedrine, amphetamine and MDMA were detected in the wastewater of three fitness centers (Aachen, Germany), where the highest concentration observed was for ephedrine (5 873 ng/L). Another case is Schiphol airport (Amsterdam, The Netherlands), where higher cocaine (1.3-times) and codeine (1.4-times) concentrations were measured at the airport in comparison to measured concentrations in the city of Amsterdam [27]. At the same time, methamphetamine was detected exclusively in airport wastewater, suggesting a relationship between its occurrence and international passengers. When the authors compared the populations of Schiphol airport and the city of Amsterdam and mass loads of drug biomarkers, a similar consumption pattern was observed. Also, weekly consumption patterns for cocaine (consumption mainly at the weekend) and codeine (continuous consumption over the week) was similar between the airport and the other four Dutch cities under investigation (Utrecht, Eindhoven, Apeldoorn, and Amsterdam). By utilizing WBE, the possible presence of drug traffickers can be shown at the airport based on the ratio of cocaine biomarkers (cocaine/benzoylecgonine). For example, the cocaine/benzoylecgonine ratio (0.85) in one wastewater sample from Schiphol airport exceeded the proposed cut-off cocaine/benzoylecgonine ratio value (0.75) from the literature [65,66], which indicates the disposal of cocaine directly into the sewage system. One reason could be drug traffickers disposing of the cocaine due to anxiety before passing customs control, but this hypothesis needs to be backed up by additional information [27].

Table 3. Overview of the reported biomarker concentrations and estimated drug consumptions in site-specific settings

Concentration (ng/L)			**Consumption (mg/day/1 000 inhabitants or mg/day)**	**Reference**
EDUCATIONAL INSTITUTIONS				
Educational institution (range) **Regular class session** THC-COOH: ND–177.1 AMP: ND–140.4 MDA: ND–173.9 MDMA: ND–3 266.0 BE: 1.9–10 COC: 2.4–13.8 COD: ND–71.4 M3G: ND–41.9 6-AM, MOR: ND	**Finals Week** THC-COOH: 4.4–372.9 AMP: ND–153.6 BE: 1.2–34.7 COC: 2.5–9.6 COD: ND–25.6 M3G: ND–565.5 MOR: ND–43.1	**Summer break** THC-COOH: ND–40.3 AMP, MDA, MDMA: ND BE: 1.3–6.2 COC:1.2–4.2 COD: 6.1* M3G: 63.6* MOR: 95.4* 6-AM: 7.7* * *one occasion*	**mg/day** **Educational institution (all samplings average);** COC: <100 mg AMP: max 3.7 mg	[25]
Dorms (range) THC-COOH: 30–2 413 THC: 22–2 070 AMP: 30–5 956 MAMP: 26–56 MDA: 348* MDMA: 30* BE: 4-350 COC: 24–184 COE: 77* EDDP: 30* COD: 14–981 MOR: 21–217 6-AM, HER, LSD, MDEA, MTHD: *ND*	**College campus (range)** THC-COOH: 152–1 373 AMP: 195–3 017 MAMP: 20–783 MDMA: 79–108 BE: 13–1 214 COC: 40* COD: 43–575 MOR: 23–491 6-AM: 44* THC, COE, EDDP, HER, LSD, MDEA, MDA, MTHD: *ND* **one occasion*		**mg/day/1 000 people** **Dorms (average)** Cannabis: 29 125 ± 24 875 AMP: 660 ± 930 COC: max 280 HER: 69 **Campus (range)** Cannabis: 1 587.5–5 750 AMP: 81–294 COC: 10–20 HER: 5.4–19.2	[32]

<u>Private college (AMP average)</u> **<u>Fall semester)</u>** *1st week*: 330 ± 4.1 *Midterms*: 480 ± 3.6 *Post-midterms:* 310 ± 3.6 *Finals:* 545 ± 0.8 ***<u>Additional information:</u>*** **Average AMP concentration normalized for creatinine *(ng AMP/mg creatinine)*** 1st week: 74 ± 51 Midterms: 240 ± 55 Post-midterms: 65 ± 51 Finals: 110 ± 50 **<u>Spring semester</u>** *1st week*: 810 ± 6.0 *Midterms*: 700 ± 5.8 *Post-midterms:* 650 ± 2.5 *Last week classes*: 810 ± 6.3 *Finals:* 2 100 ± 8.9 1st week: 75 ± 51 Midterms: 120 ± 51 Post-midterms: 110 ± 50 Last week classes: 190 ± 50 Finals: 570 ± 51	Not applicable	[22]
<u>College campus</u> No data on AMP concentration **<u>Additional information:</u>** **<u>Average AMP concentration normalized for creatinine - ng AMP/mg creatinine:</u>** *1st week*: 74 ± 7 *Midterms*: 240 ± 60 *Finals:* 111 ± 6	Not applicable	[28]
Not applicable	**<u>mg/day/1 000 people (average)</u>** AMP: 256 ± 12 MDMA: 88 ± 35 COC: 551 ± 49 HER: 474 ± 32	[33]

	COD: 50 ± 4 MOR: 18 ± 3 MTHD: 72 ± 8	
Cities where schools were located (range) ***Turin*** THC-COOH: 148–294 BE: 22–147 COC:34–241 MOR: 58–382 6-AM: 39* **one occasion* ***Milan*** THC-COOH: 37–147 BE: 57–3 516 COC: 23–421 ***Palermo*** BE: 4–205 THC-COOH: 7–40 *AMP, MAMP, MDMA, MEPH, KET: never detected in any of the school samples* ***Verona*** THC-COOH: 121–255 BE: 3–74 COC:1.3–2.2 MOR: 14–216 ***Rome*** THC-COOH: 14–46 BE: 2–6.1 COC: ND–2.5 ***Florence*** THC-COOH: 0–16.5 BE: ND–3.4 COC: ND–3.8 **Naples** THC-COOH: 40–109 BE: 12–1 398 COC:7–145 ***Bologna*** THC-COOH: ND–19.7	**mg/day/1 000 people** **Classic, scientific or artistic education (range and median)** Cannabis: 663–2 893 (1 201) COC: 5.2–187 (45) **Professional or vocational education (range and median)** Cannabis: 49–1 062 (106) COC: 0–7.1 (0.6)	[24]
University (range and average) THC-COOH: <20–32.9 (16.9) MDMA, BE, COC: <1 EtS: <1 700 **The capital, Mytilene (range and average)** THC-COOH: 49.5–90.2 (70.7) MDMA: <1–6.2 (3.2) BE: <1–37.5 (9.4) COC: <1–7.5 (3.9) EtS: 2 190–12 243 (5 283) **Villages (range and average)** THC-COOH: 22.5–50.0 (34.9) MDMA, BE, COC: <1 EtS: 2 316–7 814 (4 023)	**Illicit drugs mg/day/1 000 people, (range and average)** **University:** cannabis: 50–220 (100) **Mytilene:** cannabis: 2 400–3 400 (2 800) MDMA: 0.2–2.2 (1.2)	[34]

EME, AMP, MAMP, MTHD, EDDP, 6-AM, MXE, butylone, ethylone, methylone, MPA, PMMA, PMA, MEPH, MDPV, KET, DHNK and nor-KET were not detected in any of the samples.			COC: 0.5–33 (9.5) **Village:** cannabis: 600–1 700 (1 200) **Alcohol** **mL/day/1 000 people** **(range and average)** University: average 3.0 Mytilene: 2.2–11.2 (5.4) Villages: Alcohol: 1.7–7.2 (3.4)	
PRISONS				
<u>Prison 1</u> **<u>(range or average)</u>** THC-COOH: 347–3 152 BE: 970 EDDP: 51–353 MOR: <LOQ BUP: <LOQ MDMA, MEPH, 4-MEC: ND	**<u>Prison 2 – building A</u>** **<u>(range or average)</u>** THC-COOH: 1 021–8 900 BE: 1 083 MDMA: 21–226 MOR, MEPH, 4-MEC: ND EDDP: 313–8 507	**<u>Prison 2 – building B</u>** **<u>(range or average)</u>** THC-COOH: 640–6 240 BE: 492 MDMA, BUP: <LOQ MOR, MEPH, 4-MEC: ND EDDP: 51–605	**mg/day/1 000 people** **<u>All sampling sites (range):</u>** Cannabis: 24 000–94 000 COC: 90–282 MTHD: 255–1 707	[35]
<u>Prison (average)</u> THC-COOH:116 ± 52 THC: 67 ± 50 AMP: 97 MAMP:87 ± 66 MDMA: 61 ± 44 BE: 556 ± 291 COC:128 ± 94 6-AM: 63 ± 37 HER: 165 ± 186 MTHD:4 704 ± 1 424 EDDP: 9 262 ± 2 288 MOR: 1 346 ± 583 LSD-OH: 22 ± 9			**mg/day/1 000 people (range and average)** Cannabis:375–2 193 (990) COC: 100–910 (300) MTHD: 1 677.5–7 060 (3 900) EPH: 87,5–3 187.5 (1 150) HER, MDMA, AMP, MAMP: *sporadic use*	[36]

LSD: 56 ± 62 EPH: 3 745 ± 2 789 THC-OH-,nor-LSD: ND		
Prison BE: <40 COC: <10	**mg/day/1 000 people (range)** MAMP: 180–960	[26]
Prison (range) THC-COOH: ND–0.04 AMP: 0.011–0.027 MAMP: 0.05–0.25 COD: 2.98–4.40 MOR: 0.19–0.28 MTHD: 0.35–1.79 EDDP: 0.57–1.24 KET: 0.017–0.043 nor-KET: 0.001–0.004	**mg/day (range)** Cannabis: <638–1 206 MAMP: 25–120 COD: 5 110–9 045	[37]
Prison THC-COOH, THC, MDMA, BE, COC, MDA, MDEA, MEPH: <LOQ	**mg/day (range)** MAMP: 2–319 COD: 1 077–12 015 KET: ND–107 MTHD: 50–3 852 BUP: 331–6 243 Methylone: 537* *one occasion*	[38]
OTHER SPECIFIC SITES		
Fitness centres (range) AMP: <2–2 340 MAMP:<2 MDMA: <2–93 EPH: 97–5 873	Not applicable	[23]
Airport (range and mean) THC-COOH: <33 **4 Dutch cities (range)** THC-COOH: 73–489	Not applicable	[27]

AMP: 51–115 (81) MAMP: 16–17 (17) MDMA: 16–85 (58) BE: 659–2 933 (1 472) COC: 171–957 (559) 6-AM: <19 MOR: <360 MTHD: <45 COD: 336–894 (536) KET: <10	AMP: 40–1 779 MAMP: <15 MDMA: <12–241 BE: 260–3 701 COC: 87–673 6-AM: <19–73 MOR: <360 MTHD: <45 COD: 73–495 KET: <10–34		

ND – not detected, 4-MEC – 4-methylethcathinone, 6-AM – 6-acetylmorphine, AMP – amphetamine, BE – benzoylecgonine, BUP – buprenorphine, COC – cocaine, COD – codeine, COE – cocaethylene, DHNK – dehydronorketamine, EDDP – 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine, EME – ecgonine methyl ester, EPH – ephedrine, EtS – ethyl sulfate, HER – heroin, KET – ketamine, LSD – lysergic acid diethylamide, LSD-OH – 2-oxo-3-hydroxy-lysergic acid diethylamide, M3G – morphine-3-glucuronide, MAMP – methamphetamine, MDA – 3,4-methylenedioxyamphetamine, MDEA – 3,4-methylenedioxy-N-ethylamphetamine, MDMA – 3,4-methylenedioxymethamphetamine, MDPV – methylenedioxypyrovalerone, MEPH – mephedrone, MOR – morphine, MPA – methiopropamine, MTHD – methadone, MXE – methoxetamine, nor-KET – nor-ketamine, nor-LSD – N-demethyl-lysergic acid diethylamide, PMA – 4-methoxyamphetamine, PMMA – 4-methoxymethamphetamine THC – tetrahydrocannabinol, THC-COOH – 11-nor-Δ9-carboxy-Δ9-tetrahydrocannabinol, THC-OH – 11-hydroxy-Δ9-tetrahydrocannabinol

4. Special events

Monitoring of drug use during special events may act as an early warning system concerning drug consumption trends. Generally, drug consumption during the event and control period (e.g., wastewater sampling during non-festival days, normal weeks or in places, where no event took place) are compared to assess changes in drug consumption (Table 4). Sampling in both periods should be carefully optimized regarding sampling protocol to make adequate conclusions (e.g., obtaining representative samples) and time (e.g. covering normal days and days during a special event) or location (e.g., places affected by special event *vs* places where no event takes place). In some cases, such as festivals and sporting events, WBE approach can underestimate levels of drug use since it does not take into account people who urinate elsewhere (outside the sewer system/portable toilets), but their percentage is probably low and should not significantly affect estimates. An overview of the available data for wastewater analysis performed during special events is presented in Tables 2 and 4.

4.1. Music festivals

Several authors have studied drug consumption patterns during music festivals [30,39–41,43–48], and show increased consumption in comparison to non-festival days. Among illicit drugs, increased consumption of cocaine [39,41,46–48], cannabis [41,46] and in some cases also amphetamine and methamphetamine [47,48] were observed during various festivals, but the most extreme case was reported for MDMA [30,39–41,46–48]. For example, during the Pohoda Festival (Trenčín, Slovakia), there was a ten-fold increase in MDMA consumption [39]. In Australia in 2010, during an annual music festival that attracts people of all ages, a higher consumption rate of MDMA was observed during the festival (320–4 600 mg/day/1 000 people) in comparison to that in the nearby urban area (80–560 mg/day/1 000 people) [40]. Also, an increase in MDMA mass loads was observed during the duration of the festival (14-times higher mass loads on the final day in comparison to the first day of the festival), suggesting an increase in MDMA consumption over the festival period. At the same festival held in 2011, the increase in MDMA mass loads was even more pronounced (final day:

510 mg/day/1 000 people, first day: 15 mg/day/1 000 people) [40]. No difference in MDMA consumption was observed during the Lodenica Festival (Piešťany, Slovakia), which can be explained by the higher average age of attendees in comparison to the Pohoda Festival in Trenčín, Slovakia (average age: 30 years), where a 10-fold increase in ecstasy (MDMA) consumption was observed in comparison to consumption during the control week [39].

The connection between the consumption of a particular type of illicit drugs and the type of music festival has also been investigated by Mackuľak *et al.* [41]. The authors found that MDMA and cocaine were the main drugs consumed at a dance (Grape Festival) and multi-genre (Skalické dni) themed festivals, while cannabis was the drug of choice at a pop/rock festival (Topfest, Pohoda Festival). No specific consumption patterns were related to folk/country (Guláš Fest) and metal (VanDaal fest) festivals. Devault *et al.* [42] explored the impact that a large outdoor event, the so-called "Music day" event, which allows amateur and professional musicians of various musical tastes to perform in the street, has on illicit drug use (e.g. amphetamine, MDMA, cocaine, and cannabis) and methadone consumption in Bordeaux, France. The authors found no correlation between a music festival and drug consumption which is surprising. The authors suggest that the results reflect the influence of a non-peer audience at the street festival, although further research is needed to support this hypothesis.

Exploring drug consumption during special events such as festivals is also important for monitoring NPSs [43], which otherwise presents a challenge due to the dynamic nature of the NPSs market, and because they are readily procured from the internet or smart shops. Several studies observed sporadic consumption of NPSs, such as benzylpiperazine [40], methylone [40], mephedrone [40], MDA [46], ketamine and pseudoephedrine [30] during various music festivals. Causanilles *et al.* [43] applied a screening approach (almost 2 000 analytes, 560 NPSs) to samples collected at the Amsterdam WWTP during the Amsterdam street festivals in 2012 and 2014. In this case, NPSs from several groups such as synthetic cathinone, phenethylamine and synthetic cannabinoids were detected during the festival.

Meta-chlorophenylpiperazine (mCPP), 2,5-dimethoxy-4-bromophenethylamine (2CB) and 4-fluoroamphetamine (4-FA) were detected for the first time.

Few WBE studies have explored licit drug consumption, such as alcohol and tobacco (nicotine), during festivals [44,45]. Mackuľak *et al.* [44] found a significant increase in cotinine (nicotine metabolite) concentrations during festivals in comparison to non-festival days in the Czech Republic and Slovakia. The highest usage was observed during the rock/metal festival – Topfest (4-fold increase) and dance festival – Grape Festival (2.5-fold increase) in Piešťany [44]. Also, Andrés-Costa and co-workers [45] observed a significant increase in alcohol consumption during the “Fallas” festival (4.35–56.11 mL/day/1 000 inhabitants; >15 years) in comparison to non-festival days (1.07–18.31 mL/day/1 000 inhabitants; >15 years) at all three investigated WWTPs in Spain. In contrast, compared to an increase in alcohol consumption observed during regular weekends [45,67,68], on average there were no differences in alcohol consumption between the weekend (20.88 mL/day/inhabitant) and weekdays (19.98 mL/day/inhabitant) during the “Fallas” festival [45].

4.2. Public holidays

Impact of various public holidays has also been the subject of WBE studies (Tables 2 and 4). The results reveal the influence of different holidays on drug consumption patterns. Table 4, shows increased cocaine consumption during Christmas and New Year [16,40] as well as during Carnival [52] and the Easter holiday [53] and specific US holidays: Independence Day and Labor Day [30,49], and the Chinese Spring Festival [51]. Also, an increase in methamphetamine consumption was observed during the US Independence Day, a Solar eclipse [31] and the summer holiday season in Italy [56]. For other drugs, such as amphetamine, MDMA, and certain opioids, the impact of holidays on consumption trends was lower. In a study performed in Zagreb (Croatia) by Krizman-Matasic *et al.* [16], a 2 to 3-fold increase in the consumption of cocaine and MDMA following New Year’s Eve celebrations was observed, while over Christmas only cocaine consumption increased (2- to 3-fold increase in benzoylecgonine mass loads). The use of amphetamine-type drugs did not significantly change during Christmas, and the data most likely reflects the life-style differences between cocaine and amphetamine-type drug users [16]. Lai *et al.* [49] performed a study of drug consumption during the Christmas holidays at specific locations in a coastal urban area, an inland semi-rural area and an island vacation area in Australia. Their results indicated that specific consumption patterns observed during holidays were related to the different study areas. Similar consumption patterns were observed in the vacation area (island) and coastal urban area, which is also a popular vacation destination with numerous nightclubs and festivals. Compared to the urban area, that showed an increase in the consumption of most of the drugs analyzed (MDMA: 31-fold, cocaine: 5.6-fold, cannabis: 2.4-fold, methamphetamine: 1.8-fold), levels of cannabis and methamphetamine use in the semi-rural area during the holiday period did not differ significantly from those in the control period. However, there was a 4- and 9-fold increase in cocaine and MDMA consumption.

Foppe *et al.* [50] observed an increase in the consumption of illicit drugs and opioids during Independence Day, a solar eclipse, and during the first week of the academic semester in the USA. Significantly, 1.3- to 2-fold increase in consumption of amphetamine, methamphetamine, cocaine, morphine, and methadone was observed on Independence Day compared to an average week in two similar-size communities. Drug consumption (including cannabis) was also significantly higher during the 2017 solar eclipse than that for a typical Monday, particularly in communities with a dynamic population (highway routes and airport). A US study performed by Centazzo *et al.* [31] investigated consumption trends during four public holidays, including Memorial Day, 4^{th} of July, Labor Day and New Year. Their results found that there was no effect on the consumption of amphetamines, nicotine and cannabis, while only opioids and cocaine varied significantly between particular holidays. For example, opioid use was higher during Memorial Day (184.7–1 942.7 ng of morphine/mg creatinine) and Labor Day (178.2–1 436.9 ng of morphine/mg creatinine) compared to New Year (82.7–674.8 ng of morphine/mg creatinine), while cocaine use was higher during Labor Day (644.8–3 346.8 ng of benzoylecgonine/mg creatinine) than during New Year eve celebration (481.4–2 132.9 ng of benzoylecgonine/mg creatinine). These results should be interpreted with caution since only grab samples were collected. The impact of public holidays on drug consumption was also explored during the Chinese Spring festival and National Day [51]. Consumption of methamphetamine and cocaine was significantly higher during National Day: 47% higher for cocaine, compared to the control period while during Spring festival week, methamphetamine consumption also increased. Increased cocaine consumption was observed as well in Brazil during Carnival (6 229 ± 219 mg/day/1 000 inhabitants) in comparison to consumption on weekdays (2 296 ± 353 mg/day/1 000 inhabitants) and weekends (3 100 ± 233 mg/day/1 000 inhabitants) in the North-Wing, while in the South-Wing cocaine use was higher on a Sunday (7 385 ± 121 mg/day/1 000 inhabitants) than on the day of the Carnival (Monday) [52]. A significant increase in cocaine loadings was also observed during the Easter holiday [53] in France. Other biomarkers analyzed in this study, such as biomarkers of cannabis, codeine, morphine and heroin, also correlate with the Easter holidays. However, the focus of their study was on the impact of drug groups, e.g., opioids and stimulants rather than individual drugs.

In Croatia, Krizman *et al.* [54] observed the impact of the summer tourist season on the coastal touristic city of Zadar and the Croatian capital Zagreb. During the control period (spring), consumption of heroin, MDMA, cocaine, and cannabis was significantly higher in Zagreb compared to Zadar. In contrast, during the summer season, intercity differences in drug consumption were less pronounced, and the consumption of MDMA in Zadar was higher than in the capital. These differences in consumption are most likely a result of pronounced changes in population since the population of Zadar increases by 16% in the summer bringing with it changes in lifestyle. Surprisingly, cannabis consumption in Zadar decreased during the summer season, which agrees with data obtained by Lai *et al.* [49]. The impact of the summer holiday season was also explored by Kim *et al.* [55], who investigated drug consumption in semi-rural, residential and vacation locations in Korea. Increased consumption of methamphetamine and mephedrone was observed, but only in the vacation area. Unlike previous research, a decrease in cocaine consumption during holidays, particularly during the summer (2nd week of June to the 1st week of September) compared to November through to March was observed in Palermo, Italy (140 *vs* 210 mg/day/inhabitants) [56]. Since the summer period coincides with the school holidays, lower cocaine use could be related to the reduced population size since many people move to their summer residence.

Except for the trends in classical illicit drug consumption, Bade *et al.* [57] has explored the impact of the Christmas–New Year period on NPSs consumption since recreational drug use in this period is also higher. Their study included a broad range of different NPSs including phenethylamines, synthetic cathinones, opioids and amphetamines (22 NPSs in total). Seven NPSs were detected and confirmed such as butylone, butyryl fentanyl, furanyl fentanyl, methoxetamine, N-ethylpentylone, pentylone and valeryl fentanyl. The highest mass loads were found for N-ethylpentylone (36.35 mg/day/1 000 people) on 20 December, while mass loadings were much lower on subsequent days (e.g., 2.94 mg/day/1 000 people on 23 December, and 1.72 mg/day/1 000 people on 1 January). Despite this, caution should be taken in interpreting consumption, since only parent compounds were included in order to determine mass loadings, which could potentially arise from the direct disposal of the target drug along with or instead of drug consumption.

The impact of holidays, including the Christmas holiday season and Australia Day, on alcohol consumption, was explored by Zheng *et al.* [58]. Their results show a higher consumption of alcohol during all investigated holidays, e.g. Christmas, New Years, Australia Day, (21.2 ± 4 mL/person/day) in comparison to average weekdays (16.9 ± 5.3 mL/person/day) in years 2015–2017.

4.3. Sporting events

Wastewater-based epidemiology was also used to study drug consumption trends during various sporting events. For example, Sodre *et al.* [59] analyzed cocaine alkaloids, metabolites and adulterants as well as amphetamine-type substances in wastewater collected during the FIFA Soccer World Cup in 2014. A comparison of the data with that of an average weekend revealed a 25% increase in cocaine consumption. Also, Gerrity *et al.* [60] observed increased benzoylecgonine loads during the 2010 US National Football League's Super Bowl (718 g/day) when compared to a typical week (494 g/day). Gul *et al.* [29] also analyzed wastewater collected from the Oxford Wastewater Treatment Plant in Oxford, (Mississippi, USA), and the University of Mississippi Wastewater Treatment Plant (Mississippi, USA) during weekends when the home football team was playing. The authors analyzed a broad range of stimulants and other drugs. The use of cocaine and amphetamine was observed at both the university and in the city. A significant increase in amphetamine (18-times) and benzoylecgonine (up to 8-times) concentrations during the two highest attended games was observed only at the University of Mississippi. Since the concentrations of amphetamine and benzoylecgonine in the City remained the same, the observed increase in the levels at the university is likely related to visitors watching the game.

Table 4. Overview of the reported biomarker concentrations, mass loads and estimated drug consumptions during special events

Concentration (ng/L)	**Mass loads (g/day)** ***or*** **normalized mass loads (mg /day/1 000 inhabitants)**	**Consumption* (g/day or mg/day/1 000 inhabitants)**	**Reference**
MUSIC FESTIVALS			
<u>Pohoda Festival – Trenčin</u> MDMA: average 239 BE: max 88 COC: max 40 **<u>Lodenica Festival – Piešt'any</u>** BE: average 24 MDMA: <LOQ **<u>Non-festival days - 8 WWTPs (range)</u>** THC-COOH: 42–140 MAMP: 79–658 MDMA: <9.4–22 BE: <4.6–124 COC: <6.1–63	**mg/day/1 000 inhabitants** **<u>Pohoda Festival – Trenčin (average)</u>** MAMP: 53 MDMA: 29 **<u>Lodenica Festival – Piešt'any (average)</u>** MAMP: 163 **<u>Normal days - 8 WWTPs (range)</u>** AMP: 2–26 MAMP: 16–169 MDMA: <LOQ–5.8	**mg/day/1 000 inhabitants** **<u>Pohoda Festival - Trenčin (average)</u>** COC: 29 **<u>Lodenica Festival - Piešt'any (average)</u>** COC: 39 **<u>Normal days - 8 WWTPs (average)</u>** COC: max 141	[39]
Not reported	**mg/day/1 000 inhabitants** **<u>Annual music festival 2010 (range and average)</u>** THC-COOH:5.8–12 (16) AMP:4.6–37 (19) MAMP:61–270 (140) MDMA:49–690 (204) BE: 28–93 (45) COC:1.3–5.9 (3.5) MEPH: <0.001–1.9 (1.3) Methylone: 1.2–4.4 (2.7)	**mg/day/1 000 inhabitants** **<u>Consumption of both festivals (average)</u>** Cannabis: 1 400 MAMP: 366 (in 2010), 91.5 (in 2011) MDMA: 1 440 COC: 145 **<u>Annual music festival 2010 (range)</u>** Cannabis: 1 000–2 000 MAMP: 152.5–701.5 MDMA: 320–4 640 COC: 72.5–290	[40]

	Annual music festival 2011 (range) THC-COOH: 2.9–12 (7.8) AMP: 1.8–18 (8.8) MAMP:5.1–67 (34) MDMA:15–510 (190) BE: 6.2–100 (37) COC: 0.3–12 (3.5) MEPH:<0.001–0.9 (0.7) Methylone: <0.001–16 (5.4)	**Nearby urban areas 2010 (range)** Cannabis: 2 400–3 200 MAMP: 213.5–457.5 MDMA: 80–560 COC: 145–580	
Days around the festival / festival days ***Pieštʼany / Topfest:*** THC-COOH: 22–56 / 65–142 AMP: <6.1–29 / 45–75 MAMP: 209–255 / 411–648 MDMA: <9 / <7.6–159 COC: <5–12 / <7–52 COD: 34–110 / 53–166 ***Pieštʼany / Grape Festival:*** THC-COOH: 29, 39 / 38, 109 AMP: 24, 26 / 32, 52 MAMP: 235, 249 / 286, 455 MDMA: <6.4 / 16, 330 COC: <6 / <6, 159 COD: 10, 20 / 35, 62 ***Skalica / Skalické dni:*** THC-COOH: 82, 169 / 62–100 AMP: 22, 32 / 22–44 MAMP: 202, 243 / 200–427 MDMA: <9.1/ <8.2–78 COC: <6.3, 58 / <5.4–25 COD: 171, 541 / 106–251	**mg/day/1 000 inhabitants** **Days around the festival / festival days** ***Pieštʼany / Topfest:*** THC-COOH:14–21 / 15–43 AMP: <7.0–11 / 10–23 MAMP: 80–165 / 93–198 MDMA: <2.3–5.8 / <2.9–49 COC: <5–22 / <7–44 COD: 13–41 / 12–51 ***Pieštʼany / Grape Festival:*** THC-COOH:16–31 / 12, 36 AMP: 6–14 / 10, 17 MAMP: 44–136 / 89, 151 MDMA: <1.6–3.5 / 5, 109 COC: <8–29 / <7, 146 COD: 5–98 / 12, 19 ***Skalica / Skalické dni:*** THC-COOH: 18, 31 / 9–17 AMP: 5, 6 / 3–7 MAMP: 44, 45 / 28–71 MDMA: <1.5, <1.6 / <1.2–13 COC: <4, 29 / 2–12	Not reported	[41]

Trenčín / Pohoda Festival: THC-COOH: 55, 114 / 159–412 AMP: <9, 32 / 33–43 MAMP: 86, 227 / 244–165 MDMA: 10, 173 / <5.7–207 COC: <4.3, 24 / 12–66 COD: 59, 117 / 81–241 ***Zubří / VanDaal fest**:* THC-COOH: 34–61 / 54–98 AMP: <16–25/ 34–52 MAMP: 267–411 / 457–616 MDMA: <6.4 / <6.4 COC: <6.3 / <6.6–12 COD: 9–43 / 16–45 ***Valašské Meziříčí / Guláš Fest:*** THC-COOH: 72–108 / 74–124 AMP: 42–68 / 69–90 MAMP: 439–681 / 614–889 MDMA: <7.0/ <6.4–13.0 COC: <8.6 / <7.2 COD: 12–69 / 45–126 ***Gypsy Fest (Bratislava)****:* THC-COOH: 94–123 AMP: 44–64 MAMP: 594–738 MDMA: 13–46 COC: 146–193 COD: 89–175 **All sites:**	COD: 38, 98 / 15–37 ***Trenčin / Pohoda Festival**:* THC-COOH: 14,15 / 20–52 AMP: <2.5, 4.1 / 4.2–5.5 MAMP: 24, 29 / 31–34 MDMA: 2.8, 22 / <0.7–26 COC: <3.3, 8.4 / 4.2–23 COD: 15, 16 / 10–31 ***Zubří / VanDaal fest:*** THC-COOH: 20–29 / 22–40 AMP: <9–12 / 14–21 MAMP: 158–193 / 189–247 MDMA: <2.8–3.3 / <2.2–2.6 COC: <3 / <2–5 COD: 5–20 / 7–15 ***Valašské Meziřiči / Guláš Fest**:* THC-COOH: 21–35 / 19–25 AMP: 12–17 / 17–19 MAMP: 125–167 / 154–184 MDMA: <1.5–2.1 / <1.4–2.6 COC: <2 / <1–2 COD: 4–17 / 11–26 ***Gypsy Fest (Bratislava):*** THC-COOH: 18–24 AMP: 7–12 MAMP: 101–138 MDMA: 2.4–7.8 COC: 76–91 COD: 17–34		

HER, 6-AM, LDS, LSD-OH, MTHD, BUP, MDEA, KET, MBDB, cathinone, mephedrone: <LOQ **non-festival days were not investigated, due to failure in obtaining the wastewater samples (flushing of the sewer pipes)			
2017 (mean) **WWTP 1:** THC-COOH: 319 ± 93 BE: 647 ± 131 COC: 191 ± 70 **WWTP 2:** THC-COOH: 256 ± 59 BE: 968 ± 537 COC: 160 ± 72 **2018 (mean)** **WWTP 1:** THC-COOH: 451 ± 48 BE: 882 ± 169 COC: 233 ± 43 **WWTP 2:** THC-COOH: 362 ± 134 BE: 473 ± 341 COC: 127 ± 106	Not reported	**mg/day/1 000 inhabitants** **2017 (average all / Music day event)** **WWTP 1:** Cannabis: 4 614 ± 1 368 / 6 252 MDMA: 50 ± 76 / 0 COC: 552 ± 194 / 572 **WWTP 2:** Cannabis: 4 384 ± 1 081 / 5 019 MDMA: 51 ± 102 / 0 COC: 978 ± 516 / 855 **2018 (average all)** **WWTP 1:** Cannabis: 8 209 ± 1 530 MDMA: 199 ± 62 COC: 933 ± 101 **WWTP 2:** Cannabis: 10 005 ± 4 409 MDMA: 124 ± 170 COC: 725 ± 453	[42]
During Youth festival (average): MDMA: 940 **Non-festival days (average):**	Not applicable	Not reported	[30]

MDMA: 89.1 **<u>2 WWTPs (range and average)</u>** AMP: 52-84 (41) MAMP: 90-557 (164) MDMA: <2-413 (207) COC: <1-52 (14) HER: <1 COD: 2 207–3 967 (3 180) KET: 8 033–138 000 (18 633) PEPH: 12 133–44 667 (22 300) GHB: <2-5.5 (3.9)			
<u>COT – festival (mean)</u> *Gypsy Fest (Bratislava):* 2 495 *Grape Festival (Pieštʼany):* 2 600 Topfest (*Pieštʼany):4 327* *Skalické dni (Skalica):* 4 657 *VanDaal fest (Zubři):* 3 927 *Guláš Fest (Valašské Meziříči):* 3 540 **<u>COT – normal days (mean)</u>** *Bratislava:* 2 016 *Pieštʼany:* 1 093 *Skalica:* 5 152 *Zubři:* 3 283 *Valašské Meziřiči:* 2 485	**g/day** **<u>COT – festival (mean)</u>** *Gypsy Fest (Bratislava):* 182 *Grape Festival (Pieštʼany):* 31 Topfest (*Pieštʼany): 54* *Skalické dni (Skalica):* 15 *VanDaal fest (Zubři):* 35 *Guláš Fest (Valašské Meziříči):* 16 **<u>COT – normal days (mean)</u>** *Bratislava:* 175 *Pieštʼany:* 16 *Skalica:* 16 *Zubři:* 31 *Valašské Meziříči:* 15	**NIC g/day/1 000 inhabitant *(additionall information - cigarettes/day/1 000 inhabitants)*** **<u>Festival (mean)</u>** *Gypsy Fest (Bratislava):* 4 (5 044) Grape Festival (Pieštʼany): 6 (7 535) Topfest (Pieštʼany): 8 (10 442) Skalické dni (Skalica): 5 (6 363) VanDaal fest (Zubři): 11 (13 366) Guláš Fest (Valašské Meziříči): 6 (6 826) **<u>Non-festival days (mean)</u>** *Bratislava:* 4 *(4 863)* *Pieštʼany:* 4 *(5 025)* *Skalica:* 9 *(10 678)* *Zubři:* 10 *(11 929)* *Valaské Medziřiči:* 5 *(6 170)*	[44]
<u>EtS – Fallas festivity (range)</u> *Pinedo I:* 9 150–19 850 *WWTP of Pinedo II:* 4 000–9 890 *WWTP of Quart-Benager:* 7 160–10 710	Not applicable	**mL/day/inhabitant** **<u>Alcohol - Fallas festivity (range)</u>** *Pinedo I:* 13.36–23.81 *Pinedo II:* 4.35–9.07	[45]

<u>EtS – normal days (range)</u> *Pinedo I:* 1 460–14 900 *WWTP of Pinedo II:* 1 580–5 920 *Quart-Benager:* 2 000–7 170		*Quart-Benager:* 26.99–56.11 **<u>Alcohol – non-festival days (range)</u>** *Pinedo I:* 1.11–18.31 *Pinedo II:* 1.07–6.44 *Quart-Benager:* 3.31–12.38	
PUBLIC HOLIDAYS			
Not reported	**g/day** **<u>1st of January (New Year) 2013 and 2014</u>** BE: 224 / 197 MDMA: 62 / 67 AMP: 42 / 60 **<u>25th of December (Christmas) 2012 and 2013</u>** BE: 166 / 130	**mg/day/1 000 inhabitants** **<u>normal days 2012 and 2013</u>** HER: 73 ± 71 /140 ± 51 COC: 213 ± 34 / 261 ± 27 AMP: 24 ± 7.1 / 37 ± 11 MDMA: 36 ± 17 / 37 ± 13 Cannabis: 7 717 ± 1 580 / 10 660 ± 2 116 MTHD: 267 ± 41 / 218 ± 31	[16]
Not reported	Not reported	**mg/day/1 000 inhabitants** **<u>holidays / control period (maximum)</u>** **Semi-rural area:** Cannabis: 1 220 / 1 480 MAMP: 192 / 168 COC: 17.4/ 4.64 MDMA: 11.2/ 1.2 **Urban area:** Cannabis: 3 400 / 1 400 MAMP: 427/ 244 COC: 537/ 96 MDMA: 800/ 26 **Vacation area:**	[49]

		Cannabis: 1 720 / 1 260 MAMP: 793 / 6.1 COC: 798 / 97 MDMA: 1 440 / 6	
WWTP$_A$ (mean) **typical week / Independence day observation week (mean) / *1st week of the semester:*** THC: 22.1 / 21.7 / ND THC-COOH: 499 / 275 / 435 THC-OH: 1 130 / 1 620 / 622 AMP: 243 / 184 / 182 MAMP: 690 / 603 / 577 MDMA: 19.7 / 47.9 / 8.36 BE: 276 / 254 / 228 COC: 105 / 88.8 / 79.6 nor-COC: 25.7 / 48.9 / 9.22 COE: 20.0 / 43.4 / 8.57 HER: 226 / 859 / ND 6-AM: 224 / 554 / 11.2 MOR: 161 / 138 / 110 MTHD: 43.4 / 55.9 / 27.9 EDDP: 166 / 166 / 97.9 MDEA: 19.2 / 42.4 / 6.85 MDA: 26.8 / 56.2 / 6.67 **WW*TP$_B$* (*mean*)** **typical week / Independence day observation week / Solar eclipse observation*:*** THC: 38.1 / 8.33 / 65.2 THC-COOH: 505 / 562 / 767 THC-OH: 176 / 98 / 263 AMP: 333 / 248 / 517 MAMP: 1 350 / 1 200 / 1 560	**mg/day** **WWTP$_A$ (mean)** **typical week / Independence day observation week (mean) / *1st week of the semester:*** THC: 312 / 379 / Not applicable THC-COOH: 6 860 / 2 540 / 7 675 THC-OH: 16 000 / 27 600 / 11 000 AMP: 3 160 / 3 320 / 3 060 MAMP: 10 600 / 13 600 / 11 400 MDMA: 306 / 874 / 154 BE: 3 720 / 4 880 / 3 930 COC: 1 580 / 1 720 / 1 480 nor-COC: 448 / 1 000 / 192 COE: 308 / 797 / 159 HER: 8 990 / 41 200 / Not applicable 6-AM: 4 640 / 11 100 / 268 MOR: 1 550 / 1 800 / 1 340 MTHD: 647 / 1 150 / 527 EDDP: 2 640 / 3 790 / 2 040 MDEA: 274 / 706 / 117 MDA: 397 / 1 020 / 119 **WWTP$_B$ (mean)** **typical week / Independence day *observation week* / Solar eclipse observation*:*** THC: 799 / 190 / 1 330 THC-COOH: 11 200 / 14 700 / 14 500 THC-OH: 3 860 / 2 500 / 4 980	**mg/day/1 000 inhabitants** **WWTP$_A$ (mean)** **typical week / Independence day observation week (range) / *1st week of the semester:*** Cannabis: 62 400 / 35 200–51 900 / 69 800 AMP: 526 / 350–706 / 510 MAMP: 1240 / 1 060–2 500 / 1 330 MDMA: 59 / 43.9–83.4 / 29.7 COC: 434 / 287–773 / 458 MOR: 2380 / 1 310–3 340 / 2 060 MTHD: 1 100 / 793–1 720 / 844 MDEA: 72 / 36.3–94.5 / 30.7 **WWTP$_B$ (mean)** **typical week / Independence day observation week (range) / Solar eclipse observation*:*** Cannabis: 81 500 / 129 000–172 000 / 169000AMP: 919 / 592–1 200 / 1 450 MAMP: 3 090 / 2 420–5 600 / 3 400 MDMA: Not applicable / Not applicable / 27.6 COC: 1 970 / 1 290–3 240 / 2 280 MOR: 2 610/ 1 630–4 610 / 3 470 MTHD: 1 520 / 1 260–2 200 / 1 750	[50]

MDMA: <LOQ / <LOQ / 6.53 BE: 987 / 959 / 1 200 COC: 160 / 197 / 201 nor-COC: 9.73 / 4.93 / 6.54 COE: 9.39 / 14.5 / 6.87 HER: ND / 385 / ND 6-AM: 5.42 / 63.6 / 2.06 MOR: 141 / 107 / 193 MTHD: 42 / 28.9 / 55.4 EDDP: 177 / 137 / 204 MDEA: ND / ND / ND MDA: <LOQ / 4.96 / ND	AMP: 6 900 / 5 920 / 8 780 MAMP: 33 200 / 34 800 / 31 500 MDMA: Not applicable / Not applicable / 126 BE: 21 100 / 23 500 / 21 000 COC: 3 680 / 5 120 / 3 730 nor-COC: 255 / 94.9 / 142 COE: 209 / 258 / 128 HER: Not applicable / 10 300 / Not applicable 6-AM: 78.2 / 1 120 / 47.4 MOR: 2 120 / 1 910 / 2 400 MTHD: 987 / 809 / 1 070 EDDP: 4 580 / 4 230 / 4 330 MDA: Not applicable / 126 / Not applicable		
All WWTPs (range) **Memorial day:** THC: ND–32.8 THC-COOH: 134.2–1 213.9 AMP: 37–237.5 MAMP: 51.6–342.1 MDMA: ND–81.7 BE: 459.4–2 811.1 COC: 141.7–1 140.7 COE: 4.6–40.3 6-AM: ND–17 MOR: 177.4–1 373.5 MTHD: 12.8–270.3 EDDP: 32–532.3 COD: 21.9–208.3 MDA: ND–14.8 nor-fentanyl: ND Fentanyl: ND COT: 276–1 467.1	Not reported	Not reported	[31]

4th of July: THC: ND–23.2 THC-COOH: 68–626.2 AMP: 15.5–285.9 MAMP: 27.3–272.3 MDMA: ND–69 BE: 266.4–2 487.8 COC: 76.8–1 546.4 COE: 3.1–46.3 6-AM: ND–12 MOR: 148.3–1 096.0 MTHD: 9.2–250.4 EDDP: 23.6–481 COD: 20.8–171.7 MDA: ND–35.3 nor-fentanyl: ND Fentanyl: ND–5.9 COT: 306.8–1 373.1 **Labor Day:** THC: ND–24.1 THC-COOH: 85.6–1 451.9 AMP: 21–203.7 MAMP: 43.9–367.8 MDMA: ND–116.8 BE: 517.2–2 456 COC: 221.7–787.1 COE: 5.6–36.3 6-AM: ND–6.4 MOR: 145.8–918.6 MTHD: 13.6–205.3 EDDP: 23.6–442.6 COD: 27.8–219.6 MDA: ND–25.6			

nor-fentanyl: ND Fentanyl: ND COT: 344.9–1 350.9 **New Year's Day:** THC: ND–33.3 THC-COOH: 140–898 AMP: 15–167.2 MAMP: 21.6–372.2 MDMA: ND–187 BE: 473.5–2 014.3 COC: 184.1–788.6 COE: 4.7–42.6 6-AM: ND–18.8 MOR: 149.8–578.5 MTHD: 12.1–157.4 EDDP: 26.8–267.2 COD: 29.3–151.7 MDA: ND–51.1 nor-fentanyl: ND Fentanyl: ND COT: 336.4–776.1 **<u>Additional information:</u>** **Concentration normalized for creatinine (ng/mg creatinine) – all WWTPs;** **Memorial Day / 4th July / Labor Day / New Year's (range):** THC: 3.2–30.4 / 5.3–24.2 / 9.1–19.9 / 3–17.3 THC-COOH: 176.7–1 354.8 / 172.6–681.2 / 177.5–2 290 / 200–630.8 AMP: 25.7–265.1 / 32.8–260.9 / 27.8–167.1 / 12.9–125.7			

MAMP: 17.4–309.9 / 34.1–283.6 / 27.3–318.6 / 15.7–340.5 MDMA: 5.9–125.1 / 11.1–62.2 / 7.3–223.8 / 4.7–167.6 BE: 389.6–3 947.2 / 604.9–2 886.1 / 644.8–3 346.8 /481.4–2 132.9 COC: 92.4–1 613.4 /184.2–1 814.8 / 198.1–1 118.1 / 155.3–829.3 COE: 6.6–57 / 7.9–53.7 / 9.1–45.6 / 5.8–22.1 6-AM: ND, 18.6 / 8, 25.4 / 4.8–9.4 / 5.2, 16.1 MOR: 184.7–1 942.7 / 151.9–1 271.5 / 178.2–1 436.9 / 82.7–674.8 MTHD: 19.6–361.2 / 23.4–290.5 / 28.2–323.8 / 17.3–135.1 EDDP: 49–655.9 / 59.9–558 / 49–698.1 / 36.4–229.4 COD: 31.5–294.6 / 43.9–199.2 / 41.6–321.1 / 34.2–132 MDA: 2.7–13.4 / 4.2–24.8 / 10–37.4 / 5.8–26.6 nor-fentanyl: ND / ND / ND / ND Fentanyl: ND / ND, 5.3 / ND / ND COT: 200.1–2 075.1 / 262.3–1 593 / 267 / 1 627.4 / 187.6–509.8			
Guangzhou (average) AMP: 17.0 ± 10.3 MAMP: 145.6 ± 100.3 MDMA: 3.2 ± 1.9 BE: 1.98 ± 1.38 COD: 4.8 ± 2.6 KET: 28.6 ± 15.6 nor-KET: 8.9 ± 5.9	Not reported	**mg/day/1 000 inhabitants** **Guangzhou (range)** MAMP: 14.7–470.7 MDMA: 1.7–18.4 COC: 0.9–9.5 MTHD: 0.6–2.6 KET: 64.9–673.7 COD: 1.8–18	[51]

North Wing (range) BE: 1 598 ± 127–5 920 ± 208 COC: 835 ± 58–1 502 ± 144 COE: 59 ± 13–188 ± 34 **South Wing (range)** BE: 1 599 ± 106–8 559 ± 141 COC: 635 ± 79–1 983 ± 40 COE: <14(LOD)–155 ± 14	Not reported	**COC** **mg/day/1 000 inhabitants** **North Wing (average)** Weekdays: 2 296 ± 353 Weekend: 3 100 ± 233 Carnival Day: 6 229 ± 219 **South Wing (average)** Weekdays: 1 707 ± 250 Sunday: 7 385 ± 121	[52]
Semi-rural area (range) AMP: 2.35–4.83 MAMP: 37.53–53.32 COD: 7.33–25.27 Meperidine: 0.48–0.73 MOR: <LOQ–4.01 **Residential area (range)** AMP: 2.32–5.64 MAMP: 25.80–40.60 COD: 3.72–14.23 Meperidine: 0.41–0.74 MOR: 3.25–5.11 **Vacation area (range)** AMP: 1.81–4.65 MAMP: 18.01–47.08 COD: 2.64–11.97 Meperidine: 0.37–1.84 MOR: ND–5.4	**g/day** **July–September 2013 (range and average)** **Semi-rural area:** AMP: 0.454–0.0909 (0.0688) MAMP: 0.725–1.142 (0.895) COD: 0.142–0.467 (0.266) Meperidine: 0.009–0.015 (0.013) MOR: <LOQ–0.075 (0.024) Fentanyl: ND– <LOQ **Residential area:** AMP: 0.766–1.743 (1.431) MAMP: 8.531–12.755 (11.097) COD: 1.233–4.400 (2.527) Meperidine: 0.129–0.233 (0.181) MOR: <LOQ–1.619 (0.1138) Fentanyl: ND– <0.014 **Vacation area:** AMP: 0.128–0.330 (0.195) MAMP: 1.279–3.343 (2.192) COD: 0.187–0.850 (0.389)	**mg/day/1 000 inhabitants** **Semi-rural area (range)** MAMP: 29.10–45.81 COD: 0.567–3.920 Meperidine: 2.273–3.686 **Residential area (range)** MAMP: 23.01–34.39 COD: 0.837–1.646 Meperidine: 2.136–3.865 **Vacation area (range)** MAMP: 13.80–36.07 COD: 0.384–1.104 Meperidine: 1.745–8.640	[55]

	Meperidine: 0.026–0.130 (0.060) MOR: ND–0.384 (0.096) Fentanyl: ND–<LOQ		
Palermo – all study period (range and average): THC-COOH: 38.15–62.75 (59.05 ± 16.3) BE: 160.69–330.19 (205.69 ± 60.82) COC: 38.45–116.09 MAMP, MDMA, MDA, MDEA <LOQ **Palermo – during summer holidays / other months (average):** BE: 154.33 ± 55.49 / 235.5 ± 30.7	Not reported	**mg/day/1 000 inhabitants** **<u>Palermo – all study period (range and average)</u>** Cannabis: 1 680–3 220 (2 929) COC: 120–250 (160)	[56]
Not reported	**mg/day/1 000 inhabitants** Butylone: ND–0.02 Butyryl fentanyl: ND–< LOQ Furanyl fentanyl: ND–< LOQ Methoxetamine: ND–1.27 N-ethylpentylone: ND–36.35 Pentylone: ND–0.08 Valeryl fentanyl: ND–<LOQ	Not reported	[57]
<u>EtS</u> **Yearly average (range 2012 – 2017):** 16 100–19 600 **Average 2015:** 19 500 ± 5 100 **Average 2016:** 19 600 ± 4 600 **Average 2017:**	Not reported	**Alcohol (range)** **mL/person/day** **Yearly average (range and median 2012 – 2017):** 8.6–50.5 (19.5) **Average 2015:** 17.8–21.5 **Average 2016:** 19.7–23.9	[58]

16 200 ± 3 400		**Average 2017:** 15.7–19.0 **4 days prior Christmas / Christmas 2015, 2016 / 3 days following Christmas (average):** 19.1 ± 4.5 / 21.7 ± 2.2 / 17.6 ± 3.2 **Christmas, New Years', Australia Day / normal weekdays (average 2015–2017):** 21.2 ± 4 / 16.9 ± 5.3	
SPORTING EVENTS			
North-Wing ***Typical weekend/ 5th place playoff / 3rd place playoff:*** BE: 2.3, 2.9 / 2.9, 4.2 / 2.5, 3.1 COC: 0.69, 1 / 0.75, 0.8 / 0.6, 0.65 AE: Not analysed / 2.5, 2.6 / 1.9, 2.0 nor-BE, nor-COC, EME:<LOQ ECG, AMP, MDBD: ND or <LOQ AEME, MAMP, MDA, MDMA, MDEA: ND **South-Wing** ***5th place playoff / 3rd place playoff:*** BE: 2.4, 3.4 / 1.9, 2.5 COC: 0.58, 0.75 / 0.46, 0.49 nor-COC:<LOQ nor-BE, AEME, EME: ND or <LOQ ECG, AMP, MAMP, MDA, MDMA, MDEA, MBDB: ND	Not reported	**COC** **mg/day/inhabitant** **North-Wing** *Typical weekend:* 1.6, 2.1 *5th place playoff:* 1.8, 2.7 *3rd place playoff:* 2.4, 2.9 **South-Wing** *5th place playoff:* 0.9, 1.4 *3rd place playoff:* 0.7, 0.71	[59]
THC <100 THC-OH: <100 HER <25	**g/day** **Super Bowl weekend (average)**	Not reported	[60]

6-AM <25	AMP: 114 ± 17 MAMP: 806 ± 132 MDMA: 106 ± 30 BE: 718 ± 142 COC: 294 ±70 nor-COC: 7 ± 2 ECG: 271 ± 52 EME: 161 ± 27 MOR: 231 ± 112 MDA: 17 ± 3 **Normal weekend (average)** AMP: 125 ± 23 MAMP: 930 ± 154 MDMA: 97 ± 43 BE: 494 ± 82 COC: 295 ± 47 nor-COC: 7 ± 2 ECG: 266 ± 40 EME: 135 ± 23 MOR: 269 ± 61 MDA: 18 ± 5		
University of Mississippi (range) AMP: 900–13 700 MAMP: ND–600 MDMA: ND–100 BE: <625–7 400 COC: ND–900 COD: 55–390 MOR: <44–250 MTHD<LOQ EDDP: <LOQ MDA: <LOQ MDEA, 6-AM, COD, PCP, fentanyl, nor-fentanyl: ND	Not reported	Not reported	[29]

City of Oxford (range) AMP: 600–1 200 MAMP: ND–400 MDMA: ND–0.50 BE: 300–2 400 COC: ND–1 200 COD:0.73–310 MOR:<LOQ–170 MTHD: <LOQ EDDP: <LOQ MDA: <LOQ MDEA, 6-AM, COD, fentanyl, PCP, nor-fentanyl: ND			

* only for alcohol: mL/day/1 000 inhabitants or mL/person/day

ND – not detected, 6-AM – 6-acetylmorphine, AE – anhydroecgonine, AEME – anhydroecgonine methyl ester, AMP – amphetamine, BE – benzoylecgonine, BUP – buprenorphine, COC – cocaine, COD – codeine, COE – cocaethylene, COT – cotinine, ECG – ecgonine, EDDP – 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine, EME – ecgonine methyl ester, EtS – ethyl sulfate, GHB – gamma-hydroxybutyrate, HER- heroin, KET – ketamine, LDS – lysergic acid diethylamide, LSD-OH – 2-oxo-3-hydroxy-lysergic acid diethylamide, MAMP – methamphetamine, MBDB – 3,4-methylenedioxy-N-methyl-butanphenamine, MDA – 3,4-methylenedioxyamphetamine, MDEA – 3,4-methylenedioxy-N-ethylamphetamine, MDMA – 3,4-methylenedioxymethamphetamine, MEPH – mephedrone, MOR – morphine, MTHD – methadone, NIC – nicotine, nor-BE – nor-benzoylecgonine, nor-COC – nor-cocaine, nor-KET – nor-ketamine, PCP – phencyclidine, PEPH – pseudoephedrine, THC – tetrahydrocannabinol, THC-COOH – 11-nor-Δ9-carboxy-Δ9-tetrahydrocannabinol, THC-OH – 11-hydroxy-Δ9-tetrahydrocannabinol

5. Ethical issue related to WBE approach

Usually, WBE is applied to general populations, where the contribution of individuals to wastewater is non-identifiable, and where no information on individual drug use can be obtained [12,13,69]. According to Human Research Ethics Committees, WBE involves little ethical risk and typically does not require an ethical review of the studies. Although the WBE approach cannot be used to target an individual *per se*, site-specific WBE studies can raise specific ethical concerns [70], and a series of ethical WBE guidelines have been developed by Prichard *et al.* [71] covering general and site-specific studies. Careful research planning is especially important when dealing with specific communities. To avoid ethical risks, researchers must obtain information on countries' regulations (regulations in some countries might still require their ethics committees' approval of the research conducted in specific catchments) and obtain approval from the relevant institutional authority (e.g., school principals and prison authorities). Also, gathering information on the reputation of institutions and stakeholders' ethical practices should be considered. For example, in prisons, WBE data could be used by authorities as a base for collective punishment of the prisoners, such as reducing family visits [37,69,70]. To avoid these problems, a careful discussion between researchers and prison authorities regarding a particular study, the results and ethics are essential [69]. During research planning, appropriate media communication protocols (e.g., determining who is the media contact person and specifying the information to be classified) and preservation of the anonymity of the studied sites needs to be considered, since sensationalizing of the study results and media attention can negatively affect the target population and institution [69, 71]. In the case of prisons, negative media reports may harm opinion about the reintegration of ex-prisoners back into society or in the case of schools may harm a schools' reputation [69,70].

6. Practical challenges and future recommendation

The WBE approach enables the rapid non-invasive and cost-effective collection of objective data relating to drug consumption patterns and delivers this data in near-real-time, which is necessary for accurately tracking drug use. However, WBE is not without its disadvantages, for example, it cannot provide information on the type of users, multi-substance use or if any of the observed changes are related to an increasing number of estimated doses or an increase in the number of users.

The main uncertainty, when applying WBE in site-specific studies, derives from the sampling stage. A common practice is to collect samples with time-proportional sampling with reported sampling frequency from 1 min to 1 hour (Table 1). From this, arise the question of representativeness of samples since irregular pulses (e.g. toilet flushes) might be missed. It is advised that preliminary analysis are performed, possibly using a tracer dye, prior to determining the sampling rate. Another way to overcome this difficulty is to use flow-proportional sampling or passive sampling; however, the use of passive sampling at site-specific studies still needs to be explored. To estimate drug consumption also requires knowing the flow rate, but since low and inconstant wastewater flow makes it difficult to measure the flow rate in site-specific studies, as an alternative, the flow rate can be estimated from the monthly water bill (e.g., by dividing used water with the number of days in the month of sampling). However, the result should be treated with caution, since additional uncertainty is introduced into the result.

In specific populations, individual's excretion profiles may also have a significant effect on the overall results. Sampling for an extended period (e.g., repeated one-week samplings) is desirable as well as sampling larger populations to reveal, more precisely, the trends in drug consumption. Also, participants in a specific community should not be aware of any research being undertaken in order to avoid bias. In studies related to special events like music festivals, holidays and sporting events, sampling is not as problematic, since most commonly wastewater samples are obtained at WWTP using established procedures. However, the sampling period should be optimized to have adequate control samples, along with the samples collected during special events, which can be compared to obtain satisfactory conclusions.

Wastewater based epidemiology when applied to the general population typically does not have any significant ethical concerns, but issues may arise when applied to specific sites, especially in institutions like schools and prisons (site-specific studies). Careful research planning and compliance of developed ethical WBE guidelines should be considered to avoid ethical risks. For example, during research planning information on countrywide regulations should be obtained, good cooperation between the researcher and target facility must be established, and it is important to preserve the anonymity of the study site and use caution when reporting the results to the media.

Despite the uncertainties mentioned above, WBE is a useful tool for monitoring time and spatial trends in licit and illicit drug consumption in specific circumstances. Also, timely information on trends in new psychoactive substance use can be obtained. However, additional guidance on sampling is needed and the way data is reported standardized to enable an appropriate comparison of the results.

Declaration of interest

None.

Acknowledgements

This work was supported by the Slovene Research Agency (ARRS Program group P1-0143 and Projects L1-9191 and N1-0143).

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