The detection of new psychoactive substances in wastewater. A comprehensive review of analytical approaches and global trends.

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Abstract:

New psychoactive substances (NPS) have made a substantial impact on the global drug market through their dynamic spread into recreational drug consumption and the challenges of developing legislative controls. Drug trends are monitored by organisations such as the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) and the United Nations Office on Drugs and Crime (UNODC), which have been utilized for monitoring the presence of NPS. In particular, improved wastewater analysis (WWA) has been used to monitor NPS use successfully. NPS detection in wastewater has allowed the observation of significant drug trends at regional, national and international levels. Approaches to the technique have evolved over time with non-targeted analysis becoming more utilized in recent years as it offers a wider-scope when searching for certain compounds due to the lack of available reference standards for many of the currently known NPS. In addition to the evolution of available analytical technology so too has the scale and complexity of wastewater investigations evolved. Multi-city and multinational studies have provided detailed insight into the complex patterns of NPS abuse over time and space. The field of wastewater analysis has provided significant advancements to our understanding of these important drug trends, but challenges still remain however, both analytical and logistical. Here we review the state of the art in analytical approaches to the analysis of NPS in wastewater, and present global NPS trends ascertained by WWA.

Keywords: Wastewater-based epidemiology; Novel Psychoactive Substances; Global Trends; Drug Consumption; Monitoring techniques

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1 – Introduction

The toxicological analysis of wastewater is a well-developed analytical process and can reveal the drug-taking habits of specific populations (Zuccato et al 2008; Bugard et al 2013; EMCDDA 2020f). In recent years, the prevalence of new psychoactive substances (NPS) has become a global issue, and the use of wastewater monitoring to assess NPS trends and the geographical variation has become more widespread and is the subject of this critical review. Wastewater analysis (WWA) studies the phenomenon of substances being consumed, then metabolised, excreted and transported through the sewage network into the wastewater treatment plant (WWTP) (Zuccato 2005).

Daughton in 2001 proposed the approach of taking samples at various points in the wastewater journey to obtain a profile of drug compounds as a form of non-intrusive drug monitoring at a population scale. Zuccato et al (2005) refined the WWA method protocols to estimate community abuse of illicit substances from representative samples. Over time advances in analytical instrumentation allowed improvements to be made in methods targeting specific analytes (Bones et al 2007; Ort et al 2010a; Ort et al 2010b; Zuccato 2011; Khan et al 2014; O'Brien et al 2014). In the last 5 years at least, WWA studies have increasingly focused on the detection of New

Psychoactive Substances (NPS) and illicit substances across different cities and at-risk areas in response to the rising market (Baz-Lomba 2016; Celma et al 2019; Fallati et al 2020). NPS are a "new narcotic or psychotropic drug, in pure form or in preparation, that is not controlled by the United Nations drug conventions, but which may pose a public health threat comparable to that posed by substances listed in these conventions" (EMCDDA 2020c). These conventions are the Single Convention on Narcotic Drugs of 1961 or the Convention on Psychotropic substances of 1971 (UNODC 2020a). There are a number of compounds previously listed as NPS which are now scheduled substances in the US, such as methylenedioxypyrovalerone (MDPV), mephedrone and methylone (UNODC 2019a; UNODC 2020c). Also, there are certain compounds that are 'newly misused/abused pharmaceuticals' rather than newly synthesised that have been scheduled as well as considered to be NPS (such as ketamine and fentanyl) (UNODC 2013; Zhao 2019; ACMD 2020).

NPS can be separated by what illicit substance class they are imitating or structurally similar to (Public Health England 2015; Public Health England 2017). Synthetic cannabinoids or synthetic cannabinoid receptor agonists (SCRAs) such as 'Spice' or 'Black Mamba' are related to the tetrahydrocannabinol (THC) component of cannabis (Tracy et al 2017; EMCDDA 2019a). Synthetic hallucinogens can split into two types; psychedelics such as N,N-di allyl-5-methoxy tryptamine (5-MeO-DALT) or the N-methoxybenzyl (NBOMe) series have effects similar to traditional agents such as lysergic acid diethylamide (LSD) or psilocybin (Baumeister et al 2015; Tracy et al 2017); dissociatives such as methoxetamine are similar to ketamine (KET) and phencyclidine (Tracy et al 2017; Schifano et al 2019). Synthetic stimulants such as those in the cathinone family e.g. mephedrone aka 'bath salts' are related to 3,4-methylenedioxy methamphetamine (MDMA) and amphetamine like structures (Karila et al 2015; Tracy et al 2017). Lastly there are synthetic depressants which split into two types also; opioids such as AH-7921 or novel fentanyls have effects similar to but stronger than traditional opioids like morphine (Tracy

et al 2017; Frisoni et al 2018); benzodiazepines such as diclazopam or flubromazepam have similar effects to compounds such as diazepam (Baumeister et al 2015). First-generation NPS such as synthetic cannabinoids have been on the European drug market since around 2008 (EMCDDA 2017) the first-generation synthetic cannabinoids are some of the earlier cannabis alternatives released and then banned later by authorities. Following the first set of banned synthetics, a second-generation of synthetic cannabinoids and other NPS were released (Valente et al 2013; Chung et al 2015; Zawilska et al 2015; Nutt 2020). There is now a third generation of SCRAs, along with an increasing number of synthetic cathinones, synthetic opioids and medicinal NPS on the market. Several European countries have reported NPS abuse levels increasing in vulnerable groups including prisoners and the homeless (EMCDDA 2017). Motivations for using NPS are varied but may be driven by difficulties in obtaining traditional illicit drugs, price and status in some countries. Routes of NPS administration differ between drug type and the population abusing the drugs, but in general popular NPS classes such as synthetic cathinones are often injected and the synthetic cannabinoids are smoked, though various administration routes are possible including snorting, tablet forms and vaping.

The detection of NPS in wastewater is challenging in part due to the way in which such drugs are abused (in addition to technical challenges which are discussed later in the review). NPS are frequently abused in the same session as other illicit drugs and are most often not the primary drug choice of high-risk drug taking groups. In this context NPS may be chosen when the preferred illicit drug is not available, or purity is low, and NPS may also be used to heighten the effect of other drugs. The composition of the compounds is ever changing and usage patterns difficult to predict. The presence of certain NPS in wastewater is therefore sporadic and unpredictable, often at very low concentrations (especially for synthetic cannabinoids which are often used in extremely small doses). A further challenge for the detection of cannabinoids in wastewater is the fact that cannabinoids are lipophilic and can be excreted in faecal matter

(Bugard et al 2019. This may lead to cannabinoid presence in the particulate fraction of wastewater and so their concentration may be underestimated if the particulate fraction is neglected during extraction (Senta et al 2013).

The European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) has kept track of the complex and dynamic NPS market in Europe. More than 700 NPS (790 approximately) have been formally notified since 1997 through the European Union Early Warning System (EWS) (EMCDDA 2020d). There is an apparent decrease in the number of newly introduced substances into the European market each year since 2015 (53 in 2019 only), however the catalogue of NPS remains vast and the market is complex; 400 previously reported NPS have been detected each year since 2015 (EMCDDA 2020d) (Figure 1).

Figure 1. Overview of the NPS number and categories reported since 2008 for the EMCDDA's EWS shown in the EU 2020 Drug Market Report by the EMCDDA

These substances have become a global issue and the development of monitoring techniques such as WWA play an important role, contributing not only to detection but providing information to assist mitigation and eventually prevention of the problem. Organisations such as the EMCDDA and United Nations Office on Drugs and Crime (UNODC) have focused on outlining the supply chain, production facilities and keeping track on legislative controls specific to this global problem (UNODC 2020d; EMCDDA 2020e).

In more recent years there has been a significant increase in new legislation created to tackle NPS (see Figure 2 for examples of European legal innovations). A range of different prevention strategies have been attempted from 2009-2016 internationally; these range from outlining product components and improving regulations to changing the legal substitute drug definitions (such as Luxembourg and Austria in 2009), to creating or altering a substance list under controlled law to outline their health risks and prohibit their use (such as Poland in 2010, Portugal in 2011,

Hungary in 2012 and the Republic of Korea (Reitox 2012; Chung et al 2014; UNODC 2013; EMCDDA 2020a). There is no single way to solve this threat, focus should be on drug use prevention and impact mitigation. The EMCDDA suggests a focus on deterring suppliers and creating criminal sanctions for possessing a substance for personal use (EMCDDA 2020a). The United Kingdom attempted to reduce NPS prevalence via their Psychoactive Substances Act (PSA) in 2016 which increased NPS prices and decreased their availability (Home Office 2018b). However, crime survey data (2017-2018) and a PSA review revealed that NPS emergence did not technically reduce following this legal innovation even with its proactive changes (Home Office 2018a; Home Office 2018b; Home Office 2018c)

Figure 2. Map showing the legal innovations across Europe available on the EMCDDA website: www.emcdda.europa.eu/topics/pods/controlling-new-psychoactive-substances

Prevention and mitigation need to be considered when addressing this threat and timely, accurate information on the nature of NPS in circulation is key to this aim. Analysis of wastewater can provide this information and as such further research efforts in this field are vital. Work is required to continue to improve substance detection and information collection on prevalence, structure and concentration. The context of this work will differ based on the use of targeted or non-targeted analysis methods, the number of target compounds detected, and the detection method (depending on biological matrices also). Thus the WWA approach is considered a key investigative tool to support the larger solution.

2 – The background to wastewater analysis

The foundation of wastewater analysis (aka wastewater-based epidemiology or WBE) is that there are traces of everything we consume (whether metabolites or parent compounds) present in our waste. This ends up in the sewage network and can be utilized to show a fingerprint of consumed

substances. The collection of samples from wastewater treatment plants (WWTPs) is an important step in the overall process of wastewater analysis; a wealth of information regarding drug residue concentrations and local population concentration rates can be extrapolated and be applied to current substance abuse investigations in the area. WWA ultimately focuses on estimating the drug load and consumption of a specific substance based on presence and concentration, in order to confirm pre-existing evidence of target substances for the population/environment of interest (Castiglioni et al 2014; Thai et al 2016). WWA is applicable to a range of populations, from a singular establishment (500-1000 people) such as a university, school or prison, to a WWTP that serves a state capital or megacity (500,000 to millions of citizens) (Khan et al 2014; Gatidou et al 2016; Van Dyken et al 2016). Therefore it can offer temporal/spatial data simultaneously at different scales. WWA allows a researcher to conduct a near real-time drug consumption estimation in a complex matrix at a range of location scales and time frames (outlined by Choi et al 2018 in Figure 3).

Figure 3 WWA Process Schematic from Choi et al 2018

Part of estimating drug load consumption and suggesting drug consumption trends for NPS compounds is the use of 'back calculations' following successful detection and quantification. The calculations require: NPS concentration, WWTP influent flow rate (on day of interest), WWTP population serves. This enables calculation of the daily mass load of a substance per 1000 inhabitants (see equations (1) and (2) below and as found in Gatidou et al 2016). A correction factor (average drug residue excretion rate) is then multiplied by the daily drug load to estimate the total amount of drug consumed by users (Kankaanpää et al 2014; Thai et al 2016; Tscharke et al 2016; Gao et al 2017). This calculation has mostly been used for traditional illicit drug use estimation in WWA rather than NPS due to the lack of human metabolic and pharmacokinetic studies for NPS (necessary for calculating the excretion rate) so they remain uncorrected. Baker et al (2014) was able to infer a low usage of TFMPP and BZP in their WWA study without this

metabolic based data to corroborate another study. Other studies calculated consumption values without the excretion rates, so use could only be tentatively estimated (Kankaanpää et al 2014; González-Mariño et al 2016b; Thai et al 2016; Bannwarth et al 2019; Fallati et al 2020).

Equations 1 and 2

Following the introduction of the technique, WWA grew in popularity and was applied to the detection of traditional illicit substances (amphetamines, cannabinoids and opiates), and certain pharmaceuticals (codeine, morphine and methadone) in wastewater (Castiglioni et al 2006; Berset et al 2010; Baker and Kasprzyk-Hordern 2011b; Jacox et al 2017). WWA techniques have been applied to detect low concentration compounds with inconsistent presence and a novel structure (i.e. NPS) in the last six years especially (Kinyua et al 2015; Hernández et al 2016; Fontanals et al 2017; Salgueiro-González et al 2019). The ability of WWA to show the evolution of NPS trends has meant it has become one of the few current methods to keep track of the spread of NPS; this information often is sourced from the EWS and the EMCDDA.

There is increasing interest in the analytical investigation of locations that may have a greater chance of detecting NPS in wastewater (and pooled anonymous urine), such as music festivals and events. These are desirable locations to investigate due to the association with high recreational drug use (Lai et al 2013; Causanilles et al 2017; Mackuľak et al 2019). When researching specific locations such as nightclubs it is typical for pooled anonymous urine to be collected for monitoring NPS abuse in smaller sample groups (Archer et al 2014). The approach was successfully demonstrated by Archer et al (2013); the researchers detected phenethylamines, amphetamine-like compounds and other NPS in a nightclub in central London. Taking this more focused approach to sampling smaller sites rather than large WWTPs circumvents one of the major analytical challenges associated with NPS determination in

wastewater which is the extremely low concentrations such compounds are usually present in large scale WWTPs. NPS concentration from pooled urine collection from a festival or nightclub for example would be expected to be orders of magnitude higher than that observed in municipal wastewater.

3 – The global NPS issue and strategies for targeting NPS in WWA

Various organizations (EMCDDA and UNODC) have been strategizing how to tackle the phenomenon that is NPS emergence over the last decade (UNODC 2018; EMCDDA 2019b). Understanding the NPS issue requires such organizations to utilize data gathered by the EWS (used by the EMCDDA) and the 'Early Warning Advisory' (used by UNODC). These systems cast a wide net to gather information, using extensive networks of forensic science laboratories, law enforcement data, global surveys and many other tactics to monitor and report trends in NPS (EMCDDA 2020b; UNODC2020b). Such large scale efforts have significant benefits, particularly through collaboration and data sharing. However, Deluca et al (2012) outline that traditional systems often rely on official national surveys and police data, which is potentially an issue. Australia's National Drug and Alcohol Research Centre suggested a smaller national EWS pilot study to systematically process and rapidly assess emerging drug trends at a more jurisdictional level (states/territories) (Peacock et al 2017).

In conjunction with monitoring techniques such as WWA, early warning systems enable organizations to identify population trends/patterns with greater accuracy and background knowledge. Recent data from the UNODC suggest that synthetic cannabinoids and cathinones appear to be the most frequently discovered NPS classes, seized and abused globally (Figures 4 and 5). The increasing prevalence of new synthetic opioid receptor agonists is also of concern

(opioid NPS) (fentanyl analogues/derivatives); their seized/reported data is lower than the above two classes, however they have been linked to growing numbers of NPS-related deaths (UNODC 2019b; UNODC 2020c)

Figure 4: Synthetic new psychoactive substances by effect group reported to UNODC in 2009-2019 from their 2020 Booklet 4 world drug report

When selecting target compounds for WWA, a parent compound, metabolite or transformation product with medium-high stability is preferred. Additionally, available excretion data (from metabolic studies) is useful as it can be used to explore drug taking habits from the known inhabitants. Common limitations with detecting NPS in wastewater are i) the lack of metabolic data ii) low stability/half-life iii) no available reference standard. Finding an ideal biomarker for an NPS can be more challenging compared to a long-existing illicit compound; instability can affect back-calculations and consumption estimations, their short market-life means reference standards and metabolic data are not always available in time before banning, therefore the creation of such a commercially available standard is financially strenuous.

However, there are some NPS that persist and grow in popularity despite the enforcement of legislative powers. This has resulted in a surge in metabolic and biotransformation studies; there is some (limited) evidence of these legislations changing abuse patterns largely relative to the release of psychoactive bills in United Kingdom, New Zealand and the United States for example (King 2013; Cooper 2016; Home Office 2018b; Ministry of Health 2019). There has been an overall decrease in global NPS seizures in recent years as a result of these strategies, however, new substances are frequently being reported as well as changing classes among globally seized products (according to the UNODC's recent world drug report 2020 (Figures 4 and 5). This global data reflects the NPS market complexity based on popularity, amounts seized and new reports; hence the legislative struggle to clamp down on this 'influx' market.

Figure 5: Global quantities of NPS (plant-based and synthetic) seized reported to UNODC in 2008-2018 from their 2020 Booklet 4 world drug report

Banning substances without further investigation can create demand in illegal markets, impact prevalence data collection and limit available data for researchers on chemical structure and manufacturing processes (Negrei et al 2017). Additionally, production/supply limitations and increased cost of drug licences (in some countries) from these impacts can burden academic studies in their investigations (Van Amsterdam et al 2013). Reactive prohibition of NPS can lead to the clandestine creation of new similar substances to meet the demand left by those popular NPS after they are banned (Feilding and Singleton 2016). Those NPS that still persist after legislative controls and are most popular in seizure/prevalence data collected from the UNODC's and EMCDDA's database will often be investigated in a WWA study. Indeed the currency of the UNODC and EMCDDA databases are driven by frequent updates from forensic findings, and this in turn has driven researchers' choice of target NPS in wastewater studies.

Suitable NPS for WWA studies are chosen based on their current impact in a population and prompted by government led crime surveys or data collection; it is desirable to obtain transformation product, metabolite and stability information on the compound to understand it better, as well as have an analytical study to target. This is further discussed below in section 4.2 and 5.2.

4 – Analytical methods for the detection of NPS in wastewater

The following sections explore each step of the WWA procedure involved in NPS detection. The general process often involves: sampling, pre-treatment, extraction and instrumental analysis. Here we provide technological detail and practical examples of their uses for NPS determination in wastewater.

4.1 – Sampling Techniques

Sampling strategies may be influenced by several factors including sample area or nature and complexity of the study. High capacity treatment plants for example may suit a strategy capturing composite samples at high frequency. Sampling strategy to create representative samples from small populations on the other hand will focus on careful selection of the sampling site within the treatment plant (Ort et al 2014). The approach can differ based on number of potential users per population, drug pharmacokinetics (metabolic/transformation pathways) and sewage system characteristics (matrix complexity) (Castiglioni et al 2013). Choice of strategy can be influenced by the desired sampling time and scope of the project.

4.1.1 – Auto-Sampling

Auto-samplers allow researchers to collect as much data as one can within each hour of a 24-hour period within a predefined schedule and amount; this varied range of sampling amount and efficiency will benefit larger studies that involve a whole city or several especially. For example, Gao et al (2017) had aliquots of 200mL taken every 2 or 4 hours (based on convenience) throughout the day to form a composite sample. Whereas Van Dyken et al (2016) programmed the sampler to collect one 20mL of wastewater for every 250L of flow, which is more appropriate

for smaller populations (where the window of substance abuse is smaller and harder to detect). The creation of composite samples is important to ensure representative samples are collected.

4.1.2 – Grab Sampling

Grab samples are often single discreet samples, or can be multiple samples taken individually over a short period of time. They should be representative of the wastewater conditions at the time. Borova et al (2015) used grab sampling successfully in their study in Santorini, Greece, where they targeted mostly synthetic cannabinoids during the seven day study with a focus on compound stability. Previous studies outlined that grab samples might be advantageous over 24h composite sampling due to the stability factor, as some compounds may not be stable for a full 24-hours. For example, Baker et al (2012) were investigating the presence of illicit compounds in wastewater samples in the Czech Republic, and found significant degradation to occur within the 24-hour window.

4.1.3 – Polar Organic Chemical Integrative Sampling (POCIS)

In comparison, it has been suggested that POCIS has certain advantages over grab sampling due to the ability to obtain time-integrated results using these relatively cheap passive samplers (Criquet et al 2017). The technique provides average concentration estimates of compounds that get trapped into the solid sorbent over a time period. It uses a device that collects the smaller illicit substance molecules and metabolites as waste passes through (Bailly et al 2013). However it does require laboratory calibration to obtain compound sampling rates for estimating concentrations (not many are published for existing compounds), and the compounds need to remain stable during sample collection; meaning the target compounds must be relatively stable (Castiglioni et al 2011; Grabicova et al 2017; Keshaviah 2017). Therefore, this technique might not be suitable for the investigation of NPS as they already have a lack of functional data for many of them. (Gracia-Lor et al 2017). Yargeau et al (2014) compared POCIS and 24h auto-sampling

and found concentrations collected the WWTPs were similar, however, the more complex and untreated the wastewater, the lower the concentrations for the POCIS results. Baz-Lomba et al (2016) are one of the only groups to use POCIS when investigating NPS in sewage alongside auto-sampling and pooled urinalysis. Passive sampling devices such as POCIS can be advantageous in WWA when auto-sampling is not used as they allow for a simpler sampling process and provide *in-situ* enrichment of chemicals, increased sensitivity and a time-integrated sample (Yeh et al 2018; O'Brien et al 2019). Verhagen et al 2020 outlined the advantages of using passive sampling for WWA for hydrophobic chemicals; they introduced a new diffusion-based sampling device that showed advantages over grab sampling, and could be a less costly alternative to using an auto-sampler.

4.2 – Sample Collection and Compound Preservation

There are common procedures established for collection and transportation of wastewater samples, and these vary depending on target compound stability and preservation requirements. The options are:

- Freeze on site, transport in chilled conditions or dark or dark chilled conditions, keep frozen or refrigerate until analysis.

- Chill on site, transport in chilled conditions or dark or dark chilled conditions, keep frozen or refrigerate until analysis.

- Acidify; chill or freeze on site, transport in chilled conditions or dark or dark chilled conditions, refrigerate or freeze until analysis.

(Table 1 presents method details for WWA including various collection and transport conditions among recent WWA NPS studies)

The most common transport conditions are: package carefully, and then transport on ice to freeze fully (-18/20 degrees celsius) at the laboratory until analysis (Carlson et al 2013; Yargeau et al 2014; see Table 1). There is a lack of biotransformation and metabolism studies for many NPS, meaning their stability is largely unknown, so keeping the compounds as preserved as possible is important in case of low stability (Couto et al 2018; Bijlsma et al 2020a; Bijlsma et al 2020b). For NPS WWA studies typical conditions involve chilled transportation if laboratory analysis is immediate, if not then the samples are frozen immediately (see Table 1 for examples).

Choice of temperature during transportation and prior to analysis may impact the stability of NPS and may influence when sample pre-treatment should begin (Chen et al 2013; Senta et al 2015; González-Mariño et al 2016a; Bade et al 2017a; Kinyua et al 2018). NPS can be preserved in wastewater further through addition of preservatives, which can extend analyte stability (Pandopulos et al 2020). Examples include acidification with HCl or H_3PO_4 (Löve et al 2018; Bade et al 2020). Bade et al (2020) found that certain NPS are stable for up to 14 days through acidification at room temperature, 4 degrees celsius and -20 degrees celsius in filtered wastewater. Previously Bade et al (2017a) found samples should be analysed within 7 days if kept at 4 or -20 degrees celsius if not acidified; if acidified they (compounds such as synthetic cathinones and phenethylamines) can stay relatively stable for 7 days at room temperature for a week. Sodium metabisulphite can also be used as a preservative agent, and has been used to preserve traditional illicit compounds such as heroin and 6-monoacetylmorphine (6-MAM) (Chen 2012; Tscharke et al 2016).

4.3 – Pre-treatment of Samples

Pre-treatment protocols are typically similar among WWA studies that involve NPS and illicit drugs; they may differ based on potential analyte loss, selectivity and recovery. Predominantly filtration, acidification (an optional step which may be utilised dependent on the physico-chemical properties of the target compounds and the type of SPE cartridges used) and extraction are conducted to prepare samples ready for injection into the desired analytical system (Baker and Kasprzyk-Hordern 2011a; González-Mariño et al 2016a; Bade et al 2019a; Sulej-Suchomska et al 2020).

4.3.1 – Filtration

Filtration is typical during WWA in order to remove solid particles from the matrix that could impact extraction (Kinyua et al 2015). Vacuum filtration is commonly employed often using glass microfiber filters (see Table 1). It is possible that filtration may lead to analyte loss, which is troublesome for NPS especially due to their typical low concentrations. Synthetic cannabinoids in particular may be lost to the commonly used glass microfiber filters. Pandopulos et al (2020) demonstrated in a controlled study that a significant number of synthetic cannabinoids showed losses after filtration, with 13 compounds showing significant losses. However, Chen et al (2013) concluded that there was no significant analyte loss through glass fibre filtration (for the targeted NPS), which is supported by Chen et al (2012) with similar results for illicit compounds. Albeit, the data for the 2013 study is not published, which makes it difficult for comparison. Bade et al 2017b investigated potential analyte loss and recovery from vacuum filtration (mixed cellulose membrane) at natural and acidified pH. Recovery rate at natural pH was relatively high for most compounds at 84-98% (besides MDPV at 55%); with acidification the recovery rate was 3-20% higher for most compounds. The recovery for the NBOMe NPS and naphyrone was under 5% when filtered at both pH levels.

Analyte loss due to filtration is not solely restricted to NPS however. González-Mariño et al 2018 investigated the sorption of a wide range of commonly prescribed and abused drug analytes onto different vacuum filter materials (glass microfiber, mixed cellulose membranes, nylon membranes and polyvinylidene difluoride (PVDF) membranes). Losses were lower than 30% regardless of the material except for methadone, EDDP metabolite, two cannabinoids and most antidepressants. Higher losses were observed with cellulose membranes followed by PVDF filters. They found that methanolic washing of the filters can improve the critically lost analytes dramatically by improving desorption. Glass fibre and hydrophilic nylon are the most promising materials showing much lower adsorption for some compounds.

4.4 – Extraction Procedures

Solid phase extraction is the most commonly employed extraction technique used in wastewater analysis for NPS (see Table 1). However there are other forms of extraction for NPS from wastewater that have been used and are argued to be a suitable alternative to SPE. The techniques and applications are reviewed below.

4.4.1 – Solid Phase Extraction (SPE)

Mixed mode, cation exchange and hydrophilic, reversed phase mechanisms have proven popular choices for SPE sorbents in WWA. Several researchers have used the Oasis MCX and HLB cartridges to cover the range of physical and chemical properties of targeted NPS in their investigations (Baker and Kasprzyk-Hordern 2011b; Senta et al 2015; Salgueiro-González et al 2019). Analyte recovery is relatively high for NPS when using such SPE cartridges. Both mechanisms can be used for simultaneous extraction effectively, MCX has a higher selectivity for basic compounds (amphetamine-like compounds) and less matrix components retained in the sorbent, whereas HLB can extract a wide range and is suitable for acidic, basic and neutral analytes (Gros et al 2006; Baz-Lomba 2016). Mixed-mode ion exchange sorbents are particularly

useful due to their ability to combine capacity and selectivity, which is advantageous considering the range of chemical and physical properties of NPS (Pascual-Caro et al 2019).

Senta et al 2015 found that extracted analytes (amphetamine-like psychoactive substances) can be stored on the cartridges and kept frozen or the extracts themselves kept frozen until analysis (on another day); they found that frozen cartridges or extracts at -20 degrees celsius can remain stable for at least 7 days prior to injection.

Van Nuijs et al (2013) assessed analyte recovery of emerging drug compounds in wastewater using MCX and HLB SPE cartridges. MCX cartridges demonstrated recovery close to 100% for ketamine and its derivatives, and 90% recovery for MDPV and mephedrone. In comparison HLB cartridges showed approximately 50% recovery for both analytes. The MCX cartridge has also been shown to enhance selectivity for the enrichment of basic compounds in addition to providing high recoveries (Kinyua et al 2015; Senta et al 2015; Chen et al 2018). The clear advantage of the MCX cartridges for such compounds lies in the higher recovery figures, but the HLB sorbent provides useful flexibility for NPS due to the wider spectrum of analyte polarity it can retain.

Synthetic cannabinoids are variable in structure and as such have posed technical difficulties for some SPE cartridge types. To overcome this it has been shown that HLB cartridges are suitable for a range of cannabinoids; their copolymeric sorbent, with both hydrophilic and lipophilic capacity allows the retention of a wide range of compounds (Pandapolous et al 2020)

Diamanti et al (2019) utilized a four-sorbent solid-phase extraction protocol to retain a wide-array of compounds with different physicochemical properties (Strata-X, Strata-X-CW, Strata-X-AW and Isolute ENV+). These sorbents and elutions with acidic and basic solutions allowed extraction of a large compound range from neutral, aromatic and acidic to basic and polar analytes. Álvarez-Ruiz et al (2015) discussed the impact of pH on NPS and traditional drug recovery during the optimization of their SPE procedure using the Strata-X cartridge (polymeric reverse phase). The

technique was applied in sediment, sewage sludge and particulate matter investigations. They found generally that adjusting the extract to pH 6 prior to SPE provided the best overall recovery. Some analytes degrade at a more acidic pH and so on balance a neutral pH demonstrated good recoveries for a wider range of analytes, this has also been demonstrated by Vazquez-Roig et al (2010). SPE has been extensively tested in wastewater studies and key benefits include low solvent consumption, ease of operation and material availability.

4.4.2 – Liquid-Liquid Extraction (LLE)

The use of LLE in wastewater analysis is not as widespread as that of SPE, but in some instances there are demonstrable benefits. Chen et al (2013) compared LLE with SPE for the extraction of synthetic stimulant analytes. Both techniques showed satisfactory sensitivity, linear range, recovery, accuracy and precision. Absolute recovery for the tested NPS (methcathinone (MC), mephedrone, methylone, MDPV, benzylpiperazine (BZP) and 3-trifluoromethylphenylpiperazine (TFMPP)) was lower for LLE, whereas the relative recovery was similar for both techniques. González-Mariño et al (2017) investigated the presence of synthetic cannabinoids in wastewater using LLE, and NPS recoveries differed substantially between absolute and relative recoveries in their work. The absolute recoveries for the JWH derivatives were lower (59-75%) than the relative recoveries (101-138%); however none of the synthetic cannabinoid metabolites were found above the method limit of quantitation (LOQ). Pandopulos et al (2020) used González-Mariño et al's study as a guideline to develop their optimised LLE technique in their investigation of the presence of synthetic cannabinoids in wastewater. The extraction efficiency of LLE in their investigation was compared to SPE; they found that both LLE and SPE extraction techniques actually recovered cannabinoids from unfiltered wastewater with minimal difference. However, the extraction efficiency tests revealed that LLE performed better in terms of accuracy, precision and R^2 values.

5 – Instrumental and Data Analysis

For instrumental analysis the investigator's scope, resources, aims and ability can decide whether targeted, non-targeted/screening, or both approaches are undertaken. The predominant technique in WWA for NPS involves a chromatographic separation coupled with mass spectrometric analysis. The following section reviews the different analytical approaches undertaken for NPS determination in wastewater.

5.1 – Chromatographic Separation

Chromatography (liquid or gas) coupled with modern mass spectrometry enables the high level of separation and sensitivity required for qualitative/quantitative analysis of NPS in wastewater. Gas chromatography – mass spectrometry (GCMS) was considered the ‘gold’ standard analytical tool but has been overtaken in this field by liquid chromatography – mass spectrometry (LCMS) due to the fact that many drug compounds are non-volatile and water soluble, making LCMS a sensible choice. The requirement for derivatization in GCMS methods for some compounds is considered a bottleneck in the process and LCMS methods benefit in this area. When determining very polar/ionic analytes, the use of hydrophilic interaction liquid chromatography (HILIC) can be beneficial (Hernández et al 2016; Been et al 2017). Kinyua et al (2015) found that HILIC was a suitable technique for the 5 NPS they detected in wastewater in Belgium and Switzerland. Such phenylethylamine-based compounds are polar and good candidates for a HILIC approach. Among the prominent NPS WWA studies, common stationary phases include the use of either C18 or pentafluorophenyl (PFP) columns. Certain alternatives such as HILIC or Synergi Polar (polar endcapped, ether-linked phenyl phase) and Synergi Hydro (polar endcapped C18 phase) columns have also been used (Borova et al 2015; Kinyua et al 2015 ; Senta et al 2015; Bade et al 2017a; Bade et al 2019; Diamanti et al 2019; Sulej-Suchomska et al 2020). Water, methanol and formic acid are commonly employed as mobile phase (in various proportions) in wastewater

NPS applications (Bade et al 2019b; Bade et al 2020). Senta et al (2015) found that the best separation was achieved with methanol as a strong solvent and formic acid as a modifier. Ammonium acetate or ammonium formate has also been used in combination with water; alternatively acetonitrile may be used with formic acid as a modifier (Van nuijs et al 2013; Lai et al 2015; Bade et al 2017a; Fontanals et a 2017; Chen et al 2018; López-García et al 2018; Kinyua et al 2018). Celma et al (2019) investigated illicit drug and NPS presence in wastewater with micro-LC-MS/MS (triple quadrupole) for their WWA study. They compared it to UHPLC-MS/MS (triple quadrupole) to assess sensitivity and reproducibility. Micro-LC-MS/MS showed a higher sensitivity than UHPLC-MS/MS (the lower flow rates in micro-LC lead to higher ionisation efficiency), reduced organic solvent usage and injection volume; however it had an extended analysis time due to the micro-LC separations, and poor retention time stability. The UHPLC-MS/MS method identified ketamine and dipentylone above the LOQ, whereas the micro-LC method was not robust enough for the determination of NPS in wastewater.

The novel technique of ultra-high-performance supercritical fluid chromatography (UHPSFC) coupled with tandem mass spectrometry has recently been applied to WWA (González-Mariño et al 2017). The authors aimed to reduce substance loss, detection limits and to improve analysis time by allowing a direct injection of organic extracts due to their modification of the mobile phase. It shortens analysis time via higher separation efficiency during the chromatographic stage (Taylor 2009). The kinetic performance of UHPSFC is reportedly better and has a significantly lower generated backpressure compared to UHPLC (Perrenoud et al 2012). This is an improvement as continuous use of HPLC columns under high back pressure can decrease column life (Kastner 1999; Synder et al 2011). González-Mariño et al (2017) used UHPSFC with a modified LLE protocol as alternative in their WWA study; however, they were unable to detect the targeted four synthetic cannabinoid metabolites in wastewater samples greater than the method LOQ. This does not exclude the presence of other synthetic cannabinoids that were not targeted.

5.2 – Qualitative and Quantitative Mass Spectrometric Analysis

Low resolution triple quadrupole mass spectrometry has been the cornerstone of WWA for many years. Such instruments provide excellent sensitivity and noise elimination, a prerequisite for NPS determination as concentrations are often exceptionally low (Geib et al 2016; Prosen et al 2017; Reinstadler et al 2018). Analysis using this form of mass spectrometry will usually be targeted, and whilst this approach has several advantages the limitation is the finite number of analytes that may be determined which again may be problematic in NPS WWA studies due to the wide range of NPS available. In addition the targeted approach often requires the use of reference standards and metabolic information for NPS identification and accurate back-calculations (Bijlsma et al 2019; Salgueiro-González et al 2019; Bade et al 2020). Internal standards associated with compounds on a target list may be employed to aid quantitation through compensation of analyte losses and matrix effect. Reference standards and metabolic data for every NPS we may wish to target are often not available due to the rapidly evolving drug market and as such the targeted approach for NPS can be limiting. It should be noted though that triple quadrupole instruments are fairly prevalent in analytical laboratories, reliable, and with excellent sensitivity they still offer WWA researchers a valuable tool.

Prior to the emergence of NPS, targeted triple quadrupole quantitative assays were commonplace in WWA research. In an effort to keep up with the rapid proliferation of NPS the WWA field has seen a significant increase in qualitative screening approaches made possible through the use of high resolution mass spectrometry (HRMS) (Quadrupole-TOF or Orbitrap systems). Such HRMS instruments capture a wide *m/z* value range at a good sensitivity without a predetermined target list (Bijlsma et al 2020a). The value of this technology is the ability to provide wide-scope screening methods for NPS in wastewater, showing us how many and which compounds are present. The downside can be the potential for low concentration analytes to remain undetected.

Where possible the combination of high sensitivity from triple quadrupole and the confident screening ability of HRMS provides a comprehensive overview of NPS and other illicit drugs in wastewater (Bade et al 2017b).

It is useful here to outline an analytical approach for NPS determination in wastewater using a qualitative screening approach and a quantitative approach. An excellent example of the two approaches was conducted by Bade et al (2017b) during a detailed investigation of licit and illicit drugs in Australian wastewater. The researchers qualitatively screened 346 compounds from influent wastewater from two WWTPs in South Australia. A subset of these compounds was included in a quantitative assessment. A combined approach as demonstrated here can provide a valuable, comprehensive assessment of the data. Following sample collection and preparation qualitative data was captured using a Sciex Triple TOF 8600 instrument in SWATH mode (Sequential Window Acquisition of all THeoretical fragment-ion spectra). SWATH allows fragmentation of every available analyte in a sample providing comprehensive MS and MS/MS data for everything detectable in the sample. Bade et al collected MS data in the *m/z* range 50 - 600, using 34 acquisition windows each with 311 cycles. One TOF MS full scan at low collision energy gave information for the $[M+H]^+$ and the 34 acquisition windows each collected a different *m/z* range with a 16.2-Da offset and 1-Da overlap between windows. This feature in a SWATH qualitative screen allowed analytes with similar fragment ions but different $[M+H]^+$ to be differentiated. This is particularly relevant in wastewater NPS analysis where matrix interferences are a constant; the narrow precursor range in each acquisition window (16 *m/z* apart) greatly aids identification. To facilitate the screening approach the authors constructed a database from standard solutions which were assessed using the SWATH technique. The database records retention time and exact mass. During sample processing any sample matching a retention time and exact mass in the database is investigated for matching confirmation fragment ions. The qualitative screen culminates with the assignment of confirmation criteria: detected compounds

were those matching one accurate mass ion and retention time (within given parameters); and confirmed compounds matched two accurate mass ions and retention time. Screening approaches can require significant time during method development to setup and test but the resulting workflow and returned data can be impressive.

If we examine the quantitative approach taken by Bade et al (2017) in the same study the breadth of data recovered is of course reduced compared to the screening approach but we gain sensitivity and of course quantitative data. Using a triple quadrupole instrument the authors targeted 22 drug compounds each of which was matched with a deuterated internal standard. A precursor - product ion transition was monitored for quantitation, and an additional transition was used for qualification. The authors confirmed or detected 100 analytes, and quantitative data revealed reductions in illicit stimulants, stable levels of opioid analgesics, and NPS were varied with no visible trends. Qualitative screening and quantitative analysis for NPS in wastewater each have their place but as shown in this example there is value in their combined use.

Several other research groups have investigated NPS in wastewater employing a variety of qualitative and/or quantitative approaches. Salgueiro-González et al (2019) collected wastewater from several European cities, subjected them to SPE followed by a full-scan HRMS analysis. Using forty analytical standards, information from data libraries and a priority list of 200 NPS (chosen based on several EWS) they were able to cautiously identify suspect compounds. They used confidence levels to assist in screening results, as supported by similar studies (Causanilles et al 2017; Salgueiro-Gonzáleza et al 2019).

Web-based NPS databases are a valuable tool in this field. Databases such as HighResNPS and NPSDatahub employ forensic laboratory data to facilitate new compound identification (Urbas et al 2018; Mardal et al 2019). These database types need to be updated and maintained, they are becoming essential for HRMS suspect screening; with this combination they provide flexibility and

accuracy for monitoring NPS via WWA (Bijlsma et al 2019). Spectral libraries and databases when combined with powerful modern identification analysis software such as MassHunter, Xcalibur,, Brukers TASQ, UNIFI, Peak View, Q Exactive Tune and MetID for LC-HRMS offer the ability to conduct qualitative suspect screening (Vuori et al 2014; Baz-Lomba et al 2016; Comtois-Marotte et al 2016; González-Mariño et al 2016; Mackuľak et al 2016; Causanilles et al 2017; Diamanti 2019).

The Q Exactive Orbitrap has been frequently used in WWA (Comtois-Marotte et al 2016; Prosen et al 2017; Mackuľak et al 2019). Mardal and Meyer (2014) made 12 tentative identifications via interpreting the microbial biotransformation products full product ion HR mass spectra in conjunction with their retention times. They identified demethylenyl-methyl MDPV as the most abundant human urine metabolite and suggested it should be a wastewater biomarker for consumption estimation. Prosen et al (2017) outlined that the Q Exactive Orbitrap is becoming more affordable than it used to be, have similar detection limits to triple quadrupole mass spectrometry and can allow for confirmation of analytes from their exact mass. However several NPS exist as isomers (XLR-11/XLR-11 degradant, JWH-015/JWH-073, and JWH-019/JWH-122 for example, in addition to several others) and as such the benefits of exact mass technology will not aid identification in such scenarios.

It is known that the metabolic excretion rates of NPS in wastewater are not always available, so chiral analysis has been used to assist. Many compounds are chiral and are subject to stereoselective human metabolism, so the presence of racemates and enantiomers can help determine the source of the NPS compounds (direct disposal or consumption (Hernández et al 2016). Castrignanò et al (2017) used chiral analysis i.e. enantioselective analysis to help identify new biomarkers for the NPS mephedrone. Mephedrone was chosen for the study due to its high stability in wastewater. Two WWA campaigns were conducted in 2014 and 2015 in the UK, and both confirmed the presence of mephedrone. The normalised mass loads showed consumption

was higher on the weekend (suggesting recreational use), enantioselective analysis revealed samples from both campaigns were enriched with the *R*-(+)-mephedrone fraction (i.e. *R*-mephedrone enantiomer). Mephedrone is distributed in Europe as a racemate and if found with enrichment of mephedrone with the *R*-(+)-enantiomer it can imply stereoselective metabolism or wastewater metabolic processes have occurred, suggesting possible consumption instead of direct disposal (EMCDDA 2011). Chiral analysis has enormous potential in the field of WWA, certainly for NPS specifically when there is a lack of metabolic or transformation data; additionally it has the potential to be applied at larger scales to a variety of traditional and new psychoactive substances.

6 – Temporal and Spatial Patterns of NPS detection in Wastewater

It has been established that assessing trends in NPS drug use in defined populations can be conducted through wastewater analysis; the studies to date have ranged in scale and experimental conditions to reflect the dynamic spread of these substances. The research offers insight into the relevance of wastewater testing and how the data obtained can elucidate NPS trends globally in addition to regional and local patterns. The majority of these studies have taken place in Europe and Australia, however in recent years NPS WWA studies have been conducted in China, the US, New Zealand and other countries. NPS trends from the larger multinational wastewater studies are discussed below in the context of temporal and spatial patterns. Table 1 in supplementary materials presents a comprehensive overview of NPS WWA studies from across the globe, reporting sampling, analytical procedures, and NPS findings from regional studies and events, single country investigations to multinational investigations.

6.1 – Multinational Studies

There are several multinational studies that conducted wastewater sampling across nations in the same continent and multiple continents simultaneously to gain insight into the prevalence of certain NPS. Kinyua et al (2015) investigated NPS presence in wastewater in Belgium (7 sites) and Switzerland (1 site) to determine if WWA is sensitive enough to detect these compounds. Methoxetamine, butylone and ethylone were detected in both nations, methylone was only detected in Switzerland. Butylone, ethylone and PMMA were also detected, however they were below the lower LOQ. A further investigation in Belgium was conducted to analyse daily trends, they observed an increase on Sunday and Monday in Antwerp-Zuid (2/3 sites). Although they proved two nations could be investigated simultaneously and WWA is sensitive enough, there needs to be more resources and time when conducting a study of this calibre (multinational study compared to a multi-city one) to fully observe comparative trends.

Multinational WWA studies in the following years expanded in scope to encompass comparisons between several countries in the same continent, to multiple continents at a time to estimate NPS prevalence and trends. Popular NPS classes such as the synthetic cathinones were commonly observed; their prevalence has been established in previous single country studies and therefore are of great interest. González-Mariño et al (2016b) investigated wastewater in the United Kingdom, Spain, Italy and Norway and detected seven NPS across these countries. Mephedrone and methcathinone were the most frequently detected (excluding Norway). They also found these NPS had an increased use on the weekends when investigating daily mass load changes in the UK, implying a recreational use of these compounds (as seen previously). Bade et al (2017a) expanded the search and targeted a mixture of synthetic cathinones and emerging phenethylamines across several countries. Mephedrone, MDPV and methylone were confirmed; mephedrone had the highest concentrations in Bristol and lowest in Brussels, Oslo, Copenhagen and Utrecht. This supports previous reports that the UK has one of the highest mephedrone

683 prevalence rates (at the time of publication) (UNODC 2014); and that methylone and MPDV are
684 lower in concentration in other European countries (such as Croatia and Switzerland) (Kinyua et
685 al 2015; Senta et al 2015).

686 Salgueiro-González et al (2019) expanded this field further by investigating 18 different nations
687 over two years with a widened scope by using an LC-HRMS system with a large database based
688 on research from the NPS-*Euronet* project. They searched for almost 200 known NPS from a
689 variety of classes (synthetic cannabinoids, synthetic cathinones, phenethylamines, synthetic
690 opioids, tryptamines and more) as well as used 40 analytical standards. Using specialised
691 software they were able to search and match compounds based on the structural information,
692 fragmentation pathways, chemical properties and metabolism data provided by this database to
693 then determine confidence levels. PMA, 3,4-DMeO-alphα-PVP, alpha-methyltryptamine (AMT)
694 and 2-phenethylamine (2-PEA) were confirmed with standards; 25E-NBOMe, 25H-NBOMe and
695 2-methoxyamphetamine (2-MA) were identified with analogue standards and spectra libraries;
696 and lastly N-methyl-2AI, 2,5-dimethoxy-4-isopropylamphetamine (DOIP), isopropylphenidate,
697 HDMP-28, diphenidine and AMB-FUBINACA were tentatively identified. The researchers also
698 pooled weekend samples collected Friday to Monday and demonstrated increased drug use on
699 the weekend. The findings reflect the dynamic NPS market showing classes other than synthetic
700 cathinones and cannabinoids are popular and growing (tryptamines, phenethylamines,
701 piperidines and isomers of them). It is necessary to investigate multiple NPS classes in these
702 multinational studies to reflect the diverse NPS market.

703 Castrignanò et al (2018) also made a significant advancement in this field by conducting the first
704 spatio-temporal enantiomeric profiling of wastewater from different European cities for illicit drugs
705 and NPS. They successfully detected mephedrone, MDA, ephedrine and pseudoephedrine.
706 Enantiomeric profiling can reveal insight into the origin of the target compound, whether it is a
707 metabolite or not, consumed or deposited. They aimed to verify the origin of MDA in the

wastewater through determining if it was the result of consumption or the metabolization of MDMA or MDEA. If MDA is consumed it will be excreted in the urine enriched with the R-(--) enantiomer, if it was produced because MDMA was consumed, then the urine would be enriched with the S-(+) enantiomer. In most samples showing MDA, the S-(+)-enantiomer was found, however for three days in Bristol, and one in Oslo and Utrecht, the R-(--) form was found, thus indicating MDA indeed could have been consumed as a separate compound in addition to MDMA/MDEA. Additionally, mephedrone was found more often on the weekend (supporting recreational use) enriched with the R-(+-) enantiomer. For mephedrone this indicates it was most likely consumed rather than directly disposed of. Enantiomeric profiling shows significant promise for NPS WWA research as the additional data provided is valuable.

Table 2 presents an overview of global temporal trends in NPS as reported detections in wastewater analysis studies. Although the studies are not always directly comparable, when NPS findings are viewed chronologically there appears to be certain temporal trends that can be observed perhaps relating to global NPS outbreaks or growth in popularity. It should be noted that whilst the detected analytes reflect NPS consumption at the time (and possibly direct disposal as well), it should be considered that positive findings are also influenced by the target compounds in each analytical assay (i.e. if synthetic cannabinoids were not detected in a nation during a certain year, the researchers may not have targeted that NPS class).

Synthetic cathinones such as mephedrone, methylone and MDPV are widespread across several nations as shown in Table 2, whereas synthetic cannabinoids have been detected more frequently in recent years due to the surge in this class popularity.

Table 2. Global temporal trends in NPS as reported in wastewater analysis studies.

7 – General Summary

Wastewater analysis is a vital investigative tool for the detection of NPS in the environment and the technique can reveal drug trends over time and space. In the last 20 years the technique has developed from profiling illicit drugs, pharmaceuticals, and contaminants in wastewater using a targeted analysis, to the ability to detect a very wide range of illicit and licit drugs, including complex and trace level NPS, both in a targeted and non-targeted manner. Significant research efforts have proven the suitability of WWA for the detection of NPS, but for these emerging drugs the discipline remains highly challenging due to a variety of factors: the presence of NPS in wastewater at extremely low concentrations; limited solubility in wastewater and particulate adsorption issues (both these factors most relevant for the popular class of synthetic cannabinoids); and sporadic use compared to their more traditional drug counterparts. As analytical instrumentation and techniques have improved over the years so has the scale and scope of WWA, with research accommodating an ever widening range of NPS in studies scaling from regional to international. Quantitative and qualitative techniques are now used to not only detect these compounds with greater sensitivity, but also to circumvent the lack of structural information and reference standards for these emerging NPS. Increased focus on the study of biotransformation processes in wastewater as well as greater utilization of chiral analysis will bring more clarity to the interpretation of NPS findings in WWA research. Future directions for NPS WWA work include research to better understand the fate of NPS in wastewater, and studies to minimize error and uncertainty in back calculations and sampling. Increased focus on the use of NPS WWA to monitor the outcome of interventions intended to reduce or disrupt drug supply or demand will be of significant benefit in some areas. Prisons for example may test the effectiveness of interventions to reduce drug influx through timely analysis of wastewater.

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