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International snapshot of new psychoactive substance use: Case study of eight countries over the 2019/2020 new year period

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ABSTRACT

There is considerable concern around the use of new psychoactive substances (NPS), but still little is known about how much they are really consumed. Analysis by forensics laboratories of seized drugs and *post-mortem* samples as well as hospital emergency rooms are the first line of identifying both 'new' NPS and those that are most dangerous to the community. However, NPS are not necessarily all seized by law enforcement agencies and only substances that contribute to fatalities or serious afflictions are recorded in *post-mortem* and emergency room samples. To gain a better insight into which NPS are most prevalent within a community, complementary data sources are required. In this work, influent wastewater was analysed from 14 sites in eight countries for a variety of NPS. All samples were collected over the 2019/2020 New Year period, a time which is characterized by celebrations and parties and therefore a time when more NPS may be consumed. Samples were extracted in the country of origin following a validated protocol and shipped to Australia for final analysis using two different mass spectrometric strategies. In total, more than 200 were monitored of which 16 substances were found, with geographical differences seen. This case study is the most comprehensive wastewater analysis study ever carried out for the identification of NPS and provides a starting point for future, ongoing monitoring of these substances.

1. Introduction

New psychoactive substances (NPS) are a constantly evolving class of psychoactive substances which were originally sold as legal alternatives to conventional illicit drugs such as ecstasy and cannabis (Peacock et al., 2019a). They emerged in the early-mid 2000s and were marketed as 'party pills', 'bath salts' and 'legal highs', intimating safe use. However, with little or no toxicity information available, (co-)ingestion of these substances led to hospitalisation and fatalities (Elliott and Evans, 2014). Governments soon intervened, with legislation passed to limit their availability and some jurisdictions enforcing blanket bans (Reuter and Pardo, 2017). Despite these bans, NPS are still synthesised, transported and consumed around the world. With new derivatives continually appearing on the market, it is important to know which substances are of most concern in the community so that hospitals and health agencies know how best to treat patients.

On a global level, the United Nations Office on Drugs and Crime (UNODC) Early Warning Advisory is the main source for NPS information and aims to monitor, analyse and report trends, as a basis for effective evidence-based policy responses

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(United Nations Office on Drugs and Crime, 2020a). This advisory collects information from a variety of sources including forensic laboratories, global NPS surveys and law enforcement data. On a national level, the frontline for identifying NPS are hospital emergency rooms and forensic laboratories, who analyse seized drugs as well as *post-mortem* samples. However, this will only show consumption by certain individuals and not necessarily the most prevalent NPS consumed in the community. Furthermore, any NPS that are not implicit in a fatality or serious direct side-effects will not show up in *post-mortem* or emergency room samples. Traditionally, measures of the prevalence of drugs within a community rely on self-reporting surveys. Only few surveys have been conducted to ascertain prevalence of NPS use and have been able to provide some useful trends (Peacock et al., 2019a). Surveys such as the Australian Ecstasy and Related Drugs Reporting System and the Illicit Drug Reporting System or the Global Drug Survey ask drug users about their preferences and the use of specific NPS (Global Drug Survey, 2021; Peacock et al., 2019b, 2019c). This is valuable for determining user profiles and demographics. However, the nature of NPS means that users may be unaware of the actual substance they are using. There are various limitations involved with surveys including its expense, self-reporting biases, consumers do not necessarily have accurate information on what they are taking and that it can only capture a small portion of the population (Khaled et al., 2016). Therefore, additional means are needed to provide better coverage of NPS use within a community.

A complementary tool to estimate community prevalence and consumption of NPS is wastewater analysis. Concentrations in wastewater influents of target residues are measured, which can then be used to calculate the mass loads per day normalised to the catchment population. This provides anonymous, community-level (or greater) consumption information and has been used to estimate licit and illicit drug consumption (Choi et al., 2018). There are currently national wastewater analysis campaigns in Finland (Kankaanpää et al., 2016), New Zealand (New Zealand Police, 2019), China (Du et al., 2015) and Australia (Australian Criminal Intelligence Commission, 2019) while there has also been a pilot program in Canada (Werschler and Andrew, 2019), which capture, for example, between 12% (China) and 80% (New Zealand) of the population. However, these national programs focus primarily on the more common illicit drugs such as methamphetamine, MDMA and cocaine with a very limited number of NPS included. Only one currently published study has investigated national samples for a large number of NPS (Bade et al., 2020c). Smaller studies have focussed on the analysis of NPS in wastewater and/or pooled urine of specific locations (e.g. (Archer et al., 2020; Bade et al., 2019c, 2017; Borova et al., 2015; Diamanti et al., 2019; González-Mariño et al., 2016; Kinyua et al., 2015; O'Rourke and Subedi, 2020; Reid et al., 2014; Senta et al., 2014; Thai et al., 2016)) and others have looked at festival events (e.g.(Bijlsma et al., 2020; Brandeburová et al., 2020; Causanilles et al., 2017b; Kinyua et al., 2016; Mardal et al., 2017)). To the best of the authors' knowledge, the only multinational studies investigating NPS were based in Europe (Bade et al., 2017; González-Mariño et al., 2016; Salgueiro-González et al., 2019). However, there has yet to be an analysis of intercontinental samples collected at the same time to determine regional differences.

The current study investigated the prevalence of NPS in 14 sites from eight countries: Australia, New Zealand, China, the Netherlands, Spain, Italy, Norway and the United States over the 2019/2020 New Year period. Influent wastewater samples were collected for up to 15 consecutive days, extracted in the country of origin following an established, validated protocol and shipped to Australia for final analysis. A total of 26 NPS were quantitatively analysed with a targeted liquid chromatography tandem mass spectrometry (LC-MS/MS) method and a further 200 qualitatively analysed with liquid chromatography coupled to high resolution mass spectrometry (LC-HRMS). This is the most comprehensive investigation of NPS in wastewater ever undertaken and provides a starting point for future collaborative, multinational wastewater projects.

2. Materials and Methods

2.1. Selection of Compounds

A total of 26 NPS were selected for target analysis (three halogenated 2-(2,5-dimethoxyphenyl)-N-[(2-methoxyphenyl)methyl]ethanamine (NBOMe) derivatives: 25B–NBOMe), 25C–NBOMe and 25I–NBOMe, 3-ethylmethcathinone (3-EMC), 3-methylbuphedrone, 3-methylmethcathinone (3-MMC), 4-fluoroamphemtaine (4-FA), 4-fluoromethcathinone (4-FMC), 4-methylbuphedrone, 4-methylethcathinone (4-MEC), AH-7921, buphedrone, butyryl fentanyl, butylone, ethylone, furanyl fentanyl, mephedrone, methcathinone, methiopropamine, methoxetamine, methylenedioxypyrovalerone (MDPV), methylone, N-ethylpentylone, pentylone, U-47700 and valeryl fentanyl). These compounds constitute a wide variety of chemical classes (phenethylamines, amphetamine-derivatives, synthetic cathinones and opioid derivatives) and some of the most popular NPS seen around the world, based on early warning systems from the UNODC (United Nations Office on Drugs and Crime, 2019) and the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) (European Monitoring Centre for Drugs and Drug Addiction, 2019) as well as forensic findings (Kraemer et al., 2019; Partridge et al., 2018).

A suspect database was also developed, which included a total of 201 NPS. Reference standards were available for 122 NPS, which allowed confirmation in case of a tentative identification. (**Table S1**). Mixed solutions of the reference standards were previously analysed (Bade et al., 2018) and retention times, accurate masses for the protonated molecules and fragment ions were added to the database. These mixed solutions were supplied by Forensic Science South Australia in quantities not sufficient for quantification. Additional NPS were included based on international forensic findings as well as early warning advisories from EMCDDA and UNODC as above.

All compounds were purchased as certified solutions or powdered salts from Novachem (Heidelberg West, Victoria, Australia) or Sapphire Bioscience (Redfern, New South Wales, Australia).

2.2. Sample Collection

Influent wastewater samples (24h composite) were collected over 9-15 consecutive days during the 2019-2020 New Year period from eight countries: Australia (AU, 4 sites), New Zealand (NZ, 4 sites), China (CN, 1 site), Spain (ES, 1 site), Italy (IT, 1 site), the Netherlands (NL, 1 site), Norway (NO, 1 site) and the United States (US, 1 site). Most sites collected daily samples from December 25 – January 2 or from December 26 – January 2. However, the site in the Netherlands collected from 30 December – 6 January (excluding 5 January) and the site in Norway collected five multi-day samples (December 20 – 22, December 23 – 26, December 27 – 29, December 30 – January 1, and January 3 – 5) and one daily composite sample on January 2. These sites included small and large communities as well as holiday destinations (**Table S2).** Flow rates and population for all sites are shown in the supporting information, **Table S2**. All participants received instructions to follow for sample collection, to reduce variability. All samples were acidified to pH 2 with HCl upon collection and stored frozen (-20°C) until

all samples had been collected. Samples were then transported to the laboratory for sample treatment.

2.3. Sample Treatment

Participants were given a validated protocol to follow for sample treatment and extraction (Bade et al., 2020a), outlined below. Samples were filtered under vacuum using glass microfibre filter paper (GF/A 1.6 µm, Whatman, Kent, U.K.). Prior to solid phase extraction (SPE), the pH of the filtered samples was adjusted to 4.5–5 using aqueous ammonia (28 %). SPE cartridges (UCT XtracT DAU, 500 mg/6 mL; UCT Inc., Bristol, PA, USA) were conditioned with methanol (6 mL) and sodium acetate buffer (20 mM, 6 mL). The samples (100 mL) were then loaded under gravity and the cartridges were successively washed with sodium acetate buffer (20mM, 6 mL), 0.1 M acetic acid (2 mL) and methanol (6 mL) and then air dried for 15 minutes. The dried cartridges were stored at -20°C until they were shipped to the University of South Australia. Stability has previously been studied for dried SPE cartridges, which showed that illicit drugs remain stable for up to 12 weeks when stored at -20°C (González-Mariño et al., 2010), while another study showed that shipping dried cartridges overseas maintained their integrity and reliability of the data (Bijlsma et al., 2016). Moreover, we have previously shown the stability of NPS in wastewater for up to two weeks (Bade et al., 2020a). In-sewer stability of several NPS have also been investigated, including methylone, mephedrone, MDPV and ketamine (Gao et al., 2017; Kinyua et al., 2018; McCall et al., 2016; Ramin et al., 2016). Most showed medium-high stability after 24h, with mephedrone the least stable (50% transformed after 24h) (Kinyua et al., 2018).

Upon arrival at the University of South Australia, the cartridges were again stored at -20°C for no longer than 48 hours before final elution was performed. Analytes were eluted from the cartridges with a mixture of dichloromethane:isopropanol:aq. ammonia (80:16:4 v/v/v, 6 mL) and evaporated to approximately 200 μL under nitrogen at 40°C, whereupon 1% HCl in methanol (20 μL) was added, then evaporated to dryness. The dry residue was reconstituted with 0.1% formic acid in methanol (20 μL) and 0.1% formic acid in ultrapure water (80 μL) to give a final concentration factor of 1000 times.

All relevant method validation information including limits of detection and quantification, recovery, linearity and matrix effects for the quantitative method can be found in the supporting information (**Table S3**) and in previously published literature (Bade et al., 2020a, 2019a).

2.4. Instrumentation

Two separate liquid-chromatography-mass spectrometry (LC-MS) methods were utilised to analyse the samples, one quantitative (LC-MS/MS) and one qualitative (LC-HRMS). The quantitative method incorporated a Sciex ExionLC coupled to a Sciex 6500 + QTrap (Toronto, Canada), fitted with a TurboSpray IonDrive source. The qualitative screening method used a Sciex ExionLC coupled to a Sciex Triple TOF 5600 spectrometer. MS data were collected over a m/z range of 50–600. Data were acquired in Sequential Window Acquisition of all THeoretical fragment-ion spectra (SWATH) mode, utilising one full scan MS (collision energy of 10 V) and 33 subsequent experiments, each of which had a collision energy of 25 V with a collision energy spread of 15 V. The first experiment gave information relating to the parent mass, while the other 33 gave information relating to the fragment ions. For specific information regarding instrumental parameters, please refer to the previous publications. (Bade et al., 2020c, 2020a)

2.5. Criteria for Quantitative and Qualitative Analysis

2.5.1. Semi-quantitative QqQ analysis

A ten-point calibration curve was constructed for the 26 targeted NPS, either from 0.1 – 36 ng/L (ethylone, methcathinone, mephedrone, methylone, MDPV) or from 0.04-100 ng/L for all other NPS. For positive identification, retention time compatibility with the reference standard in solvent (± 2%) as well as both the quantitative and qualitative transitions being found, was necessary. Concentrations were calculated using the slope of the peak area vs concentration. Not all parties had access to the same internal standards, so quantification was based on standard response curves alone. As internal standards were not used, no correction was used for matrix effects and recovery. However, all participants followed the same validated protocol, for which it has been shown that SPE recovery was satisfactory even without internal standard correction (Bade et al., 2020a) and therefore relative site differences would be accurate.

Excreted mass loads were calculated according to equation 1:

$$\text{Excreted mass load}\,(\text{mg/d}/1000\,\text{people}) = \frac{concentration\ \left(\frac{mg}{ML}\right) x\ daily\ flow\ (ML)}{population} \times 1000$$

Equation 1: Calculation for excreted mass loads

Although many wastewater based-epidemiology studies include correction factors for excretion rates to back-calculate consumption, there is not enough information available for NPS to make the same estimation. Therefore, in this work, the mass loads were calculating only using the concentration of the parent drugs.

2.5.2. Qualitative QTOF Screening

Compounds could be tentatively identified, detected or confirmed applying the following criteria, based on previous works (e.g. Hernández et al., 2015; Partridge et al., 2018):

Tentative identification (no standard available): presence of at least two accurate mass ions (mass error < 2 mDa), supported by literature mass data on the suspect compound.

Detection (standard available): presence of one accurate mass ion (protonated molecule; mass error < 2 mDa) and retention time agreement with the reference standard (± 2%).

Confirmation (standard available): presence of at least two accurate mass ions (mass error < 2 mDa), one of which being the protonated molecule, and retention time agreement with the reference standard (± 2%).

3. Results and Discussion

3.1. Quantification of NPS

A total of 10 NPS were semi-quantified across all sites: 3-methylmethcathinone (3-MMC), 4-fluoroamphetamine (4-FA), 4-methylethcathinone (4-MEC), ethylone, methylenedioxypyrovalerone (MDPV), mephedrone, methcathinone, methylone, N-ethylpentylone and pentylone. Structures of these compounds are in the supporting information (**Table S4**). Fatalities resulting from the (co-)consumption of these substances have been reported (Jamey et al., 2016; Zaami et al., 2018) and such compounds are thus important to monitor. **Figure 1** shows the excreted mass loads (mg/d/1000 people) for the sites in which these substances were found. To aid in the readability of the figure, for countries with more than one site (AU and NZ), mean values are given. However, individual mass loads for all sites are given in the supporting information, **Table S5**. There was no single NPS found in all sites although methcathinone was identified in all sites except NO. N-ethylpentylone and 3-MMC were the next most prevalent

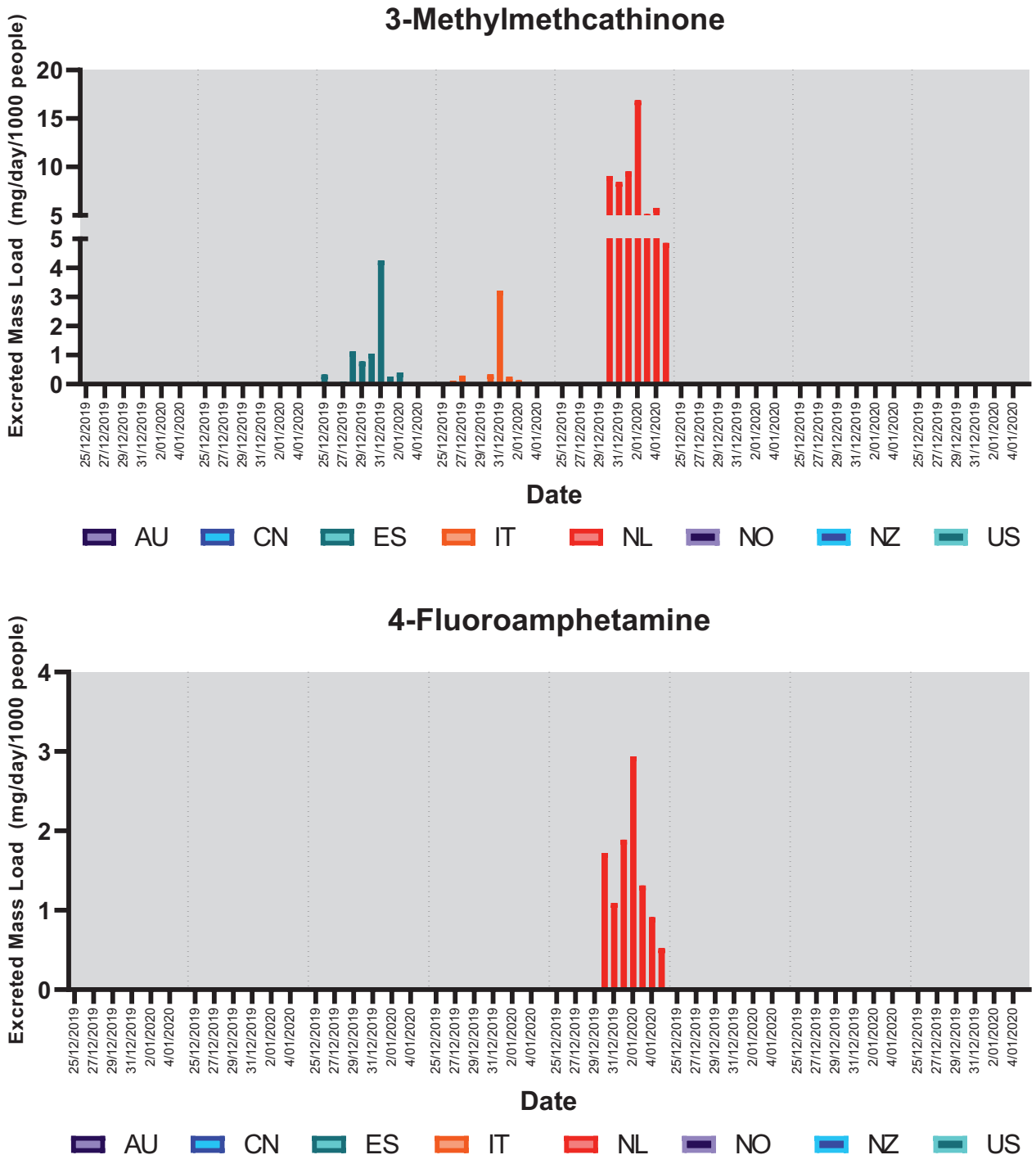

Fig. 1. Excreted mass loads of all quantified NPS. For specific mass loads on each day, refer to Table S2. For all compounds, every second day is shown on the x-axis to aid readability. Y-axes are broken for some substances to better show the lower levels found in various locations.

compounds and each was found in three countries. Results for individual substances are described in alphabetical order, in separate sections below.

3.1.1. 3-MMC

3-MMC was found in ES, IT and NL, with increases seen over the New Year's Eve, particularly in ES and IT. In NL there was generally higher mass loads across all days sampled, with a peak on 2 January. 3-MMC is an isomer of mephedrone, which is one of the most popular NPS. However, some countries in Europe have seen a move from mephedrone to 3-MMC since 2012 (Bäckberg et al., 2015), after being first detected in Sweden (World Health Organization, 2016). The findings from this work provides supporting evidence, with mephedrone found only in AU and NZ sites and not in any of the European sites that were sampled.

From 2013-2015, 3-MMC was the most commonly seized NPS in Italy (Odoardi et al., 2016), while Hondebrink *et al* showed that it was detected in forensic drug samples, consumer drug samples and/or poison centre exposure in NL from 2013-2017 (Hondebrink et al., 2020). To the best of the authors' knowledge, there is no published data of 3-MMC use in Spain while it has never been reported in wastewater anywhere before, yet our re-

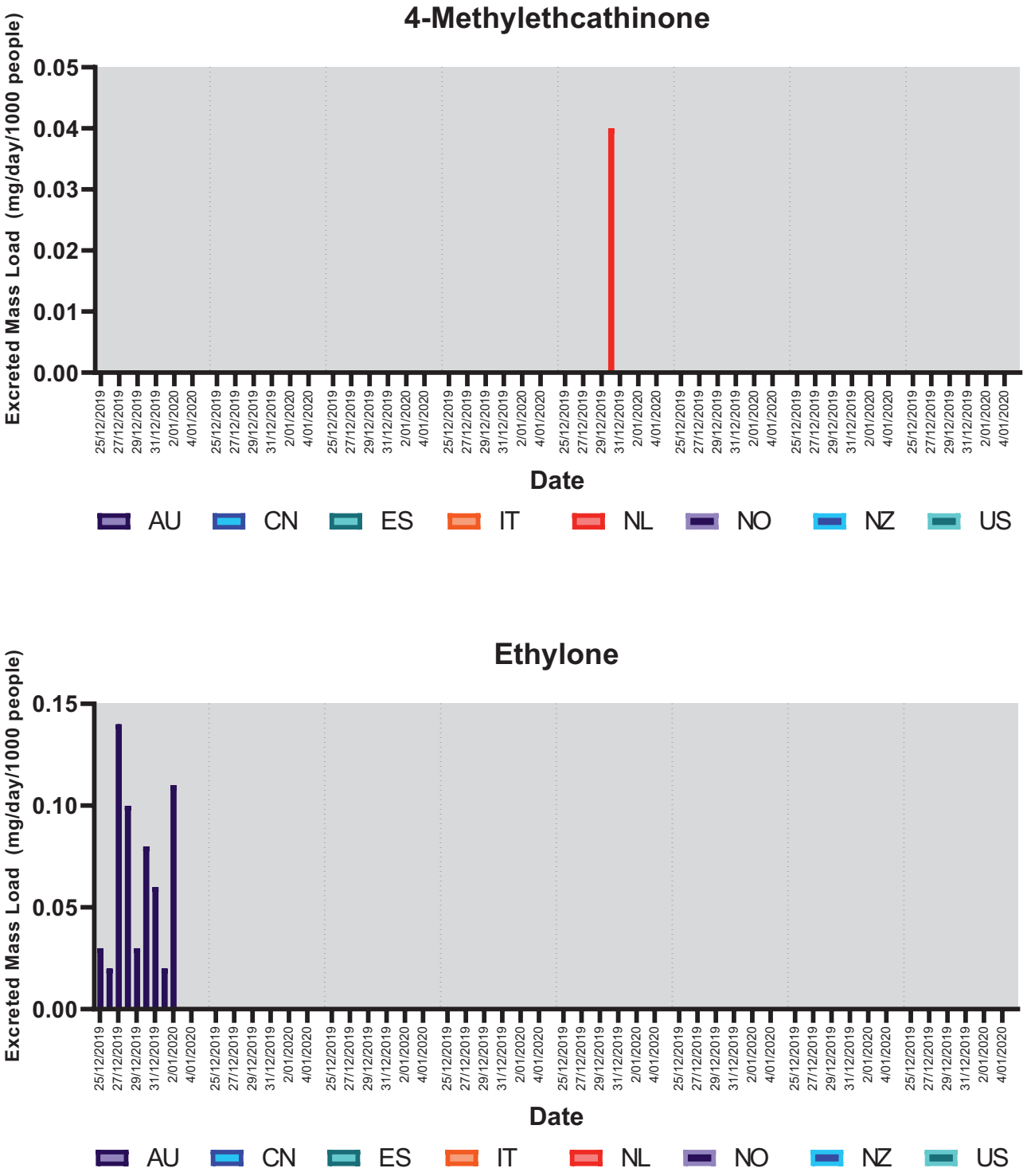

Fig. 1. Continued

sults show that it is one of the most popular NPS from this list in the sites we sampled.

3.1.2. 4-FA

4-FA was only found in NL, consistent with previous publications (Blankers et al., 2019; Brunt et al., 2017; Wijers et al., 2017). As with other NPS in NL, the highest mass load was seen in the weekend following the New Year (2.9 mg/d/1000 people on January 2), although mass loads across the sampling week were much higher than other NPS detected in this study – with a minimum of 0.5 mg/d/1000 people on 6 January.

3.1.3. 4-MEC

A low mass load of 4-MEC was found in NL on one day, 30 December, with an excreted load of 0.04 mg/d/1000 people. It has been reported in multiple forensic sources between 2013 – 2017 (Hondebrink et al., 2020) and has been declared illicit in NL since 2018. The finding over the New Year period confirms that some

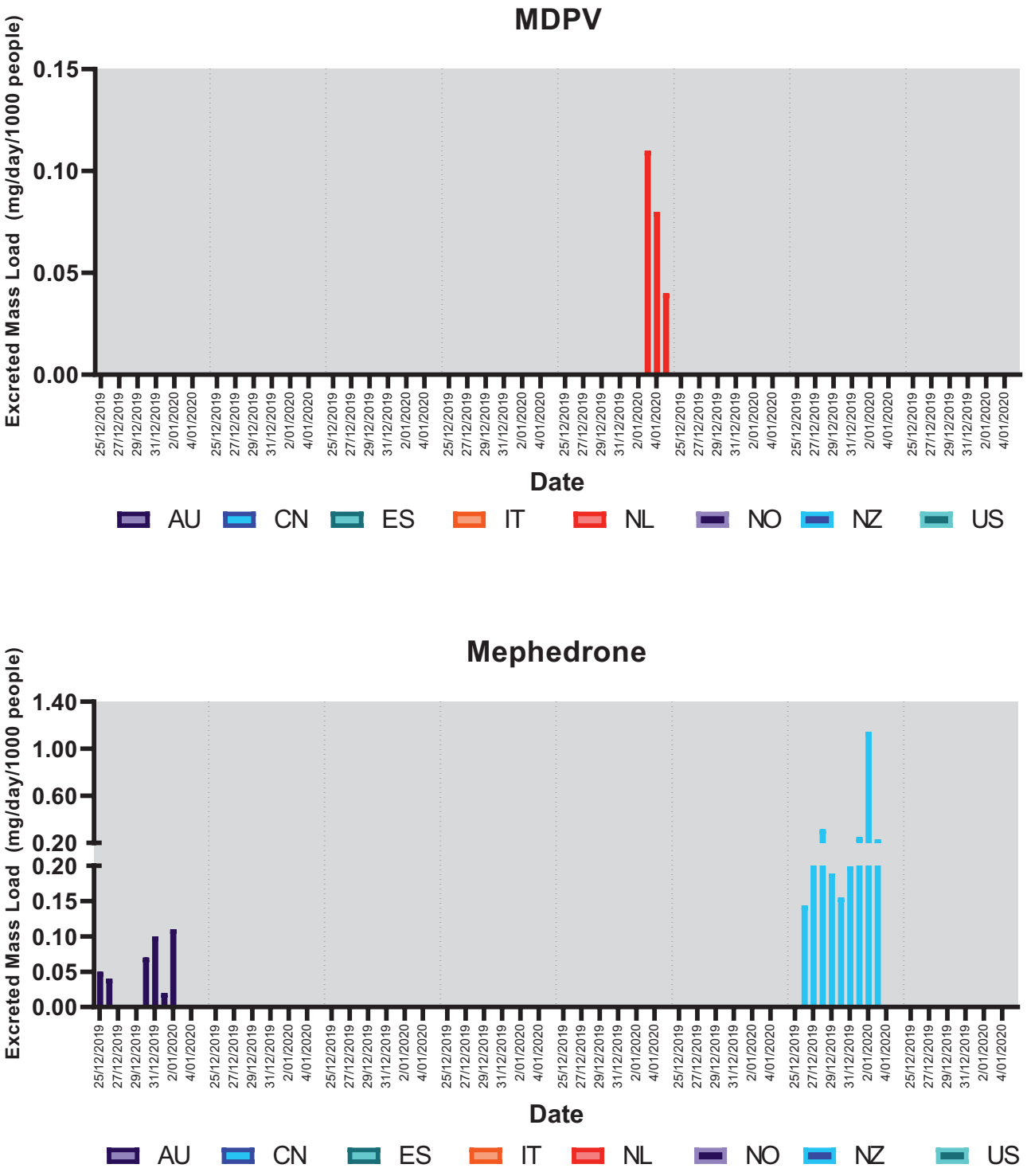

Fig. 1. Continued

users in the NL have a preference for this substance, or access to this drug.

3.1.4. Ethylone

Ethylone was not detected anywhere except in AU. It has been seen in Australia since 2014 in both wastewater and forensic samples (Bade et al., 2020c, 2019b, 2019c). In this work, ethylone had no clear consumption trend, with variable mass loads seen over the days sampled, with a peak on 27 December of 0.14 mg/d/1000 people, which was a Friday after three consecutive days of work closures (i.e. public holidays).

3.1.5. MDPV

MDPV was quantifiable in the NL on three days: January 3, 4 and 6, with mass loads between 0.04 – 0.11 mg/d/1000 people, with the highest load on January 3. Much like 4-MEC, MDPV was banned in 2015 in NL, but has been seen since, albeit at a lower level than before the ban (Hondebrink et al., 2020).

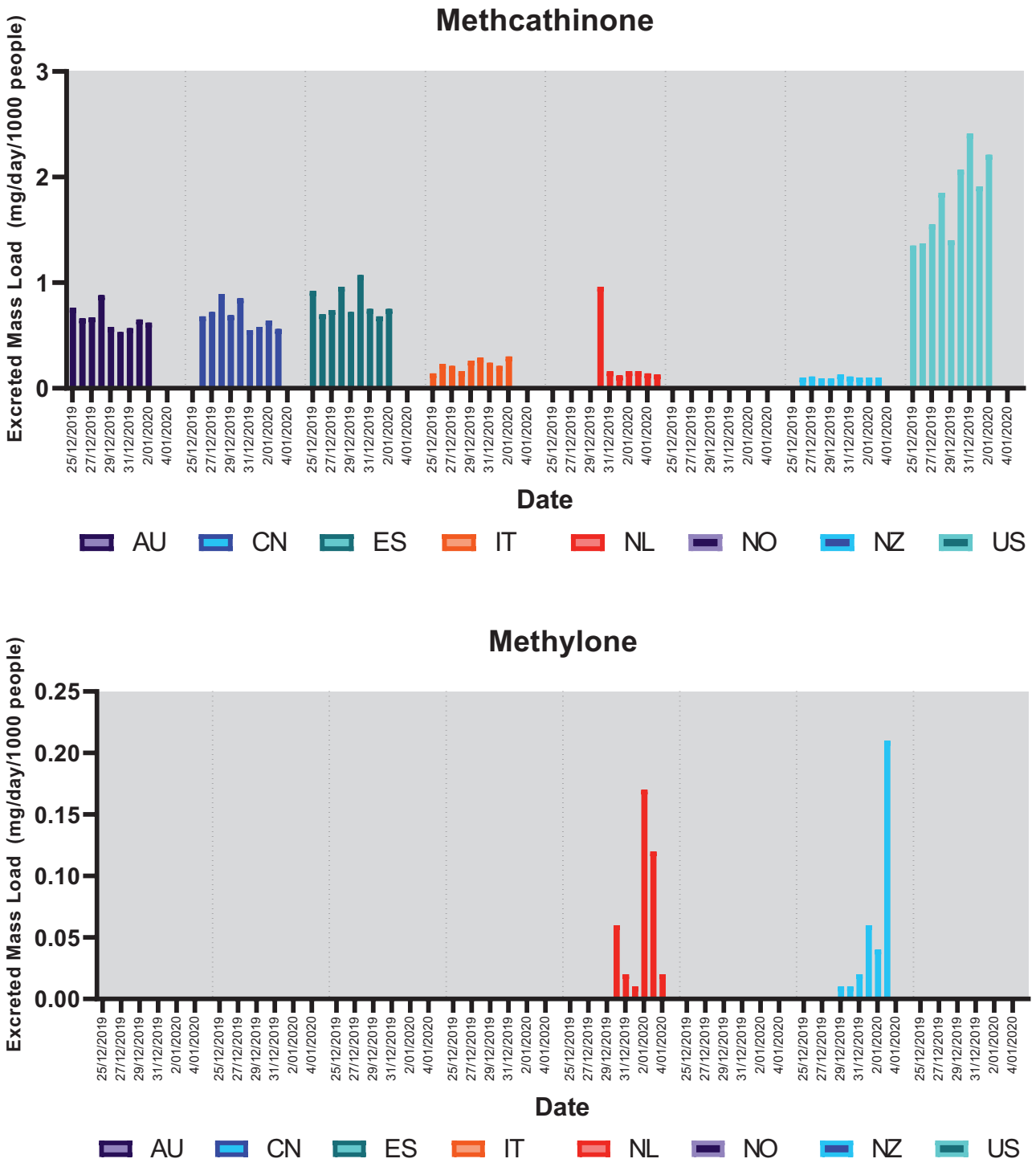

Fig. 1. Continued

3.1.6. Mephedrone

Along with 3-MMC, mephedrone was the NPS that showed significant increase in consumption over New Year's Day, particularly in NZ 1 and NZ 4. Mass loads increased up to 20 times over New Year's Eve. This substance was only found in AU and NZ, predominantly over the New Year, although NZ 1 showed consumption throughout the sampling week.

Mephedrone is one NPS that has remained popular in spite of legislation restrictions and is one of the most popular NPS, particularly in the United Kingdom (Hutton, 2020). In Australia, mephedrone has been detected in wastewater since 2009, while it increased in consumption when a supply shortage reduced MDMA (ecstasy) use in 2010 (Chen et al., 2013). It is still being detected around the country (Bade et al., 2020c). Survey data also showed it was the most popular NPS in Australia in 2012-2013 (Matthews et al., 2017), while more recent data from 2019 showed it is still used amongst ecstasy and stimulant users (Peacock et al., 2019b). The most recent survey data from New Zealand from the Illicit Drug Monitoring System, which surveys frequent drug users, is from 2014 (Wilkins et al., 2015), which showed that mephedrone

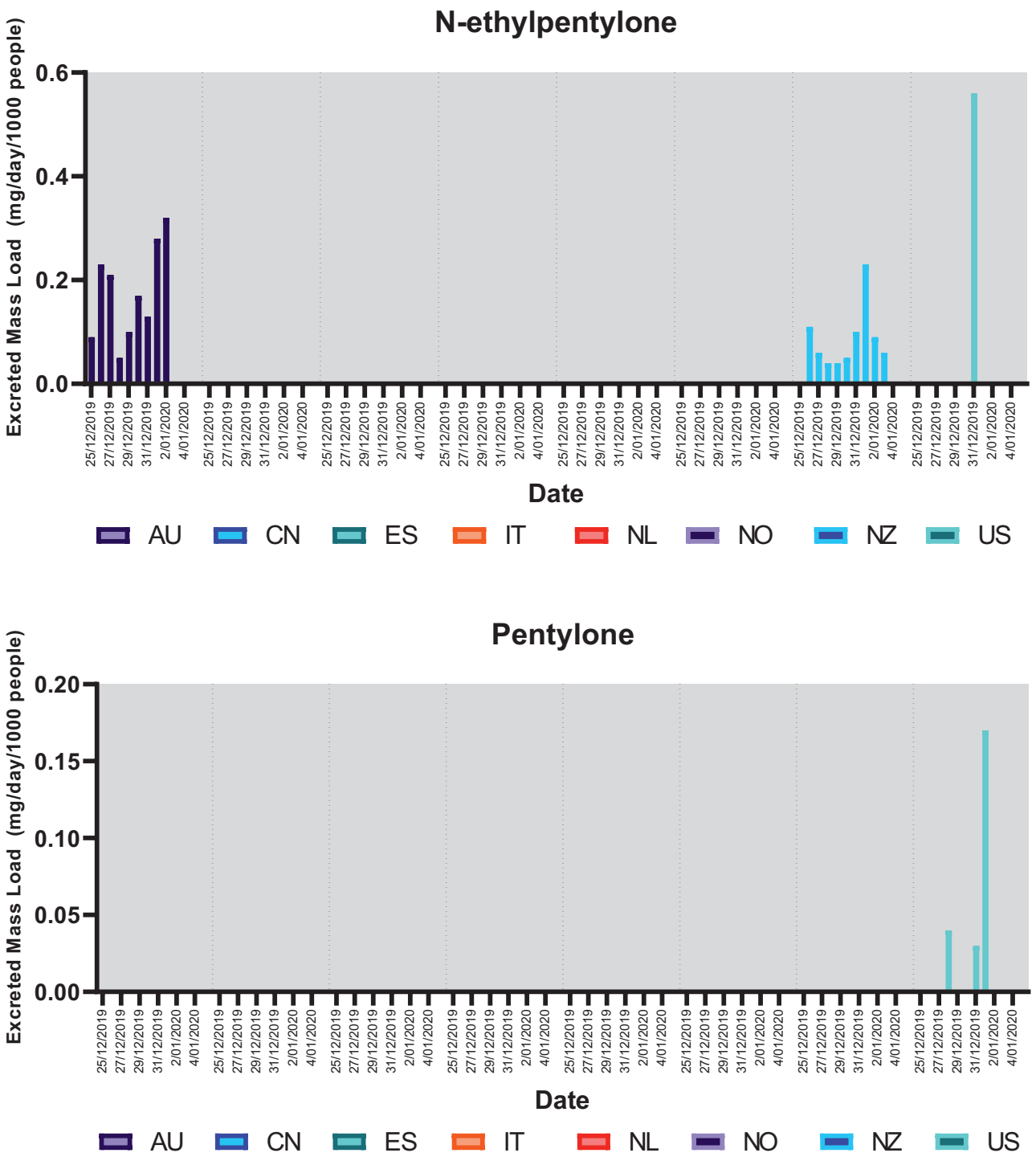

Fig. 1. Continued

was one of the most popular NPS of this user group. Unfortunately, more recent survey data are not available. One pilot wastewater study in New Zealand included mephedrone in the method, but none was found (Lai et al., 2017). However, this could be due to the sampling being carried out in the winter (May – July), when there are fewer recreational events.

3.1.7. Methcathinone

Methcathinone was found in all sites except NO, with the highest mass loads seen in US followed by ES, CN and AU. The popularity of methcathinone may be partly attributed to its ease of preparation from ephedrine (Liu et al., 2021). Although this drug has been found in previous wastewater studies in Australia (Bade et al., 2020c, 2019c), Slovakia (Brandeburová et al., 2020), Italy (González-Mariño et al., 2016), and the United States (Montgomery et al., 2021; O'Rourke and Subedi, 2020), results need to be treated with caution. Aside from use as a stimulant on its own (Zhao, 2020), methcathinone has been found as an intermediate in illegal pseudoephedrine/ephedrine production (Zhu et al., 2020) and may find a route into the sewer other than through consumption. However, the results reported in **Figure 1** show quite a variable pattern across all sites, inconsis-

tent with dumping or use in illegal manufacturing. Only one large peak was seen – on 30 December in NL – of around 1 mg/day/1000 people. This is similar to what has previously been found in Dutch waterways (van der Aa et al., 2013), albeit only in effluent wastewater.

3.1.8. Methylone

Methylone was one of the more interesting geographical findings – only being seen in NL and NZ. There were similar consumption trends in the sites in both countries, with the highest mass loads seen on January 2 and 3, which was the weekend following New Year's Eve. Methylone has not previously been found in NZ while it has been detected in NL since 2013, particularly in forensic cases (Hondebrink et al., 2020).

3.1.9. N-ethylpentylone

N-ethylpentylone was detected in three countries: NZ, AU and US. Although the US site had the highest mass load (0.56 mg/d/1000 people), it was only found on one day (31 December). However, it was seen on all days in NZ (particularly sites NZ 1, NZ 2 and NZ4), with mass loads up to 0.52 mg/day/1000 people. Additionally, three sites in AU had low levels of N-ethylpentylone. It was detected on every sampling day in AU 1, while it was only found on the weekend of December 28 (AU 2), December 29 and 30 (AU 4) in the other two sites. This substance was first reported to the EMCDDA in 2016, having been found in Slovenia (EMCDDA, 2016). Since then, it became prevalent in various parts of the world. In the United States, it was the most seized synthetic cathinone by the Drug Enforcement Administration (DEA) in 2017 and 2018, and accounted for almost 20% of all seizures of synthetic cathinones in the country in 2019 (Drug Enforcement Administration, 2019). This substance has known toxicity and has been implicated in fatalities in the United States (Krotulski et al., 2018), while the UNODC released a report in early 2018 warning of it being sold as ecstasy (United Nations Office on Drugs and Crime, 2018). It has been detected in forensic samples in Australia since 2017 (Bade et al., 2019b) and in wastewater since 2018 (Bade et al., 2020a, 2020c) as well as at music festivals in Australia and New Zealand (Johnson et al., 2020). For the most part, there did not appear to be any increase in N-ethylpentylone consumption over the New Year period in most sites, although NZ 3 and US both showed an increase over 31 December and 1 January, indicative of consumption over New Year's Eve, while AU 1 had a general increase across the sampling week. Interestingly, New Year's Eve was the only day this substance was detected in the US site.

3.1.10. Pentylone

Pentylone was only seen on 3 days in US – December 28, 31 and January 1, with the highest mass load of 0.17 mg/d/1000 people on January 1. The substance was not detected at any other site, suggesting that supply or demand for the drug is low.

Of the eight countries studied in this work, seven had NPS at semi-quantifiable levels, with only NO not having any NPS. NL had the greatest number of NPS (six) followed by NZ and AU (four each) and US (three). ES and IT had two, while only methcathinone was found in CN.

3.2. Qualitative Screening of NPS

A qualitative screening method was applied to all samples, using a database of more than 200 priority NPS. Seven additional NPS were found with this approach, either tentatively identified, detected or confirmed, according to the criteria of Section 2.5.2 (**Table 1**): 4-chloromethcathinone (confirmed), 4-fluoromethamphetamine (confirmed), acetyl fentanyl (detected), mitragynine (confirmed), eutylone (tentatively identified then confirmed), ketamine (confirmed) and norketamine (confirmed)). The structures of these compounds are in Table S4. Ketamine was included as it is considered an NPS by the UNODC. Four compounds were only found in one location: IT (4-chloromethcathinone), NL (4-fluoromethamphetamine) and US (acetyl fentanyl and mitragynine), perhaps indicating geographical preference or supply meeting demand.

3.2.1. Most prominent NPS

Ketamine and its metabolite norketamine were found in every site on all days analysed. Although it is considered an NPS by the UNODC, ketamine has been around for decades as a human and veterinary anaesthetic. However, it has become a common recreational drug, particularly in Europe and Asia. Although a qualitative study as this cannot compare sites, the largest chromatographic peak heights were seen over the New Year in NZ, AU, CN, IT and US, while in NL, NO and ES, the largest peak heights were on December 28 (Saturday) or January 2-5 (Thursday – Sunday), potentially indicating recreational use in all catchments.

Eutylone was seen in AU, NZ, US and NL. This substance has previously been detected in wastewater in Australia (Bade et al., 2020c) and in forensic samples in Poland and the US, both as a lone stimulant and in conjunction with N-ethylpentylone (Krotulski et al., 2020). The results in **Figure 1** agree with the latter, with N-ethylpentylone also found on the same days as eutylone in NZ, US and AU sites. However, this was not the case in NL, where eutylone was detected on its own.

3.2.2. NPS qualitatively found in specific countries

Mitragynine and acetyl fentanyl were only found in US. Mitragynine is the primary alkaloid found in the 'kratom' plant. The drug was involved in almost half NPS-related deaths in 2019 reported to the UNODC Early Warning System, with a large number from the US (United Nations Office on Drugs and Crime, 2020b). Kratom has grown in popularity in the US, with life-time prevalence estimated at 1.3% and prevalence of use over the last 12 months of 0.8 % (Schimmel et al., 2020). It therefore came as no surprise that mitragynine was found across all days at the US site. Acetyl fentanyl is one of the most common synthetic opioids in the US and was the second most identified by the DEA in 2019 (Drug Enforcement Administration, 2019). It has been implicated in fatalities since 2013 and during the opioid crisis in the US (Noto et al., 2019). It was therefore a cause for concern that this compound was detected on every sampling day at the US site. A recent study from rural communities in Southern Illinois found the presence of additional synthetic opioids carfentanil, furanyl fentanyl and methoxyacetyl fentanyl in influent wastewater (O'Rourke and Subedi, 2020), indicating the range of opioid derivatives used in the United States.

4-Chloromethcathinone was only found on one day in IT (1 January), which is consistent with the substance having been consumed on New Year's Eve. Although this substance was one of the top NPS reported to the UNODC Early Warning Advisory in 2015 – 2016, there have been few reported cases of 4-chloromethcathinone-induced intoxications (World Health Organization, 2019). 4-Fluoromethamphetamine was only found on one day in NL (3 January).

4. Limitations and Future Possibilities

This work provides an interesting insight into NPS use in eight countries around the world over the New Year period – a time synonymous with increased party drug use. However, only between 1 and 4 locations were investigated per country and therefore the results described in this work are not necessarily indicative of consumption on a national scale. Although every effort was made to

Table 1
All NPS found with the qualitative screening method based on LC-HRMS.

Compound	Confidence in Identity	Country/Site	Day(s) found	Day of highest level[a]
4-Chloromethcathinone	Confirmed	IT	Jan 1	Jan 1
4-Fluoromethamphetamine	Confirmed	NL	Jan 3	Jan 3
Mitragynine	Confirmed	US	All	Jan 1
Acetyl Fentanyl	Detected	US	All	Dec 31
Eutylone	Tentatively Iden-ti-fied/Confirmed*	AU 1	Jan 1-2	Jan 2
		AU 2	Jan 1-2	Jan 1
		AU 3	Jan 1-2	Jan 2
		AU 4	Jan 1-2	Jan 1
		NL	Dec 30, Jan 1-4	Jan 1
		NZ 1	Dec 26-28	Dec 26
		NZ 2	Dec 31, Jan 1-2	Jan 1
		NZ 3	Jan 1-2	Jan 1
		NZ 4	All	Jan 2
		US	Dec 28, Dec 31, Jan 1-2	Jan 1
Ketamine	Confirmed	AU 1	All	Dec 30
		AU 2	All	Dec 31
		AU 3	All	Dec 30
		AU 4	All	Jan 2
		CN	All	Dec 30
		ES	All	Dec 28
		IT	All	Dec 30
		NL	All	Jan 2
		NO	All	Jan 3-5
		NZ 1	All	Dec 26
		NZ 2	All	Jan 2
		NZ 3	All	Jan 1
		NZ 4	All	Jan 3
		US	All	Jan 1
Norketamine	Confirmed	AU 1	All	Dec 30
		AU 2	All	Jan 3
		AU 3	All	Dec 28
		AU 4	All	Jan 2
		CN	All	Dec 30
		ES	All	Dec 28
		IT	All	Jan 1
		NL	All	Jan 2
		NO	All	Jan 2
		NZ 1	All	Dec 26
		NZ 2	All	Jan 1
		NZ 3	All	Jan 1
		NZ 4	All	Jan 2
		US	All	Jan 1

[a] based on chromatographic peak height
* Eutylone was initially tentatively identified. However, subsequent purchase of the reference standard enabled confirmation.

preserve samples (i.e. acidifying and freezing upon collection and priority shipping of cartridges), some compounds may have been lost under these conditions. One potential solution would be the inclusion of internal standards during the extraction to cater for any losses. Cases in point are the designer benzodiazepines and synthetic cannabinoids – some of the NPS classes of most international concern – which were not found in any sample. This is likely due to the acidification of the samples upon collection and then filtration prior to sample extraction. Previous studies have shown that benzodiazepines and cannabinoids are not quantifiable in wastewater at acidic pH (Bade et al., 2020b; Causanilles et al., 2017a). Furthermore, the lipophilic characteristics of cannabinoids lend themselves to being primarily in the solid phase which was filtered off in this work (Pandopulos et al., 2020). Regarding the specific findings of this study, as only parent compounds were targeted, direct disposal of the NPS could have contributed to the calculated mass loads.

Much like the "COVID-19 WBE Collaborative" which has recently been launched to aid in the coordination of research groups undertaking work related to SARS-CoV-2 detection in wastewater (Bivins et al., 2020), future international NPS wastewater analysis collaborations would help to determine NPS prevalence in a wider number of sites and countries. The HighResNPS database is another example where laboratories from around the world contribute HRMS data to produce an ever-increasing database of NPS suitable for qualitative methods (Mardal et al., 2019). Moreover, the sharing of samples and reference standards, development and expansion of analytical methods to incorporate more substances of a wider number of classes will aid future analyses.

5. Conclusion

This work has shown the potential of wastewater analysis and collaboration to provide international levels of NPS. Samples were collected over the New Year period to coincide with expected increased use of drugs. A total of 16 substances were found, with methcathinone (seven countries), N-ethylpentylone and 3-MMC (three countries each) having the highest wastewater loads. NL and US had the highest number of NPS detections, while NO and CN had the least. With sampling undertaken over the New Year period, it was evident that many NPS showed increases over New Year's Eve, particularly mephedrone and 3-MMC. It is also worth noting that some NPS were only detected in one specific site, such as ethylone in AU, 4-chloromethcathinone in IT, pentylone, acetyl fentanyl and mitragynine in US and 4-FA, 4-fluoromethamphetamine and 4-MEC in NL. Despite the number of 'new' NPS reportedly de-

clining in recent years, this work shows that they are still used within communities and across countries, emphasising the need for continued surveillance and monitoring.

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

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.watres.2021.116891.

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