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New psychoactive substances in several European populations assessed by wastewater-based epidemiology

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ABSTRACT

Wastewater-based epidemiology (WBE) can be a useful tool to face some of the existing challenges in monitoring the use of new psychoactive substances (NPS), as it can provide objective and updated information. This Europe-wide study aimed to verify the suitability of WBE for investigating the use of NPS. Selected NPS were monitored in urban wastewater by high performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS). The main classical illicit drugs were monitored in the same samples to compare their levels with those of NPS. Raw composite wastewater samples were collected in 2016 and 2017 in 14 European countries (22 cities) following best practice sampling protocols. Methcathinone was most frequent (>65% of the cities), followed by mephedrone (>25% of the cities), and only mephedrone, methcathinone and methylone were found in both years. This study depicts the use of NPS in Europe, confirming that it is much lower than the use of classical drugs. WBE proved able to assess the qualitative and quantitative spatial and temporal profiles of NPS use. The results show the changeable nature of the NPS market and the importance of large WBE monitoring campaigns for selected priority NPS. WBE is valuable for complementing epidemiological studies to follow rapidly changing profiles of use of drugs.

1. Introduction

Urban wastewater reflects the lifestyle of a population as it represents anonymous urine samples from thousands of people. When an individual ingests a substance, it can be excreted in urine or faeces as the parent substance or a metabolite, and enters the urban sewage system. Wastewater-based epidemiology (WBE) is a promising approach for estimating the use of a substance in a population by chemical analysis of urban wastewater for selected urinary metabolites (biomarkers) (Gracia-Lor et al., 2017). WBE was developed to estimate the use of illicit drugs more than a decade ago (Zuccato et al., 2005, 2008; van Nuijs et al.,

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2011), and is now regularly applied worldwide to assess the use of cocaine, amphetamine-like stimulants and cannabis (González-Mariño et al., 2020). With its advantage of providing objective and updated information on a population in a short period of time, WBE is considered a new indicator of drug use, complementary to the established drug monitoring tools (EMCDDA, 2016) . WBE can be useful to tackle some of the challenges in monitoring the use of new psychoactive substances (NPS).

NPS are an extremely heterogeneous group of substances originally designed as legal alternatives to the more established illicit drugs, and they have challenged traditional approaches to drug monitoring, surveillance, control and public health response (Peacock et al., 2019). These challenges include the large number of substances and the speed with which they enter and exit the market, the lack of awareness of the exact content of NPS products, and their often unknown potency and effects (Peacock et al., 2019). By the end of 2019, the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) was recording about 790 NPS in Europe (EMCDDA, 2020), and the Early Warning Advisory (EWA) of the United Nations Office on Drugs and Crime (UNODC) reported more than 950 NPS worldwide (UNODC, 2020). In Europe, the number of new substances identified for the first time reached a peak in 2014-2015 with 100 newly-reported NPS per year, and dropped to 53 in 2019 (EMCDDA, 2020). The NPS market is highly dynamic depending on the substances availability over time that may vary because of the changing laws, i.e. when a substance from licit become protected, illegal producers create rapidly an alternative substance with the same or similar effect. NPS are now increasingly manufactured and marketed as counterfeit prescription medicines or mixed with established illicit substances, but their composition is usually mostly unknown (EMCDDA, 2015).

Surveys of self-reported use and seizure data are not suitable alone to assess the prevalence of NPS use, and the current best option is to adopt monitoring systems that triangulate different information sources, including forensic and toxicology analyses of human biological or drug samples (Peacock et al., 2019). WBE can be used as a new additional tool to monitor NPS use by the general population or in specific sub-populations (e.g. at festivals) as for established illicit drugs (Benaglia et al., 2020; Bijlsma et al., 2020; EMCDDA, 2016; González-Mariño et al., 2020).

The main limitation when assessing NPS use by WBE is the very low concentration levels expected in urban wastewater because of the lower prevalence of use. This in combination with the often unknown excretion pattern that hinders focusing on specific metabolic products, and the large number of different substances that should ideally be investigated because they were recorded in the market (Bade et al., 2019a; Bijlsma et al., 2019; Salgueiro-González et al., 2019) makes monitoring NPS use very challenging. These limitations can be partially overcome using advanced analytical techniques based on mass spectrometry, which reaches very good sensitivity for detecting a substance in wastewater even at trace levels, and screening the presence of large number of substances (Hernandez et al., 2016). Some recent studies investigated urban wastewater for NPS in different countries, addressing a selected set of substances (Bade et al., 2020, 2017; Gonzalez-Marino et al., 2016; Reid et al., 2014) or screening hundreds (Bade et al., 2019b; Diamanti et al., 2019; Salgueiro-González et al., 2019). Although relatively few substances were detected in wastewater at low levels (few ng/L), literature did identify temporal trends of synthetic cathinones use in Australia (Bade et al., 2019a; Chen et al., 2013; Tscharke et al., 2016), and spatial trends of use in three countries in Europe (Gonzalez-Marino et al., 2016). Nevertheless, most of previous studies were limited to a single country and very few cities.

As far as we know, the present work is the most extensive monitoring study of NPS use in the general population ever done through WBE, and it was part of the NPS Euronet project funded by the European Commission. The aim was to test the suitability of WBE for investigating the use of NPS in the general population in an extensive monitoring campaign. This study covered two years in 14 European countries and 22 cities. A selected number of NPS including mostly synthetic cathinones and phenethylamines were quantified in urban wastewater to assess their spatial and temporal profiles of use. The main classical illicit drugs (amphetamine, methamphetamine, MDMA, and cocaine) were analysed in the same samples to compare their levels with those of the NPS.

2. Material And Methods

2.1. Compounds selection

Thirty NPS were selected according to their frequency of citation in alert reports from the Early Warning Systems of the EMCDDA and UNODC, the availability of analytical standards, their reported presence in urban wastewater in previous studies, and stability in wastewater. The selected NPS were 19 synthetic cathinones, 7 phenethylamines, 1 synthetic cannabinoid, 1 tryptamine, 1 aminorex derivative, and 1 arylcyclohexylamine/ketamine analog (Table 1). In total, thirteen deuterated compound analogues of eleven NPS plus amphetamine-d6 (AMPH-D6) and methamphetamine-d9 (METH-D9) were used as internal standards (Table 1). All the substances are parent drugs as for most NPS the metabolism is unknown, and very few analytical standards of metabolites are available. Furthermore, the majority of the NPS considered are synthetic cathinones that are mainly excreted as parent compounds (Uralets et al., 2014). The main classical drugs were included in the analysis to compare their use and were: amphetamine (AMPH), methamphetamine (METH), 3,4-methylenedioxy-methamphetamine (MDMA), ketamine (KET) as parent substances, and benzoylecgonine (BE) as the main metabolite of cocaine.

2.2. Chemicals and Materials

Analytical reference standards of NPS and classical drugs were supplied by LGC (Teddington, UK), Cerilliant (Round Rock, TX, USA) and Cayman Chemicals (Ann Arbor, MI, USA) as 0.1, 0.4 or 1 mg/mL solutions in acetonitrile (ACN) or methanol (MeOH). Working solutions containing either the 30 analytes or the 13 deuterated compounds were prepared in MeOH before each analytical run and stored in the dark at -20 °C up to two months. Reference standards of classical drugs were purchased from Cerilliant (Round Rock, TX, USA) as 0.1 or 1 mg/mL solutions in ACN or MeOH. Working solutions were prepared as described for NPS. HPLC grade ACN and MeOH, formic acid (98%), hydrochloric acid (HCl, 37%) and ammonium hydroxide solution (NH_4OH, 25%) were purchased from Fluka (Buchs, Switzerland) and Carlo Erba (Italy). Milli-Q water (HPLC grade) was obtained directly from a MILLI-RO PLUS 90 apparatus (Millipore, Molsheim, France).

2.3. Wastewater sampling

Composite 24-hour raw wastewater samples were collected for 3 days over the weekend (in 2016) and for-7 consecutive days (in 2017) from wastewater treatment plants (WWTP) in different cities in Europe. The sampling period was March-May in 2016, and May-June (plus one city in October) in 2017 (Table S1). The sampling in 2017 was randomly stratified over four weeks taking one/two samples per week to ensure better representability of samples (Ort et al., 2014). Each sample was collected as a daily composite sample and 0.5-1 L aliquots were received by our laboratory.

Table 1
NPS selected for investigation, with their classes and abbreviations. Labelled deuterated analogs listed in the right column were used as internal standards (IS).

NPS classes		Deuterated compounds (IS)
Synthetic cathinones	Phenethylamines	
buphedrone (BUPH)	25-B-NBOMe	amphetamine-D6 (AMPH-D6)
butylone (BUTL)	25-C-NBOMe	butylone-D3
4′-chloro-α-pyrrolidinopropiophenone (4-Cl- α -PPP)]	25-I-NBOMe	25-B-NBOMe-D3
N,N-dimethylcathinone (DCAT)	25-iP- NBOMe	25-C-NBOMe-D3
3,4 dimethylmethcathinone (3,4-DMMC)	N-ethyl-1,2-diphenylethylamine (NEDPA)	25-I-NBOMe-D3
ethcathinone (ETHC)	para-methoxyamphetamine (PMA)	mephedrone-D3 (MEPH-D3)
ethylone (ETHL)	para-methoxy-N-methylamphetamine (PMMA)	methamphetamine-D9 (METH-D9)
4-fluoromethcathinone (4-FMC)	**Synthetic cannabinoid**	3,4-methylenedioxypyrovalerone-D8 (MDPV-D8)
mephedrone (MEPH)	5-fluoropentyl-3-pyridinoylindole (5-Fpentyl-3-pyr)	methylone -D3)
methcathinone (METC)	**Tryptamine**	methoxetamine-D3 (MXE-D3)
methedrone (METD)	5-methoxy-N-isopropyl-N-methyltryptamine (5-MeO-MiPT)	naphyrone-D5
methylenedioxypyrovalerone (MDPV)	**Aminorex derivative**	ρ-methoxymethamphetamine-D3 (PMMA-D3)
4-methylethcathinone (4-MEC)	4,4-dimethylaminorex (4,4-DMAR)	alpha-pyrrolidinovalerophenone-D8
methylone (METL)	**Ketamine analog**	
naphyrone (NAPH)	methoxetamine (MXE)	
1-naphyrone (1-NAPH)		
pentedrone, (PENTD)		
pentylone (PENTL)		
α-pyrrolidinovalerophenone (α –PVP)		

Pooled weekend and weekday samples were prepared for analysis mixing fixed aliquots from each sample (50-100 mL). Pooled weekend samples were created by mixing aliquots from Saturday, Sunday and Monday, and pooled weekday samples by mixing aliquots from Tuesday to Friday. This design was adopted to optimize the analytical effort to achieve our objectives, i.e. evaluate the use of NPS and investigate their weekly pattern of use (week-weekend).

The investigation included 20 cities from 14 European countries (21 WWTPs with approximately 6.9 million people connected) in 2016, and 7 cities in 5 European countries (7 WWTPs with approximately 3.4 million people connected) in 2017. Unfortunately, the sampling capacity was not the same in 2016 and 2017, but five cities were included in both campaigns in order to follow the time trends (weekend pooled samples from 2016 and 2017 were compared). Information on population size and WWTP characteristics including the daily flow rates are reported in the Supplementary Material (SM) (Table S1).

The sampling scheme was designed according to best practice protocols (Castiglioni et al., 2013), in order to limit sampling uncertainty to 5-10%. Samples were collected volume- or time-proportionally, ensuring the collection of at least one aliquot per hour. For substances with a low prevalence of use as NPS, sampling errors may be higher than for substances used more widely (cocaine, alcohol, nicotine), but this can be partially balanced by the large populations investigated, which lowers the uncertainty (Ort et al., 2010).

2.4. Chemical analysis

The analytical method used to measure NPS was adapted from a previous study (Gonzalez-Marino et al., 2016), including additional substances. Samples were solid-phase extracted and analysed by high-performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS). The analytical procedure and method validation are described in detail in the SM. Briefly, 50 mL of wastewater samples were filtered and spiked with deuterated labelled internal standards to compensate for matrix effects and potential loss during analysis. Samples were acidified to pH 2, and solid-phase extracted on Oasis® MCX (6 mL, 150 mg) cartridges (Waters, Milford, MA, USA) to clean up the sample and enrich the concentrations of the target analytes. Cartridges were conditioned by washing with 12 mL MeOH, 6 mL Milli-Q water and 6 mL Milli-Q water acidified to pH 2 and were eluted with 2 mL of MeOH and 2 mL of 2% NH_4OH in MeOH. Chromatographic separation was carried out at room temperature using an Atlantis® T3 (100 × 2.1 mm; 3 μm) column (Waters, Milford, MA, USA), and analyses were done with a triple quadruple mass spectrometer TripleQuad 5500 ABSciex (Concord, Ontario, Canada). Analytical parameters are reported in Table S2. Quantification was done using the isotopic dilution method with six-point calibration curves prepared freshly before each analytical run. Instrumental and procedural blanks were included in each analytical batch to check for contamination.

For classical illicit drugs, samples were prepared and analyzed using solid phase extraction (SPE) and HPLC-MS/MS, as previously described (Zuccato et al., 2016).

2.5. Method validation and quality control

The analytical method was validated for accuracy, precision, linearity and sensitivity according to international guidelines (United Nation Industrial Development Organization, 2006). Results are reported in Table S3. Recovery and repeatability of the analytical method were tested in raw wastewater (n=3) by spiking 50 mL aliquots with 100 ng/L of each analyte. A non-spiked wastewater sample was analysed as well, to correct the recoveries for the background levels. Recoveries ranged between 75 and 116%, with few exceptions, and variability was lower than 14% (Table S3). Limits of detection (LOD) and quantitation (LOQ) of the whole method were calculated from raw wastewater samples as the values corresponding to signal-to-noise ratios (S/N) of 3 and 10, respectively. LOQs were all in the low ng/L concentration range (0.06 -15 ng/L) (Table S3).

2.6. Mass loads and statistical analysis

All instrumental data were acquired and processed using Analyst® 1.6.1 and MultiQuantTM 2.1 software (AB Sciex). Concentrations (ng/L) were multiplied by wastewater daily flow rates (m^3/day) to obtain the mass load of each substance. The loads were then normalised to the population served by the WWTP to obtain the mg/d/1000inh (d=day; inh=inhabitants), which allowed the comparison of results for different cities. Normalised mass loads were used as an indicator of NPS use per day in the population. The back-calculation normally done considering the illicit drug metabolism to estimate drug consumption (Castiglioni et al., 2013; Gracia-Lor et al., 2016), was not done in the present study

Table 2
Mean, median and range concentrations of NPS in wastewater, and number of cities and countries where each NPS was found.

Sampling 2016 (20 cities, 14 countries)	Concentration (ng/L)			Number of cities	Number of countries
Synthetic cathinones	**Mean**	**Median**	**Range**		
BUTL	2.51	2.51	-	1	1
3,4-DMMC	0.64	0.64	-	1	1
ETHC	3.22	2.97	2.4-4.3	3	1
MDPV	0.65	0.65	-	1	1
MEPH	13.90	16.11	1.6-23	5	5
METC	3.49	2.29	0.9-10.4	13	8
METL	13.89	13.89	4.4-23.3	2	2
PENTL	1.98	1.98	0.8-3.1	2	2
α -PVP	5.26	6.35	1.3-8.1	3	3
Phenethylamines					
PMA	41.01	28.80	20.4-106	6	5
Sampling 2017 (7 cities, 5 countries)	Concentration (ng/L)			Number of cities	Number of countries
Synthetic cathinones	**Mean**	**Median**	**Range**		
BUPH	3.35	1.30	0.9-7.8	2	2
MEPH	18.31	9.76	2.5-60	4	4
METC	3.30	2.27	1.2-8.3	6	4
METL	4.38	4.38	4.2-4.6	1	1
Phenethylamines					
25-iP-NBoMe	3.06	3.06	2.8-3.3	1	1

because of the lack of information on NPS metabolism. This currently impedes the development of specific correction factors for back-calculation. The comparison with classical drugs took account of the mass loads too, without back-calculation, for a more realistic comparison.

3. Results And Discussion

3.1. NPS occurrence in urban wastewater

Table 2 reports the mean, median and concentrations ranges of the NPS quantified in the different cities: only synthetic cathinones and phenetylamines were found in wastewater. Twenty-six NPS were searched in 2016, and 10 were detected (38%), while 30 NPS were targeted in 2017 and 5 were detected (17%). METC was the substance most frequently detected, as it was found in 13 cities (65% of the cities investigated) and 8 different countries (57% of the countries investigated) in 2016, and 6 cities (86%) and 4 countries (80%) in 2017. MEPH was also frequently found (5 cities from 5 countries in 2016, and 4 cities from 4 countries in 2017) (Table 2). MEPH, METC and METL were the only substances found in wastewater in both years. METC was also one of the substances found most frequently over the years in Australia, with a steady profile of use (Bade et al., 2019a; Chen et al., 2013; Tscharke et al., 2016). In Europe it was found in previous investigations in Italy and the UK (Gonzalez-Marino et al., 2016), but not in Croatia (Senta et al., 2015).

NPS concentrations were generally in the low ng/L range (1-20 ng/L), except for PMA, a phenethylamine, that was found up to 100 ng/L in 2016. PMA was not found in 2017, but we detected another phenethylamine, 25-iP-NBoMe, which was not identified in 2016, suggesting the variability of the NPS market and the interchangeable nature of this group of psychoactive stimulants. Concentrations above 20 ng/L were also found for MEPH and METL in 2016 (up to 23 ng/L), and for MEPH in 2017 (up to 60 ng/L). These levels are generally in agreement with previous investigations where NPS were found at trace levels in wastewater, but there were some differences among countries for some substances. For instance, α-PVP was found in Greece at similar levels to the present study, while MEPH in Greece was not detected (Borova et al., 2015). In Belgium PMA was not detected, but methoxetamine was detected very often (Kinyua et al., 2015). In Croatia, METL and MEPH were found at lower levels than in the present study, and MDPV was not found (Senta et al., 2015), but it was found in China (Gao et al., 2017) and in Finland where the levels were quite high in a specific area (Kankaanpaa et al., 2014). In previous European studies, MEPH was found at the highest levels in the UK suggesting a decrease of MEPH use in that country (Bade et al., 2017; Castrignanò et al., 2016; Gonzalez-Marino et al., 2016), and METL was found also in Denmark (Bade et al., 2017).

Results from the literature give only a snapshot of NPS use in different countries, because different substances were investigated over the years and studies mainly focused on single countries. In view of the complex and dynamic market of NPS, it would be useful to apply WBE for repeated monitoring campaigns focused on a core group of relevant substances to identify temporal and spatial trends of use, especially compounds that have an established niche market and/or are highly potent.

3.2. Spatial profiles of NPS use

Figs. 1 and 2 reports population-normalized mass loads (mg/d/1000inh) of the NPS found in this study. Detailed values are set out in SM (Tables S4-S5). The highest loads (> 30 mg/d/1000inh) were for PMA in 2016 in Novi Sad (Serbia) and Bratislava (Slovakia) (Fig. 1). MEPH was second highest, with considerably lower loads: 8.8 mg/d/1000inh in Krakow (Poland), and 5 mg/d/1000inh in Cluj Napoca (Romania) and Ljubljana (Slovenia). METC was the substance found most frequently, but at lower mass loads, with maximum values of 3 mg/d/1000inh in Bucharest (Romania) and Novi Sad (Serbia) (Fig. 1). METL and α-PVP were detected less frequently and were found only in Ljubljana (Slovenia) (5.5 mg/d/1000inh) and Utrecht (The Netherlands) (0.7 mg/d/1000inh) for METL, and Lugano (Switzerland) (3.3 mg/d/1000inh) and Porto (Portugal) (0.3 mg/d/1000inh) for α-PVP (Table S4).

In 2017 (Fig. 2), the highest loads were found for MEPH in Krakow (Poland) (up to 11 mg/d/1000inh) and Ljubljana (Slovenia) (3 mg/d/1000inh), and for METC and BUPH up to 2 mg/d/1000inh in all the places where they were found. All the other substances detected (BUTL, 3,4-DMMC, ETHC, MDPV, PENTL, BUPH, and 25-iP-NBoMe) were at levels lower than 1 mg/d/1000inh and were found only in one city or country each (Table S5). MEPH was already reported in Krakow few years ago (2012) where it was found in sewage effluents at comparable loads (3.6-7.1 mg/d/1000 inh) (Styszko et al., 2016). In the same study 4-MEC was also reported

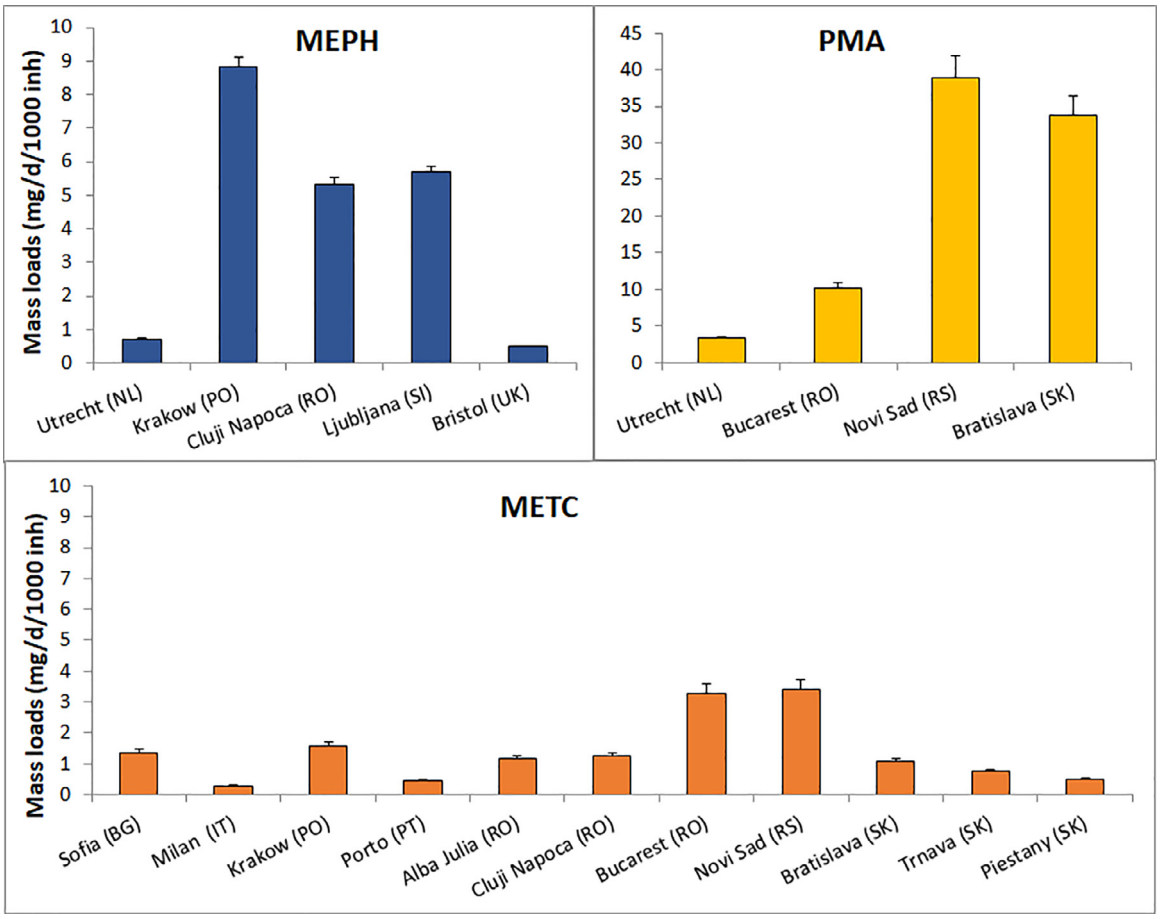

Fig. 1. Mass loads (mg/d/1000 inhabitants) of the NPS detected most frequently in 2016.

at similar levels (4.8-5.8 mg/d/1000 inh), but it was not anymore detected in this study.

Generally, the loads found in this study were similar to those in previous investigations in Australia (Bade et al., 2019a; Chen et al., 2013), Norway (Baz-Lomba et al., 2016), UK, Italy and Spain (Gonzalez-Marino et al., 2016), China (Gao et al., 2017) and Greece (Diamanti et al., 2019). Nevertheless, there are some interesting differences for certain substances such as PMA that was found in this study at the highest levels ever found before, but only in a few cities. PMA was reported previously only in Greece (up to 11 mg/d/1000inh) (Diamanti et al., 2019), but not in Belgium (Kinyua et al., 2015), indicating a scattered profile of use.

METL was the first synthetic cathinone appearing in the market in Europe in 2005 (UNODC, 2013) and was found in Europe at levels <10 mg/d/1000inh in a few studies [this study and (Baz-Lomba et al., 2016)], while an increasing trend was seen a few years ago in Australia (Chen et al., 2013; Thai et al., 2016), where it reached loads up to 40 mg/d/1000inh. MDPV was found at levels of 1 mg/d/1000inh in Italy [this study and (Gonzalez-Marino et al., 2016)], Australia (Chen et al., 2013) and China (Gao et al., 2017), but increased consumption was recorded a few years ago in Finland, where it ranged up to 19 mg/d/1000inh (Kankaanpaa et al., 2014). These results were confirmed by unusually high rates of MDPV-related crimes and MDPV-positive biological specimens from clinical sources in the same region (EMCDDA, 2014; Kankaanpaa et al., 2014). The ketamine analog methoxetamine was not found in the present study, but was recorded in Belgium (about 1-2 mg/d/1000inh) (Kinyua et al., 2015), Norway (0.8 mg/d/1000inh) (Baz-Lomba et al., 2016) and, more recently, in Australia (1.3 mg/d/1000inh) (Bade et al., 2020) and the UK (up to more than 100 mg/d/1000inh) (Rice et al., 2020).

3.3. Temporal trends of use of NPS

Temporal trends were evaluated on the five cities investigated in 2016 and 2017. MEPH, METC and METL were the only substances found in both years (Figure S1 and Tables S4, S5). No major differences were seen between the loads found in the two years, though there were differences for the single cities (Figure S1). The only exception was PMA that was found only in 2016 and disappeared in 2017, indicating a potential change in the market/use of this drug over the investigated period.

MEPH was higher in 2017 than 2016 in Krakow (Poland) and Porto (Portugal), and lower in Ljubljana (Slovenia), while METC was generally higher in 2017, except in Bratislava (Slovakia). METL was found only in Ljubljana and loads decreased from 5.5 mg/d/1000inh in 2016 to 1 mg/d/1000inh in 2017 (Table S4, S5). MEPH mass loads in Italy and UK resulted in lower levels than in previous studies (Gonzalez-Marino et al., 2016; Rice et al., 2020), particularly in the UK where mass loads dropped from about 30 mg/d/1000inh in 2015 to 0.5 mg/d/1000inh in 2016. This trend was seen also in other countries such as Australia where MEPH use peaked in 2010-2011 (Chen et al., 2013) then dropped rapidly remaining very low in the next few years (Bade et al., 2019a; Thai et al., 2016). MEPH was one of the first NPS to become popular in the market in the mid-2000s, especially in the UK and Ireland, and its use increased for a few years (2008-2010), reaching the levels of MDMA in the general population, and cocaine among

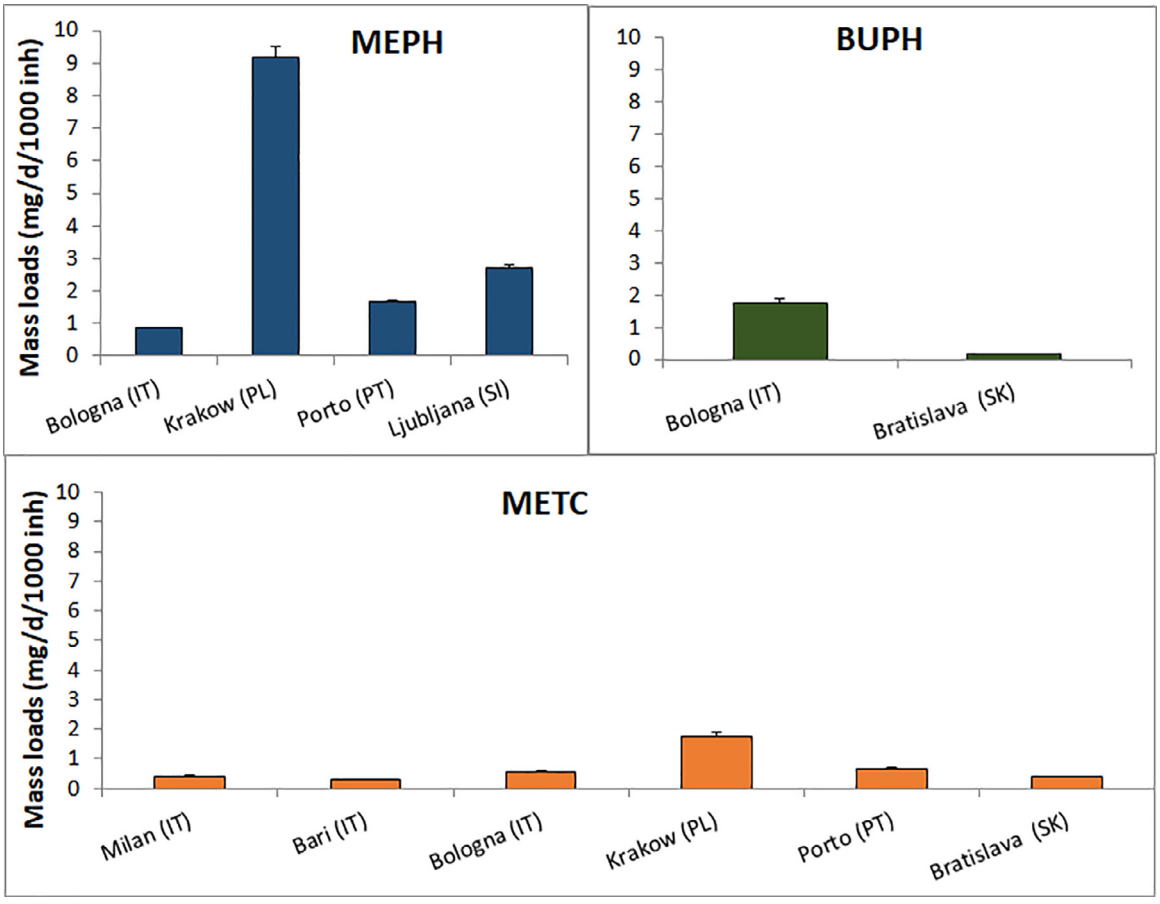

Fig. 2. Mass loads (mg/d/1000 inhabitants) of the NPS detected most frequently in 2017.

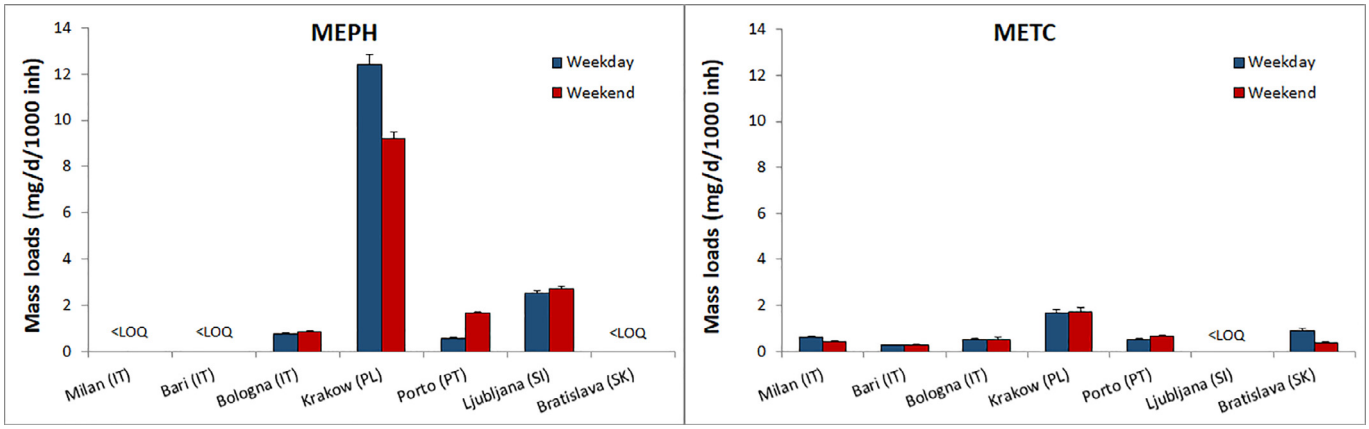

Fig. 3. Weekly profiles of use of MEPH (left) and METC (right) in the 2017 sampling campaign. Weekday= Tuesday to Friday; Weekend= Saturday to Monday.

young people (EMCDDA-Europol, 2013), until it was put under control and its use gradually decreased (EMCDDA, 2014).

No particular patterns of use have been identified so far in Europe for other substances, but an interesting trend was recently noted in Australia where different substances were preferentially used over the few last years (even if not always in the same area), i.e. MEPH in 2010-2011, METL in 2012-2013, ETHL in 2014-2016 and, more recently (Bade et al., 2019a), PENTL. It would be useful to start periodic monitoring campaigns also in Europe for a "priority" group, i.e. those substances for which preliminary information is available in some countries, in order to identify patterns of use better. Selecting this group of "priority" substances is not easy, due to the high numbers of NPS on the market and the speedy changes in their profiles of use, but combining different information such as their presence in wastewater, seizures, epidemiological and forensic data will help to establish a list of selected substances. A prioritization approach was proposed recently by our research group for selecting "priority NPS" based on wide screening by HRMS, using a database of substances selected on the basis of their reporting frequency by the Early Warning Systems of different agencies and previous detection in wastewater in several European countries (Salgueiro-González et al., 2019). Future strate-

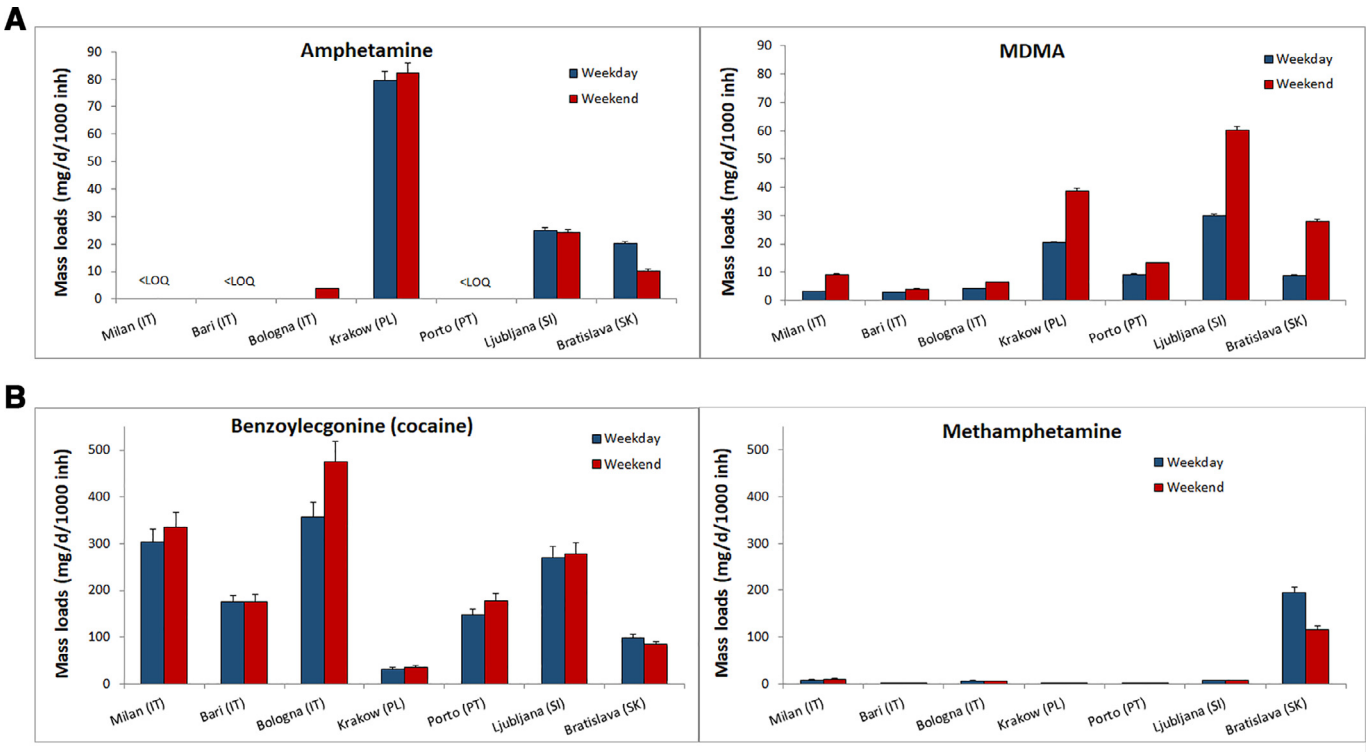

Fig. 4. Weekly profiles of amphetamine and MDMA (A) and benzoylecgonine and methamphetamine (B) in the 2017 sampling campaign. Weekday= Tuesday to Friday; Weekend= Saturday to Monday.

gies could make use of a first HRMS screening, using broad lists of compounds, followed by triangulation with information from other sources to build up a panel of "priority NPS" for target quantitative methods.

3.4. Weekly profiles of NPS use

Pooled samples from weekdays and weekends collected in 2017 were compared to assess any changing pattern, but no noteworthy differences were found (Fig. 3). This may be due to the small number of samples investigated that were too limited to identify potential changes properly and thus future investigations should expand the sample size. However, the weekdays vs weekend comparison was also done for classical drugs measured in the same samples, giving results in line with the known profiles of use (Fig. 4 A and B): increases during the weekends for MDMA and cocaine, i.e. BE (Been et al., 2016; Kankaanpaa et al., 2016; Lai et al., 2016; Thomas et al., 2012; Zuccato et al., 2016), but usually not for METH and AMPH (Kankaanpaa et al., 2016; Zuccato et al., 2016). This boosts the reliability of the results also for NPS, which probably have less marked differences in their weekly profiles of use between weekdays and weekends. For instance, METC shows a constant profile of use during the week (Chen et al., 2013; Gonzalez-Marino et al., 2016; Tscharke et al., 2016) and this was confirmed in the present investigation. On the contrary, in previous investigations MEPH and METL have shown an increase in use during the weekend (Chen et al., 2013; Gonzalez-Marino et al., 2016; Tscharke et al., 2016); this was also seen here, but only in Porto (Portugal) (Fig. 3).

3.5. Comparison of the use of classical (established) illicit drugs and NPS

Mass loads of classical illicit drugs were measured in the same samples to compare results and identify differences of use. Table 3 reports the means and ranges of the mass loads for each substance in 2016 and 2017. With the exception of PMA, mean mass loads of NPS ranged between 0.2 and 4.2 mg/d/1000inh, thus resulting 20 to 50 times lower than classical illicit drugs such as cocaine (expressed as its metabolite BE) or AMPH. PMA instead had higher loads (up to 62 mg/d/1000inh) in 2016, at a comparable level with some classical drugs such as AMPH (Table 3). Ketamine (KET), a dissociative anesthetic used in veterinary medicine and to a lesser extent in human medicine, thus not classified as an NPS, was also monitored because of its illegal use as a recreational drug for its psychoactive effects (EMCDDA, 2002). KET use was lower than for the other classical illicit drugs, and the mean

Table 3
Mean population-normalized mass loads and ranges (mg/d/1000 inhabitants) of NPS and classical illicit drugs.

Sampling 2016					
Classical drugs	Mean	Range	NPS	Mean	Range
AMPH	77.3	LOQ-279	BUTL	0.8	0.8
METH	68.6	LOQ-233	3,4-DMMC	0.2	0.2
MDMA	26.8	LOQ-206	ETHC	1.0	LOQ-1.3
BE	114.8	0.4-304	MDPV	0.2	0.2
KET	5.2	LOQ-52	MEPH	4.2	LOQ-9
			METC	1.4	LOQ-3.4
			METL	3.1	LOQ-5.5
			PENTL	1.0	1.0
			α -PVP	1.8	LOQ-3.3
			PMA	24.0	LOQ-62
Sampling 2017					
Classical Drugs	Mean	Range	NPS	Mean	Range
AMPH	35.0	LOQ-79	BUPH	0.7	LOQ-1.8
METH	26.0	0.3-195	MEPH	3.8	LOQ-12.4
MDMA	17.1	3.3-60	METC	0.7	LOQ-1.7
BE	210.4	32-476	METL	0.9	0.9
			25-iP-NBoMe	0.7	LOQ-0.8

mass load (5.2 mg/d/1000inh) was comparable to those of the NPS in this study and to those already found in Italy in a previous investigation (Castiglioni et al., 2015).

These results showed that, with the exception of the phenethylamine PMA, which might be used at a similar level to AMPH, the consumption of NPS is normally much lower than classical drugs in the general population, confirming observations from previous studies. Moreover, among the 30 NPS selected only a few were found in wastewater, suggesting that many of them were not used anymore or were used at very low extent not detectable by wastewater analysis. This, in addition to the fact that several substances identified in 2016 were not found in 2017, confirms the changing patterns of these substances. Data provided by wastewater analysis are referred to the general population and complement the epidemiological information that is scanty for NPS and mainly focused on specific populations (e.g. festival attendees, students and young people) (EMCDDA, 2020, 2015; UNODC, 2020).

4. Conclusions

The present study demonstrates the suitability of WBE for investigating NPS use in the general population as this approach was able to identify spatial profiles and temporal trends for selected substances in 14 European countries. Despite the limitations still related to monitoring NPS in wastewater, WBE can provide quantitative and qualitative information on the use of these substances in a population. This is particularly difficult for other epidemiological tools such as population surveys, because consumers often do not know exactly which drug or mixture of drugs they are taking. This study gives information on the use of NPS in Europe, confirming that it is lower than classical drugs, but also highlights the changeable nature of the market and consequently of the profiles of use. This is a useful contribution to epidemiological studies, in view of the difficulties of following these rapid changes.

This study also highlighted the scattered nature of the current WBE information for NPS, at least in Europe, where studies so far have addressed different substances and few locations. It would therefore be very useful in the next few years to implement large international NPS monitoring campaigns (e.g. yearly), employing appropriate strategies for choosing a set of "priority" substances and monitoring them over time. The best strategy for selecting NPS will be the triangulation of different sources of information, including epidemiological surveys, forensic and toxicology analyses, analysis of seized drugs, and WBE results from previous studies. Despite its focus on a limited number of substances, target analysis of selected priority substances has the advantage of providing qualitative and quantitative information on use, facilitating comparisons among different areas.

For WBE studies on NPS, the main current limitation is related to the scant information on human metabolism that prevents back-calculation of drug consumption, as is done for the classical illicit drugs. Further studies in this field are required in order to help identify the best biomarkers of use (parent drugs or urinary metabolites), and refine WBE back-calculation.

Declaration of Competing Interest

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

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Supplementary materials

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