CREW CENTRE OF EXPERTISE FOR WATERS

Review of psychoactive substances wastewater monitoring approaches and recommendations for the feasibility of applying different approaches in Scotland

Lisa Avery, Kristin Ceniccola-Campos, Claus-Dieter Mayer, Wakene Negassa, Zulin Zhang, Sebastian Sprick, Eulyn Pagaling

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Executive Summary

Introduction

This report provides the findings of the above study to evaluate the feasibility of applying wastewater monitoring to enhance data on psychoactive substances in Scotland.

Scotland faces a critical public health crisis with one of the highest drug-related death rates in the developed world. Polysubstance use further complicates this issue, creating unpredictable health risks for users. Efforts to address this crisis include the National Drugs Mission Plan (2022-2026), which emphasizes reducing drug-related deaths through improved data collection and harm reduction strategies. The RADAR system (Rapid Action Drug Alerts and Response) plays a pivotal role in providing early warnings about emerging drug trends, such as the rise of potent synthetic opioids like nitazenes, enabling timely policy responses. However, existing approaches to monitor psychoactive substance use are limited by their time-consuming nature and inability to provide real-time data on drug consumption dynamics. Wastewater-Based Epidemiology (WWBE) offers a promising solution by analyzing psychoactive substance residues in wastewater to deliver robust, dynamic, and timely insights into drug use patterns. This project explored the feasibility of leveraging Scotland's existing wastewater monitoring infrastructure to enhance early warning systems like RADAR and support public health initiatives.

Objectives and Approach

The objectives of the project were to address the following questions:

1. Which specific target substances should be monitored to address the highest public health threats for Scotland? For which are there recognised analytical strategies internationally?
2. What are the characteristics of (a) existing infrastructure and (b) different analytical approaches available internationally and in Scotland for supporting the monitoring of target psychoactive substances and their metabolites?
3. What are the characteristics of existing early warning reporting systems on drug use – internationally and in Scotland – that the different monitoring activities feed into?
4. What are the options for monitoring and reporting of target psychoactive substances and their metabolites that are currently a) feasible and b) infeasible to do in Scotland and why, based on: – existing infrastructure for influent sampling of wastewater and its capacities – speed of turnaround to fit in with current reporting times (e.g. in RADAR updates) – availability of licenced laboratories with the appropriate skillset and technology in Scotland and the UK – restricted funding environment – capacities in relation to laboratory analysis – existing reporting systems?
5. What are the potential benefits afforded by such a recommended monitoring approach to support the existing systems of early warning surveillance data to inform Public Health Scotland action and international public health organisations?
6. What is the most feasible recommended approach to implement post-project (through limited trials or nationwide use of all appropriate Scottish Water sampling sites)?

Objectives were addressed through systematic literature review, evidence mapping, engagement of key stakeholders (Scottish Water, Public Health Scotland, European Drugs Agency) and experts through informal discussion, and a formal focus group.

Key Findings

1. Target Psychoactive Substances

Target substances which should be monitored to address the highest public health threats for Scotland were broadly grouped into drug classes including, but not limited to, amphetamine-type stimulants, benzodiazepines, cannabinoids, depressants, novel synthetics, other pharmaceuticals, and other stimulants.

2. Analytical Strategies

Internationally recognised analytical strategies were identified through literature searches and evidence mapping for all target substances. Identification was most often achieved using tandem liquid chromatography – mass spectrometry (LC-MS/MS) for known compounds

that are non-volatile and water-soluble. By contrast, high resolution MS (LC-HRMS) and database comparison was suitable for volatile compounds or situations where the identity of substances is unknown or novel.

Sampling infrastructure and laboratories for sample analysis

Scottish Water sites are sampled for regulatory purposes and additional projects (e.g. Chemical Investigation Programme and COVID monitoring) between four times per week and quarterly, with larger works being sampled more frequently. Most works are equipped with autosamplers which collect 3-5L composite samples over 24 hours. An internal courier system is used for transporting samples. Additional resources would be required for any significant additional sampling effort. At least 15 organisations in Scotland were identified as carrying instrumentation applicable for wastewater monitoring.

Data Analysis Pipelines

In the literature reviewed, there was no specific detail on how long it would take to develop a pipeline for routine analysis of psychoactive substances, or what the time period would be from sampling through analysis and formatting of data for reporting. We estimate that that this is likely to be ~ 1 week for routine monitoring once pipelines are established.

Potential Benefits of Wastewater Monitoring

Overarching benefits of applying wastewater monitoring of psychoactive substances in Scotland would include: 1) informing a public health response 2) informing national policies 3) Understanding use of psychoactive substances in Scotland in the international context. Specific potential benefits are:

- Additional and timely intelligence of changes in both the types and levels of consumption of psychoactive substances.
- Reveal Scotland-specific drug trends geographically and over time.
- Communicating actions to other sectors, for example, if a new drug is found in the wastewater, this could be added to toxicology screening in the clinical setting.
- Provide population level data not readily available by other means.
- Provide consumption estimates at catchment and national level.
- Detect a wider array of psychoactive substances.
- Able to be applied in a targeted approach for known substances and a non-targeted way as a screening method for unknown or novel substances.
- Be applied and compared from local to international scale.
- Be used to monitor the outcomes of interventions.

Recommendations

- Pilot schemes should be trialled in Scotland to establish and develop practical implementation.
- Existing sampling platforms should be built upon to facilitate monitoring of psychoactive substances.
- Monitoring should emphasise identification of both established and novel substances using both targeted and non-targeted analytical screens.
- Given the restricted funding environment, wastewater-based epidemiology (WWBE) trials should focus on a select number of samples/ target substances prior to expansion at a national level.
- Trials should involve the major stakeholders from public health and water industry and would benefit from academic and statistical input into the development of sampling regimes, optimisation of detection methods and development of data analysis pipelines.
- The following could be reasonably trialled in further pilot study; cocaine, diamorphine, methadone, diazepam, and amphetamines through low-resolution LC-MS analysis and synthetic cannabinoids, synthetic opioids, and novel benzodiazepines through high-resolution LC-MS.
- To respond to the focus group outcomes, a matrix of target substances based on reason for inclusion should be produced based on this study and target substances narrowed down for trials at selected locations before up-scaling.

1 Introduction

Scotland faces a significant public health challenge with one of the highest levels of drug-related deaths in the developed world. In 2023, there were 1,172 drug use deaths registered in Scotland. In 2022/23, the estimated number of people with opioid dependence in Scotland was 43,400 (95% CrI: 41,900 to 45,100); this represents an estimated prevalence of 1.23% (95% CrI: 1.19% to 1.28%) of 15- to 64-year-olds. Evolving drug supply and use of multiple drugs simultaneously, known as polysubstance use, coupled with social/health inequalities, are part of the complex equation leading to this public health crisis (Public Health Scotland, 2022, (Artigiani and Wish, 2020). Rapid responses to emerging or changing drug use patterns (as provided by RADAR – Scotland's Drugs Early Warning System) allow policy makers to make timely decisions and take action to reduce drug-related deaths and harm (Public Health Scotland, 2022). Delivering to the National Mission to reduce drug deaths and harms (National Drugs Mission Plan 2022-2026) requires high quality data. In Scotland, there is limited population-level prevalence data relating to psychoactive substance use. Existing approaches to assess psychoactive substance use provide critical public health information, but these approaches can be time consuming and may not deliver the most up-to-date information about the dynamics of drug supply.

The application of Wastewater-Based Epidemiology approaches (WWBE) to monitor these substances could augment existing monitoring programmes to allow a public health led, harm minimisation strategy. Analysis of psychoactive substance residues in wastewater has been demonstrated to provide dynamic and robust drug monitoring, with the potential to provide timely information on drug consumption patterns ((Health Scotland, 2024) and references therein). Indeed, Bijlsma et al. (Bijlsma *et al.,* 2024) noted that monitoring temporal trends on cannabis use through WWBE is a particular strength of the approach. Targeting a range of psychoactive substances and their metabolites, including cocaine, heroin and methamphetamines, studies have demonstrated consistency with other drug use monitoring approaches (Sims and Kasprzyk-Hordern, 2020). They are reliant, however, on the availability of suitable monitoring platforms, analytical techniques and statistical analysis of data.

This project aimed to establish the feasibility and benefits of using the current Scottish Water influent monitoring infrastructure to monitor psychoactive substances and their metabolites in Scotland and, particularly, how it would benefit existing early warning reporting systems and other health monitoring programmes.

Project objectives

The project objectives were to determine:

1. (a) Which specific target substances should be monitored to address the highest public health threats for Scotland and (b) For which are there recognised analytical strategies internationally.
2. The characteristics of (a) existing infrastructure and (b) different analytical approaches available internationally and in Scotland for supporting the monitoring of target psychoactive substances and their metabolites.
3. The characteristics of existing early warning reporting systems on drug use – internationally and in Scotland – that the different monitoring activities feed into.
4. The options for monitoring and reporting of target psychoactive substances and their metabolites are currently a) feasible and b) infeasible to do in Scotland and why, based on: – existing infrastructure for influent sampling of wastewater and its capacities – speed of turnaround to fit in with current reporting times (e.g. in RADAR updates) – availability of licenced laboratories with the appropriate skillset and technology in Scotland and the UK – restricted funding environment – capacities in relation to laboratory analysis – existing reporting systems.
5. The potential benefits afforded by such a recommended monitoring approach to support the existing systems of early warning surveillance data to inform Public Health Scotland action and international public health organisations.
6. The most feasible recommended approach to implement post-project (through limited trials or nationwide use of all appropriate Scottish Water sampling sites).

Approach

As outlined in **Box 1**, these objectives were addressed through a combination of systematic approaches to literature review and evidence mapping with the addition of engagement with

key stakeholders and experts through informal discussion, and a formal project workshop, as well as reciprocal interaction with the Project Steering Group (PSG) throughout the project.

Limitations of approach

As the objectives of the project required a broad understanding of the literature in this area, this could only be achieved through a focus on existing review papers with less reliance on original studies. Further, to complete the study within time and budgetary scope, reviews with largely duplicated information were removed. Although this has the potential of reducing the depth of the review, it facilitates the breadth needed to meet the project objectives. We also mitigated this where possible by including additional reviews or original studies through a snowballing approach. There was no scope within the study to undertake a detailed analysis of drug detection and usage trends across databases (nationally or internationally); therefore, our findings relating to this are based on literature searches and discussions with knowledgeable stakeholders. It was evident that a different approach would be required to prioritise drugs in terms of risk of harm, due to the divergent nature of the substances, their usage and the mixed level of knowledge about their effect or harms and the different ways in which these might be categorised.

Box 1: Outline of project tasks

Task		Purpose	Objective
1i	Discussions with stakeholders	To understand the existing wastewater sampling infrastructure in Scotland (Scottish Water)	O2a
ii		To identify the psychoactive substances of concern in Scotland (Public Health Scotland (Chief Pharmacist, Drugs Team), Toxicology and Pathology Information Network)	O1a
iii		To understand current approaches to identifying use/ emerging use of psychoactive substances (Public Health Scotland (Chief Pharmacist, Drugs Team), Toxicology and Pathology Information Network)	O3
iv		To understand benefits of wastewater monitoring for drugs and how it has been undertaken in Europe (European Union Drugs Agency (EUDA)	O5
2i	Narrative literature review	To underpin list of psychoactive substances of importance in Scotland through wider understanding of literature (international)	O1
ii		Characterise data analysis pipelines	O4
iii		Understand benefits of wastewater monitoring of psychoactive substances	O5
3i	Evidence mapping literature review	To understand for which substances there are internationally recognised analytical detection techniques	O1
ii		What are the characteristics of those approaches (e.g. multiplex tests)	O2a
iii		What are characteristics of monitoring infrastructure nationally/internationally (e.g. grab sampling, autosamplers)	O2b
iv		What are the characteristics of drug use early warning systems nationally and internationally	O3
4	Analytical capability search	Identify laboratories in Scotland/UK with capability to run the required analyses	O4
5		Final Focus Group to communicate findings and identify next steps towards trialling this approach in Scotland	

Task 1: Stakeholder Discussions

Task 1 was to undertake discussions with relevant stakeholders to elicit their knowledge on the following:

- Existing influent wastewater sampling infrastructure in Scotland (O2a)
- Substances of public health concern (O1a) and current approaches to identifying and reporting on illicit substance use, multi drug use and emerging illicit substance use (O3) (e.g. PHS, RADAR and community organisations working with addiction such as the local authority alcohol and drugs partnerships).

Wastewater sampling infrastructure in Scotland

Online meetings were held with Scottish Water on 4th September 2023 and 20th January 2025. Both were attended by the Technical Logistics Manager and the latter one also by the Wastewater Sampling Team Manager.

Sampling infrastructure

Wastewater treatment works (WWTW) have permanent autosamplers in place that take 24h composite samples to a maximum volume of 5L. The field team also have poles to allow them to take grab samples.

The samples are transported in a van to the receiving depot or lab in either the in-built fridge or a cool box containing cool packs. Scottish Water laboratories are located in Edinburgh and Inverness. If samples are taken to a Scottish Water depot, they are transported to the appropriate Scottish Water laboratory by internal courier. Samples from the islands are flown in or in some cases transported to the mainland by ferry. An internal courier team go on scheduled runs for this. Samples are delivered to each lab according to proximity and testing requirements/capability.

Sampling Officers are based at Scottish Water depots across the country. Officers collect the samples from WWTW and may be responsible for taking samples at multiple sites within a day.

Sampling frequency ranges from four times a week to quarterly, dependent on the size of the WWTW and the monitoring requirements for each site. Larger works (e.g. those serving cities) tend to be subject to more frequent sampling, and remote, smaller sites tend to be less frequent. Priority is given to regulatory sampling requirements but additional projects are undertaken, for example the Chemical Investigation Programme and COVID monitoring (the latter includes 200 sites). Island WWTW sampling is performed by Operational staff in some cases. Approximately 10% of sampling takes place at the weekend for a specific programme, but this does not cover all WWTW. This may be an issue for sampling psychoactive substances where particular psychoactive substance use occurs predominantly over the weekend. It should be noted that no pharmaceuticals are analysed in-house at Scottish Water laboratories.

Infrastructure required for additional sites

Additional resource or infrastructure needed to monitor psychoactive substances will likely be driven by the purpose of the monitoring and desired outputs, but likely requirements are:

- Recruitment of further personnel to increase capacity in the field team, depending on the scale of the monitoring to be undertaken.
- Advance notice of new work being required to commence. This is because for any new work is to be undertaken, there would then be a 3-4 week lag between while that new work is logged, information is sent out to the field teams and documentation, and sampling bottles are prepared. Scottish Water Laboratory Information Management System (LIMS) facilitates efficiency by coordinating sampling effort across regulatory and project sampling requirements. This requires work to be logged several weeks before it becomes a "live" part of the sampling effort, or longer should recruitment be required.
- If larger volumes are required (i.e. >5L), then more time is required to collect a further 24h composite sample.

There is no capacity to expand chemical analysis in the labs. Scottish Water Scientific Services do not have expertise in detecting and quantifying psychoactive substances, so their role in any wastewater based monitoring of these substances would be sample collection.

Sampling during COVID

A new team was established by Scottish Water to collect samples for SARS-CoV-2 (COVID-19) WWBE studies. There are specific sampling visits required to collect COVID samples over and above visits made for regulatory compliance and internal monitoring by Scottish Water.

Substances of public health concern in Scotland

An online meeting was held with representatives from Public Health Scotland (Principal Pharmacist, Drugs team) in which researchers undertook an informal discussion to determine which psychoactive substances should be considered for monitoring in Scotland for the purposes of this application. A researcher also attended a Toxicology & Pathology Investigation Network meeting to gain further understanding from a toxicological viewpoint. The recommendations from these discussions were that the list of target substances initially suggested should be expanded significantly, to account for psychoactive substances commonly encountered globally as well as novel compounds identified in Scotland through forensic case-working units and medical examinations. Discussion focused on the potential for screening entire drug classes in addition to individual substances to maximise the impact of WWBE. Furthermore, the ability to identify both known and unknown targets was highlighted as a critical element of WWBE implementation, so that substances were not limited only to those of public health concern today.

The project specification suggested targeting opioids, benzodiazepines, gabapentin or pregabalin, cocaine, and NPS more generally, such as nitazenes. The specification also requested a ranked list of these substances in order of importance or the greatest threat to public health; however, following discussions with stakeholders, the research team determined that would not be realistic for several reasons. Principally, the current state of drug use in Scotland is heavily influenced by polydrug mixtures, in which several substances are taken in combination. The identity of these substances is not always known to the user, and their toxicological effects may subsequently be impacted as a result of the combination. Secondly, as the drug market and landscape are constantly in flux, substances which are of concern at one moment may have drastically changed in a few months. Furthermore, many of the substances listed can be legally prescribed to treat clinical conditions, though they are also associated with significant harm when consumed above prescribed levels or by those who have not received them through legal channels. Finally, drug preference differs by geographical location in Scotland, and a ranked list might unevenly highlight the importance of one or more particular substances at the detriment of others.

Following the conclusion of stakeholder discussions, a final list of target substances included the following drug classes: cannabinoids, amphetamine-type stimulants (ATS), benzodiazepines, opioids, anticonvulsants, other stimulants and anaesthetics. A list of known parent compounds (i.e., the original compound as consumed) and their associated wastewater analytical targets (i.e., either parent compound, breakdown product, or metabolite that would be detected) are detailed in **Table 1**. Psychoactive substances which are currently unknown, or those that emerge in the future, can be identified via a non-specific, non-targeted screen. These substances include but are not limited to nitazenes, additional synthetic opioids, synthetic cannabinoids, synthetic cathinones, and novel street formulations.

Table 1: Target psychoactive substances and their metabolites for monitoring in wastewater influents.	
Parent Compound	**Suspected Wastewater Target(s)**
Amphetamine	Amphetamine
Bromazolam	Bromazolam
Buprenorphine	Buprenorphine Norbuprenorphine Norbuprenorphine glucuronide
Cannabidiol	CBD-7-OH CBD-7-COOH
Cocaine	Cocaine Benzoylecgonine Ecgonine Methyl Ester (EME) Norcocaine Anhdroecgonine Methyl Ester (AEME) Cocaethylene
Codeine	Codeine Morphine Norcodeine
Delta-9-THC	11-nor-9-carboxy-delta9-THC (THC-COOH) THC-OH
Diazepam	Oxazepam
Etizolam	Etizolam
Fentanyl	Fentanyl Norfentanyl 4-ANPP
Flubromazepam	Flubromazepam
Gabapentin	Gabapentin
Heroin	Heroin O6-MAM Morphine
Hydrocodone	Hydrocodone Dihyrdocodeine
Ketamine	Ketamine Norketamine
MDA	MDA
MDMA	MDMA HMMA HMA
Methadone	Methadone EDDP EMDP
Methamphetamine	Methamphetamine
Morphine	Morphine Normorphine
Oxycodone	Oxycodone Noroxycodeine Oxymorphine
Pregabalin	Pregabalin
Tramadol	Tramadol N-desmethyltramadol O-desmethyltramadol

Task 2: Literature Review

Rapid review approaches have been developed to address the need for policymakers, decision makers and stakeholders to access contextualised resources that succinctly and methodologically address a broad scope of scientific evidence quickly (e.g. (Khangura *et al*., 2012)). This approach was suited to answer some of the questions posed in this project whereby the multiplicity of questions would make it difficult to undertake a full systematic review within the timeframe. We adopted rapid literature review approaches to answer the following specific questions. This was carried out by co-constructing inclusion and exclusion criteria with the PSG (e.g. geographic and temporal boundaries), trialling and optimising search terms within Web of Science and then reviewing inclusion/exclusion criteria and search terms to obtain a defined and agreed number of papers for in-depth review. Papers were reviewed and the solicited information was summarised to answer the questions:

"Which of the listed target substances present the greatest threat to public health in Scotland?" **(O1)**

Literature was used to develop a narrative review, focussing on prevalence and level of harm attributed to different substances or substance classes in order to assess them as public health threats.

Search methods

Search terms were developed through a series of iterations among the research team with input from the project steering group to produce a number of hits feasible to review while also capturing the breadth and depth of information required to be as comprehensive as possible within the bounds of the project resources. The final search terms were applied in Web of Science as follows:

"**Psychoactive** and (**substances** or **drugs**) and (**illicit** and **important** or **frequent** or **common** or (**public health**) or **importance**)"

Document type: **Review Article**
Years selected: **2020, 2021, 2022, 2023, 2024**
No geographic constraints were applied.

This yielded 363 results which were imported into Mendeley for review. All abstracts were scanned. Articles related to the following were excluded from further consideration:

- Neurological research
- Therapeutics
- Clinical trials
- Cognitive effects
- Non-human animals
- Societal perception

A second refinement of the resulting ~60 articles was conducted and articles pertaining to the following were excluded from this aspect of the review:

- Laboratory testing methods
- WWBE sampling methods
- Drug-checking services
- Papers with duplicate content, i.e. multiple papers on synthetic cannabinoids

Information was extracted from the resulting list related to substance groups and names arising, effects and health risks and prevalence. Where additional information was sought, a "snowballing" approach was used, acquiring and reading references within review articles and using additional searches in Google Scholar to find the required information.

Results

The inclusion of the word "psychoactive" in the search terms led to a strong bias towards Novel Psychoactive Substances (NPS), such that few or no papers focussed strongly on diamorphine, methadone, cocaine, or amphetamine-type stimulants. However, this was deemed acceptable since there is already substantial understanding of trends, prevalence and detection in more "conventional" drug classes.

Novel Psychoactive Substances (NPSs) are defined by the United Nations Office on Drugs and Crime (UNODC) as "substances of abuse, either in pure form or in preparation, that are 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" (Grafinger, Bernhard and Weinmann, 2019). Over 1200 NPS were reported to UNODC between 2012 and 2022 (Fitzgerald, Cottler and Palamar, 2024).

NPS classes summarized by Gent and Paul (2021) are shown in **Box 2**.

Box 2: NPS classes

- Synthetic cannabinoids or synthetic cannabinoid receptor agonists
- Synthetic hallucinogens – two types: 1) 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 2) dissociatives e.g. methoxetamine are similar to ketamine and phencyclidine
- Synthetic stimulants such as those in the cathinone family e.g. mephedrone (related to 3,4-methylenedioxymethamphetamine – MDMA) and amphetamine-like substances
- Synthetic depressants including
  Opioids such as AH-7921 and novel fentanyls (with similar but enhanced effects to naturally occurring opioids like morphine)
- Designer drugs, including benzodiazepines such as diclazepam or flubromazepam (with similar effects to diazepam)
- Synthetic cannabinoids or "Spice," such as JWH- or HU- compounds

Major themes

Several key themes were repeated throughout multiple publications that were reviewed, which are outlined in **Box 3**. Multiple papers emphasised that NPS were an emerging public health issue. Authors frequently highlighted the fact that the number of psychoactive substances identified is increasing.

People who use drugs are at risk as a result of taking substances where they do not know the content. This can occur where "known" substances are substituted with alternatives, where a given drug is mixed with another substance or where contamination with other substances has occurred during the production of the substance. For example, it is unknown whether people taking etizolam are consuming the drug knowingly or because it is in counterfeit drugs e.g. diazepam/alprazolam or other licenced medications (Khangura *et al.*, 2012).

There remains an "arms race" in which the development of novel psychoactive substances is driven by legislation or seizure of particular substances, though the Psychoactive Substances Act 2016 makes it an offence to produce, supply, offer, possess, import, or export any substance not intended for human consumption. This is exemplified by synthetic cathinones and synthetic or semi-synthetic cannabinoids (de Oliveira, Vieira and Santos, 2023b; Kuropka, Zawadzki and Szpot, 2023; Caprari *et al.*, 2024). Legal status may appear complicated for substances based on or from natural substances, such as with novel synthetic cannabinoids, where there may also be confusing messages around health benefits and perceived legal status.

Of critical importance and seen widely in Scotland, polydrug use is the norm and not the exception. This means that multiple substances are being used simultaneously, either by design to enhance effects of other substances or to inhibit side effects, or unknown to the user.

Box 3: Major themes

- Increasing number of psychoactive substances appearing
- NPS developed to circumvent legislation
- Users often do not know the content of the substance
- Polydrug use is the norm (intentional or otherwise)

NPS of concern

Opioids, synthetic cathinones, phenethylamines/amphetamines and cannabinoids were the NPS classes that are an emerging public health issue and are most associated with fatalities – (Ferrari Jr *et al.*, 2022). Cannabis/marijuana, ecstasy/MDMA, nitrous oxide and cocaine used by most students who use drugs at some point (Boden and Day, 2023). Synthetic cannabinoids and cathinones most frequently seized/used globally (Gent and Paul, 2021). Growing drug classes include synthetic cannabinoids, synthetic cathinones, phenethylamines, synthetic opioids, tryptamines, piperidines and their isomers (Salgueiro-González *et al.*, 2019) and overall, the number of psychoactive substances being sold and used is increasing.

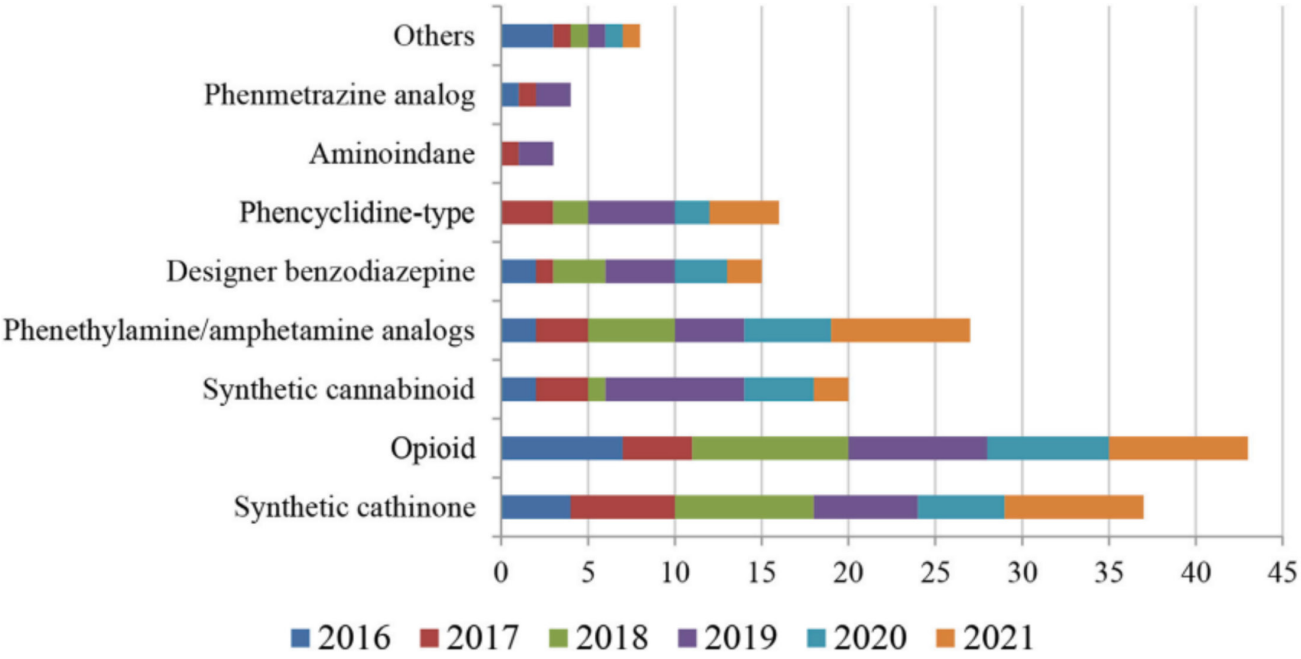

Figure 1. (from (Ferrari Jr et al., 2022)). Main NPS classes reported in reviewed publications from 2016 to 2021. Others: piperazine (2016), tryptamine (2017–2021), indole alkaloids found in kratom (2018 and 2019); methaqualone analogue (2020). Amphetamine analogues included in phenethylamine data. Opioids includes fentanyl and related analogues. No geographic constrains to the publication search were reported in the publications.

Ferrari Jr *et al*., (2022) undertook a review to identify fatal cases involving NPS for which there were chromatographic analyses. **Figure 1** summarizes the main NPS classes reported in their final list of 96 publications reviewed. Blood is the most widely used biological fluid for detection of NPS as it correlates best with toxicological and pharmacological findings. In blood samples analysed in the literature, 28 opioids, 26 synthetic cathinones, 12 synthetic cannabinoids, 8 phenethylamine/amphetamines, 5 designer benzodiazepines and 5 phencyclidines were detected. Designer benzodiazapines, "others", phenmetrazine analogue and aminoindanes were also reported in that order of frequency, respectively.

The substances detected, which were presumed causative, are listed in **Appendix 1**.

Appendix 2 provides a detailed overview of each substance group and references the review papers used to derive findings, rather than the individual references within those reviews.

Scotland-specific findings

The most recent RADAR Quarter 9 report (Public Health Scotland, 2024) identified polydrug use as a continued main driver of harms. The most frequent combinations causing harm involved benzodiazepines (primarily diazepam and bromazolam), cocaine and opioids. New synthetic drugs have exhibited an increasing role in harms, in particular nitazene-type opioids and xylazine. In terms of hospital admissions (March-May 2024) the most detected drug was cocaine, followed by temazepam and desmethyldiazepam.

The most detected drug classes in post-mortem toxicology reports (January-March 2024) were opioids and benzodiazepines, associated with 72% and 60% of deaths, respectively. The most common individual substances detected were cocaine (36%), heroin/morphine (31%), diazepam (30%) and methadone (28%). Nitazenes were detected in 4% of deaths (an increase since the previous quarter).

The drug classes noted in the RADAR report and in conversations with Public Health Scotland also appear in the literature. Some specific substances of concern within those classes differ from those discussed in the global literature. However, it is clear from the literature that changes in legislation in other countries as well as changes in localised legislation are likely to drive emergence of different novel psychoactive substances, alongside changes in user preferences. The cross-over between literature and localised information in Scotland in terms of drug classes of importance leads us to direct our appraisal of technical approaches at a drug-class level. This is also more practical in generating information about detection of target compounds and approaches for screening for "unknowns".

Data analysis pipelines

Methods

This component of the literature review set out to answer the following question:

"What are the timelines for developing and routinely running pipelines for analysis of data derived from wastewater monitoring of psychoactive substances? How long does it take from acquisition of raw laboratory data to provision of data in a suitable format for reporting to early warning system managers? " **(O4)**

In order to answer this question, a literature search was run within the Web of Science using the keywords "Wastewater based Epidemiology, Psychoactive substances, Monitoring" as search terms. 134 publications were found to match this search. Those abstracts were scanned to check whether the articles described a monitoring scheme of a wider regional (or national) scale and were not just restricted to one drug or assay. 13 articles remained after this.

Results

None of the 13 articles gave any information on how long it had taken to develop a routinely run analysis pipeline, neither did any of them state an exact period it would take from sampling to producing raw data and then turning these into a suitable reporting format. Various articles made qualitative statements in this respect though. Several papers (Verovsek *et a*l., 2020; Huizer *et al*., 2021; Jaunay *et al.*, 2023) used the expression "near-real-time" to describe the timescale on which interpretable data can be obtained within a monitoring scheme. In Zarei *et al*., (2020) WWBE is described as a "real-time-measure," whereas Salgueiro-González *et al*., (2019) say, WWBE can be used as early-warning tool, that provides data in a "timely manner."

The fact that none of the studied publications gives an exact statement on timelines is not so much due to a lack of detail in these articles, but rather because such timelines will very much depend on the circumstances: what drugs are being monitored, what assays and lab procedures are being used to obtain, what capacity do labs have for this?

Within a Scottish context, the most relevant source of information is the experience of the Wastewater Monitoring for COVID-19 from 2020 onwards. The PHS evaluation study of the program states that it can take as little as three days from sampling to interpretable data summary (Health Scotland, 2024). Regarding sampling and transport to a laboratory, we would not expect much change between the COVID-19 monitoring and a potential psychoactive substance monitoring scheme. Lab techniques, however, are quite different with chemical analysis (like LC-MS) replacing the molecular techniques (qPCR) used for COVID-19 detection. Once the results of such analysis have been captured in a standardized data format and passed on for data analysis automated analysis and reporting scripts allow to produce routine reports within hours. Overall, we estimate that with sufficient lab capacity and analysis resources a psychoactive substance monitoring system should not take more than a week from sampling to data reporting, which aligns with the (near) real-time description of schemes described in the literature.

Benefits of wastewater monitoring

This component of the project was to determine ***"the potential benefits afforded by such a recommended monitoring approach to support the existing systems of early warning surveillance data to inform Public Health Scotland action and international public health organisations"*** **(O5)**

Methods

Literature for this part of the review was taken from selected publications from the substances of concern literature review and the data analysis pipeline review. We also identified and contacted key practitioners and researchers in wastewater-based detection of psychoactive substances to capture relevant information.

Results

Caprari *et al*., (2024) highlighted the critical importance of a close collaboration between science and policy to develop and disseminate the knowledge base in order to help underpin the reduction of public health risks from psychoactive substances and increase societal wellbeing.

Psychoactive drug use is traditionally monitored through police seizures, hospital toxicology and post-mortem reports, surveys of sentinel populations and prevalence estimates, drug checking, hospital admissions, treatment and support services and interviews with people who use substance . These methods, however, are limited in their spatial and temporal population information, are subject to user self-reporting bias and misinterpretation of toxicology or post-mortem results. (Huizer *et al*., 2021; Fitzgerald, Cottler and Palamar, 2024; PHS personal communication). This is compounded by

the rapid evolution of novel substances reaching the market and changes in habits of substance use over time. Many of the most recent literature reviews on psychoactive substances identify WWBE as a way of improving existing data, for example, by noting that "harmonization of multiple data sources can help present a more complete picture" of NPS trends to better inform public health responses.

Huizer *et al*., (2021) summarize the role of WWBE for monitoring use of psychoactive substances. WWBE approaches for detecting psychoactive substances quantify and/or detect substances consumed or metabolites of those substances are excreted from the human body and pass into the wastewater. The most investigated substances globally using WWBE are cocaine, methamphetamine, MDMA and amphetamine and localised, international trends have been identified as having increased incidences of some of these substances associated with events, for example urban vs. rural locations, weekly trends, festivals.

Quantitative information can be used to back-calculate the amount of the original substance entering the wastewater as a daily load. Combined with information on in-sewer transformation rates, consumption per day from the population served by the wastewater treatment plant can be derived. This does not provide information about individual inhabitants, and therefore is protective of identity of individuals, which is seen as beneficial in the context of the current criminal legislative framework and protecting people's rights. It is, however, helpful in identifying substance use patterns and changes at population level. It also provides near-real time information, providing insight into specific geographic areas as well as temporal trends. It also shows potential for determining the size of the psychoactive drugs market. The utility of WWBE in this context is dependent on adopting the same (validated) techniques for sampling and analysis – for example as per the Sewage analysis CORe group Europe (SCORE) – to ensure quality control and allow comparison of results. SCORE data represents around 70 European cities and are collated and used by the EMCDDA.

Gent and Paul, (2021) note that the development of analytical methods over the last 20 years is such that there are now both quantitative and qualitative techniques that can provide increased sensitivity of detection for novel compounds and can overcome the lack of available reference standards and paucity of structural data. They also note the potential to use WWBE to monitor interventions to interrupt NPS supply in specific locations.

A senior analyst from the European Union Drug Agency (EUDA, formally EMCDDA) responsible for the wastewater monitoring programme offered a high-level perspective on monitoring benefits, challenges, and areas for improvement. With over 10 years of historical data the programme has proven outstanding success in monitoring trends and patterns in drug use. As an example, wastewater monitoring of cocaine from 2017-2018 was the first reporting of a significant spike in usage in Europe, but traditional testing services did not see the same increase until two years later. The portfolio at present investigates patterns of community drug use via the SCORE network, whereby worldwide partners sample and analyse wastewater at a national level one week per year in the early spring and data are passed to EUDA for quality reporting. The timing is chosen so that testing is conducted during a "routine" week with no festivals or holidays, and additional proficiency testing is conducted during the summer. Samples are obtained from both cities and rural areas, which allow for a comparison between population locales. Currently, the targeted substances are cocaine, cannabis, amphetamine, ketamine, MDMA, and methamphetamine. Importantly, although wastewater analysis provides almost real-time data, it is only possible to determine that substances are found, not the number of people using or the frequency in which they are using. Despite this, it is possible to differentiate between drug disposal versus drug use through enantiomeric selection (i.e., as with the case of amphetamine and MDMA) or direct analysis of metabolites (i.e. benzoylecgonine for cocaine consumption).

As this is a free programme, network partners are keen to participate where possible, though as it is also voluntary the EUDA recognises the volume of workload on participants and difficulty in obtaining more frequent samples. A further difficulty lies in encouraging new partners to join the programme. Despite some member states having well-established monitoring services, drug epidemiologists are not always keen on including wastewater as they fear it may replace traditional epidemiological approaches. Some states have limited analytical capacities and must outsource the samples for testing. A hope is that by increasing the budget for networking in 2025, the portfolio may begin checking for non-routine substances such as synthetic opioids and cathinones. NPS are not currently reported on as laboratories don't have the capacity or reference materials available, plus concentrations in wastewater are typically very low and difficult to detect. Hair analysis has since been piloted in a small number of countries

at music festivals and drug checking services as a means of obtaining additional information about polydrug use and NPS consumption. Ethical considerations of monitoring have also been taken into account. All network participants receive their data back and are able to request that it not be included in the final publication; this has occurred in approximately 2-3 cases out of approximately 120. In other situations, the submitting laboratory is unable to release information about the cities where wastewater was sampled. While wastewater cannot be traced back to individual people, there is a risk of bringing stigma to a community (or prison, or school, or other small sampling pool), although no major issues have been cited with respect to this.

The overarching benefits of applying wastewater monitoring of psychoactive substances in Scotland would include:

1. Informing a public health response – to add to our public health surveillance system to monitor established and new/emerging trends of substances.
2. Informing national policies – to better understand the prevalence of psychoactive substance use in Scotland beyond the current focus on opiates.
3. Understanding use of psychoactive substances in Scotland in the international context using comparable data (for example, Scotland could feed into the European Yearly monitoring – SCORE Network).

Based on reviewed literature (including Katz *et al.,* 2003; Saran and White, 2018) and informal and focus-group interactions with experts in the field, the following specific aspects were identified as benefits of applying wastewater monitoring of psychoactive substances to enhance existing drug use data:

- Greater and more timely intelligence of changes in the drug market and population
    - E.g. detect complex NPS E.g. intelligence on emerging drugs, especially ones that are marketed as a different drug
    - In a specific example, wastewater monitoring detecting a spike in cocaine use in Europe during 2017-18 which was not seen through traditional analyses until 2 years later.
- Reveal Scotland-specific drug trends geographically and over time.
- Communicating actions to other sectors, for example, if a new drug is found in the wastewater, this could be added to toxicology screening in the clinical setting.
- Provide population level data not readily available by other means.
- Provide consumption estimates at catchment and national level.
    - E.g. mass per capita usage.
- Detect a wider array of psychoactive substances.
- Able to be applied in a targeted approach for known substances and a non-targeted way as a screening method for unknown or novel substances.
- Be applied and compared from local to international scale.
- Be used to monitor the outcomes of interventions.
    - Impact of seizures on communities – which areas and the resilience of the drug network.

Task 3: Evidence Mapping

Evidence maps facilitate the systematic identification and reporting of the range of research activity in broad topic areas or policy domains. They provide stakeholders with tools and guidance to inform research priority setting. This is ideally suited to this project where we seek to determine a way forward for identifying approaches to be the focus of subsequent research trials. Evidence mapping literature review was applied to elicit information to address the following objectives:

O1: *For which of the listed substances or their metabolites are there recognised analytical strategies internationally*

O2a: *What are the characteristics of different analytical approaches available internationally and in Scotland for supporting the monitoring of target psychoactive substances and their metabolites (to include multiplex tests, capacities and different analytical strategies and approaches)*

O2b: *What are the characteristics of existing infrastructure available internationally and in Scotland for supporting the monitoring of target psychoactive substances and their metabolites (to include but not exclusive to sampling platforms, autosampling, grab samples, passive samples)*

O3: *What are the characteristics of early warning reporting systems on drug use –internationally and in Scotland that the different monitoring activities feed into.*

Similar to the literature review carried out in Task 2, inclusion and exclusion criteria were established by trialling and optimising search terms within Web of Science. A defined number of papers were selected for in-depth review. To ensure the quality of evidence identified, team members included a critical evaluation of the benefits or detriments to the studies as they relate to implementation of identified analytical approaches in Scotland.

Analytical strategies search

This search answered the questions noted above in objectives O1, O2a, and O2b. The search was used to gather quality information on studies previously conducted on wastewater samples for the substances of concern outlined in Task 2 (refer back to **Table 1**, above). Search terms were developed with input from the research team to identify a reasonable number of papers for a scoping review, while maintaining sufficient breadth so as to capture as much information needed for a comprehensive understanding. Several target psychoactive substances were grouped according to the following logic:

- **Amphetamine:** This term returns any papers discussing amphetamine or amphetamine-type stimulants (ATS), including MDA, MDMA, methamphetamine, and synthetic cathinones.
- **Cannab*:** This term returns any papers discussing cannabis, natural cannabinoids (i.e., those found in the cannabis plant itself), and synthetic cannabinoids (i.e., laboratory- or clandestinely-manufactured substances).
- **Opioid:** This term returns any papers discussing a substance related to a naturally-occurring or synthetic opioid or opiate such as fentanyl, diamorphine, or tramadol. While, in theory, nitazenes should also be captured by this term, it was included separately as well due to their relative novelty.
- **Benzodiazepine:** This term returns any papers discussing a pharmaceutical or street formulation such as diazepam or flubromazepam.

Conversely, target substances that could not be grouped into a class, such as cocaine and ketamine were explicitly labelled in the search. This approach minimised the number of search terms while maximising the chance of capturing target-specific information. **Appendix 6** details the final search terms applied in Web of Scholar, additional search parameters, and exclusion criteria to narrow down the list of articles identified.

The final list contained 19 results, plus 2 additional hand-picked studies from outside the scope of this search but directly relevant to the question at hand, for a total of 21 results. A detailed breakdown of information extracted from the 21 papers can also be found in **Appendix 7**.

Mapping results and limitations

Country

WWBE techniques for biological sampling – for example COVID-19 testing – have been broadly implemented worldwide. However, the results from this search suggest that while many countries have employed wastewater testing for one-off occasions of psychoactive substance use at festivals or special events, widespread monitoring or long-term surveillance of a sweeping number of psychoactive substances is largely restricted to Australia, mainland Europe, and the United States. It is unclear whether this may be due to a shortage of resources or a lack of infrastructure. A small sampling of studies from Canada and China also complements the research as highlighted by Huizer *et al.* (2021), although these represent few overall by comparison.

Target substances

All substances of concern and/or associated metabolites noted in **Table 1** (above) have recognised analytical strategies that have been successfully implemented for wastewater samples. The comprehensive list is outlined in the Excel file included in **Appendix 7**.

Of particular interest was the first known wastewater identification of protonitazene in samples collected in the U.S ; this methodology can be expanded to include other nitazenes of interest. Overall, these findings confirm the availability of methodology and analytical techniques which can be adapted to wastewater monitoring in Scotland.

Water sources

Nearly all studies employed wastewater influent samples for analysis with two exceptions. A 2024 U.S. study by Acosta *et al*., (2024) investigated surface water for a semi-quantitative analysis of total drug concentration, while Hehet *et al*.,

(2021) utilised a combination of 95% wastewater influents plus 5% sludge in a 2021 German study. The authors were successful in the identification of synthetic cannabinoid receptor agonists (SCRAs) in the 95%/5% influent/sludge combination, though it should be noted that SCRAs have also been successfully identified in pure influent samples (Bade *et al.,* 2023; de Oliveira, Vieira and Santos, 2023a). Sample collection sites were chosen to highlight/investigate drug consumption in a particular region (e.g., the New York/New Jersey waterways) or to support Early Warning System surveillance systems already in place.

Sample collection technique and transport

Wastewater samples were collected by several means, including grab samples (in which samples were manually collected just below the surface), autosamplers (fixed sampling devices), and composite samples (an autosampler configured to collect a set amount of sample which accumulated over a given interval of time), and much less often using a passive sampling device known as a Polar Organic Chemical Integrative Sampler (POCIS). All studies reviewed in this search utilised either grab or composite samples using an autosampler with subsequent storage on ice or in a refrigerated unit until arrival at the analysing laboratory or temporary storage unit. Approximately 200-600 mL of wastewater was collected for analysis. Often samples were stabilised immediately with acid upon collection to reduce degradation of the suspected substance(s). Discussions with stakeholders at Scottish Water confirmed that autosamplers are currently in place at water sampling locations and that they collect composite samples over the course of 24 hours; the sampling infrastructure itself would not need to be modified in order to support drug monitoring.

There was little information in the papers reviewed about the development of sampling strategies. This is likely to be at least in part because some of the major wastewater monitoring networks for detection of psychoactive substances were developed prior to the last 5 years on which the reviews focused. For example, the EU SCORE group have monitoring strategy was established over ten years ago. They adopt an approach of sampling for one week per year in a particular city (often only one city per country) at non-festival or holiday times. This is replicated in a number of studies. While there are other models within the wider literature (e.g. 6-8 weeks of daily (24h composite) sampling in which weekly patterns were detected Chen *et al.,* (2023), there was no clear discussion of the benefits of e.g. 1-week intensive sampling vs. monthly sampling (as currently undertaken by the Scottish Water sampling team, with variation at some sites). Gent and Paul, (2021) note that for NPS, the presence is sporadic and unpredictable and often at low concentrations. They may therefore not be detected with enough frequency to establish trends if less intensive sampling approaches are employed. Sampling strategies may also depend on the nature of the geographic area – large treatment works may be suited to high frequency composite samples, whereas at smaller works the focus might be on selecting the right sampling point within the treatment works to best capture representative samples for a small population. Castiglioni *et al.,* (2013) note that the choice of strategy will depend on:

- Potential users of psychoactive substances per population
- Preferred sampling time
- Nature of the project
- Drug transformation pathways
- Complexity of the sewage system

Concentration method

Due to the low relative abundance of parent compounds or metabolites in wastewater, samples were often concentrated using Solid Phase Extraction (SPE) prior to instrumental analysis (Bade, Abdelaziz, *et al.,* 2020; Bade, Ghetia, *et al.,* 2020; O'Rourke and Subedi, 2020; Boogaerts *et al.,* 2021; Steenbeek *et al.,* 2022; Adhikari *et al.,* 2023; Campo *et al.,* 2023; Frankenfeld *et al.,* 2023; Acosta *et al.,* 2024; Bade, Nadarajan, *et al.,* 2024; Bade, van Herwerden, *et al.,* 2024; Salgueiro-Gonzalez *et al.,* 2024). However, SPE was not used in situations of direct injection onto the instruments or when no sample preparation was trialled, as reviewed by de Oliveira et al., (2023a) and proven by Bade et al., (2023) and Gracia-Marín et al., (2024). The evidence map suggests that while SPE concentration is predominantly used in wastewater analysis, it is not definitively required, and further research could be completed to explore alternative sample preparation methods.

Analytical instrumentation

Gent and Paul, (2021) comprehensively reviewed and highlighted the mainstream analytical techniques employed for identifying psychoactive substances in wastewater:

4. **Liquid Chromatography – Mass Spectrometry (LC-MS, LC-MS/MS):** Low-resolution MS is most often the choice for targeted identification where the substances are known. Also, a common option for non-volatile and water-soluble compounds. SPE is typically required for concentration prior to analysis. Identification is made by comparison to a reference library.
5. **Mass Spectrometry (GC-MS, HRMS):** Suitable for volatile compounds. High resolution MS is capable of non-targeted, widespread screens where the identity of substances is unknown or novel. Direct injection with no sample pre-treatment is possible. Identification is made by comparison to open-source databases.

A simplified overview of these techniques is illustrated in **Figure 2**.

Hydrophilic interaction liquid chromatography coupled to mass spectrometry (HILIC-MS) is another, relatively new method explored by Steenbeek *et al*., (2022). When used in combination with SPE it can offer both qualitative and quantitative information with good sensitivity and resolution. However, it is most suited for very polar or ionic analytes such as cocaine and related alkaloids.

Some of the newest (albeit expensive) analytical instrumentation available on today's market allows for simultaneous targeted and untargeted LC-MS/MS analysis. While this feature is certainly beneficial, it should be noted that there is no right or wrong answer as to which analytical technique is chosen. Both provide clear benefits for wastewater monitoring and with technological advances over the past several years, both LC-MS/MS and LC-HRMS offer comparable resolution and sensitivity. It could be argued that LC-MS/MS should be employed routinely for most of the substances in **Table 1** (above), such as cocaine, heroin, amphetamine, and delta-9-THC; these targets have been and will likely continue to be seen in the Scottish drug market for years to come and have established reference materials for comparison. Simultaneously, LC-HRMS should be employed for other NPS such as benzodiazepines, nitazenes, and synthetic cannabinoids. The variability in these compounds and fluctuating availability on the drug market continues to evolve rapidly, making it wise and more cost-effective to monitor for prospective or novel formulations.

Turnaround time

No studies reported on either the total time taken for analysis, or the number of samples processed per day. However, multiple studies noted that samples were frozen at -20C until analysis (up to one year later) with no apparent loss in stability (Bade, Abdelaziz, *et al*., 2020; O'Rourke and Subedi, 2020; Boogaerts *et al*., 2021; Adhikari *et al*., 2023; Campo *et al*., 2023; Acosta *et al*., 2024; Bade, Nadarajan, *et al*., 2024; Gracia-Marín *et al*., 2024; Salgueiro-Gonzalez *et al*., 2024). An older review by Hernández et al., (2018) reported that influent

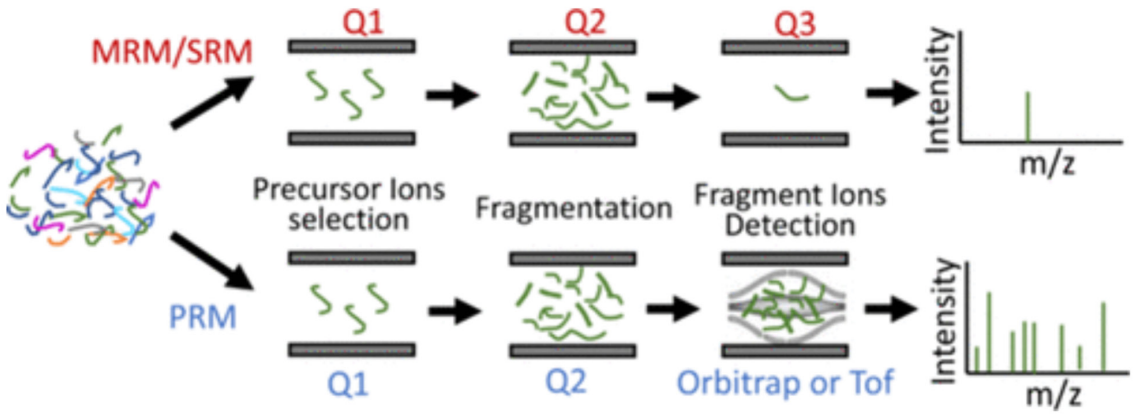

Figure 2. An illustration of the differences between LC-MS/MS and LC-HRMS as adapted from the Journal of Agricultural and Food Chemistry. TOP ARROW: A triple quadrupole (QQQ) or quadrupole-ion trap (Q-Trap) MS/MS selects precursor ions of a particular mass-to-charge ratio in Q1. The precursors are fragmented into Q2 to produce product ions, which are selectively detected in Q3 or the ion trap. BOTTOM ARROW: By comparison, Orbitrap or Time-of-Flight (TOF) high-resolution MS analyses all the fragmented product ions that have been produced in Q2. This allows for all ions to be evaluated, as in the case of widespread drug detection, as opposed to just the one that has been selected.

samples could be stored for up to three weeks at -20C and 4C for several days; however, a more recent assessment by Bade, Abdelaziz, *et al*., (2020) identified that long-term acidic storage conditions were ideal, and samples could be refrigerated following collection with little degradation for up to 14 days. The understanding is therefore that samples could be stored and re-analysed if necessary for up to 1-year post-collection.

Level of analytical understanding

The use of LC, LC-MS, LC-MS/MS, GC-MS, and HRMS instrumentation requires specialised analytical laboratory training to conduct wastewater analysis. A strong knowledge of analytical chemistry is required for method optimisation, instrument operation and data analysis. Qualitative (i.e. identification), quantitative (i.e. mass load and consumption estimate), and/or semi-quantitative information can be obtained from these techniques.

Benefits and drawbacks to techniques used

As noted, targeted analysis using traditional LC-MS/MS techniques are generally restricted to compounds whose identity is known by comparison to reference materials. This precludes novel NPS or unknown compounds from being identified through routine screening; however, when combined with HRMS untargeted screening and database comparison, LC-MS/MS techniques can be used for secondary confirmation of substance identity. Boogaerts *et al*., (2021) successfully combined the standard addition method with LC-MS/MS analysis to counteract matrix effects. A review by de Oliveira, Vieira and Santos, (2023) emphasised the analytical challenges associated with chemical diversity, which requires the development of either new cartridges used in SPE or new sample preparation approaches, but both of which can be avoided by using a direct injection HRMS setup. For quantitation purposes, the difficulty for all techniques lies in the unknown pharmacokinetic profiles of most NPS. Unlike established psychoactive substances or prescribed drugs, excretion rates can be complex to estimate and potentially lead to imprecise mass loads or consumption estimates. It is still entirely possible to estimate mass loads without the reference material or correction factor for a novel substance, though caution should be taken as the resulting figure may have a larger uncertainty.

Implementation in Scotland

A proposed workflow that could be implemented in future pilot studies is illustrated in **Figure 3**. This workflow details possible collection and analysis options as well as estimated timeframes for completion.

Early Warning Systems

To address the question posed in O3, "What are the characteristics of early warning reporting systems on drug use – internationally and in Scotland that the different monitoring activities feed into (O3)", a third literature search was undertaken. **Appendix 8** details the final search terms applied, additional search parameters, and exclusion criteria to narrow down the list of articles identified.

However, the resulting list (Camilleri *et al.,* 2021; Graziano *et al.,* 2021; de Morais *et al.,* 2023, 2024; Grp *et al.,* 2023; Syrjanen *et al.,* 2023) failed to capture information on EWS from several known organisations including SATA (EWS of the Americas), RADAR, FEWS (UK Home Office Forensic EWS) or UNODC, which is not surprising as EWS alerts are not necessarily peer-reviewed, published literature. Therefore, a direct search of these organisations' websites was conducted to gather additional details as documented in **Table 2**.

Psychoactive substance EWS are typically supported by a framework which includes the monitoring and reporting of public health threats, the release of information, and a resulting regulatory response. Data sources usually include analytically-supported confirmatory analysis, although self-reporting or community surveys have also proven to be successful. This has been particularly seen in the South Australian Drug Early Warning System (SADEWS). Among the challenges noted were reporting timeliness and a need for more accurate NPS identification methods (Artigiani and Wish, 2020). Scotland's RADAR reporting system publishes quarterly reports and ad-hoc alerts; it could be argued that this robust timeframe, coupled with HRMS analysis may offer a solid means of reporting on NPS presence in local wastewater.

Though not directly related to psychoactive substances, the World Health Organisation's Health Emergencies Programme monitors high-threat diseases as part of their surveillance system. Similar to the EWS of the European Union, member states contribute regional surveillance of threats to public health based on routine data collection and automated thresholds for action.

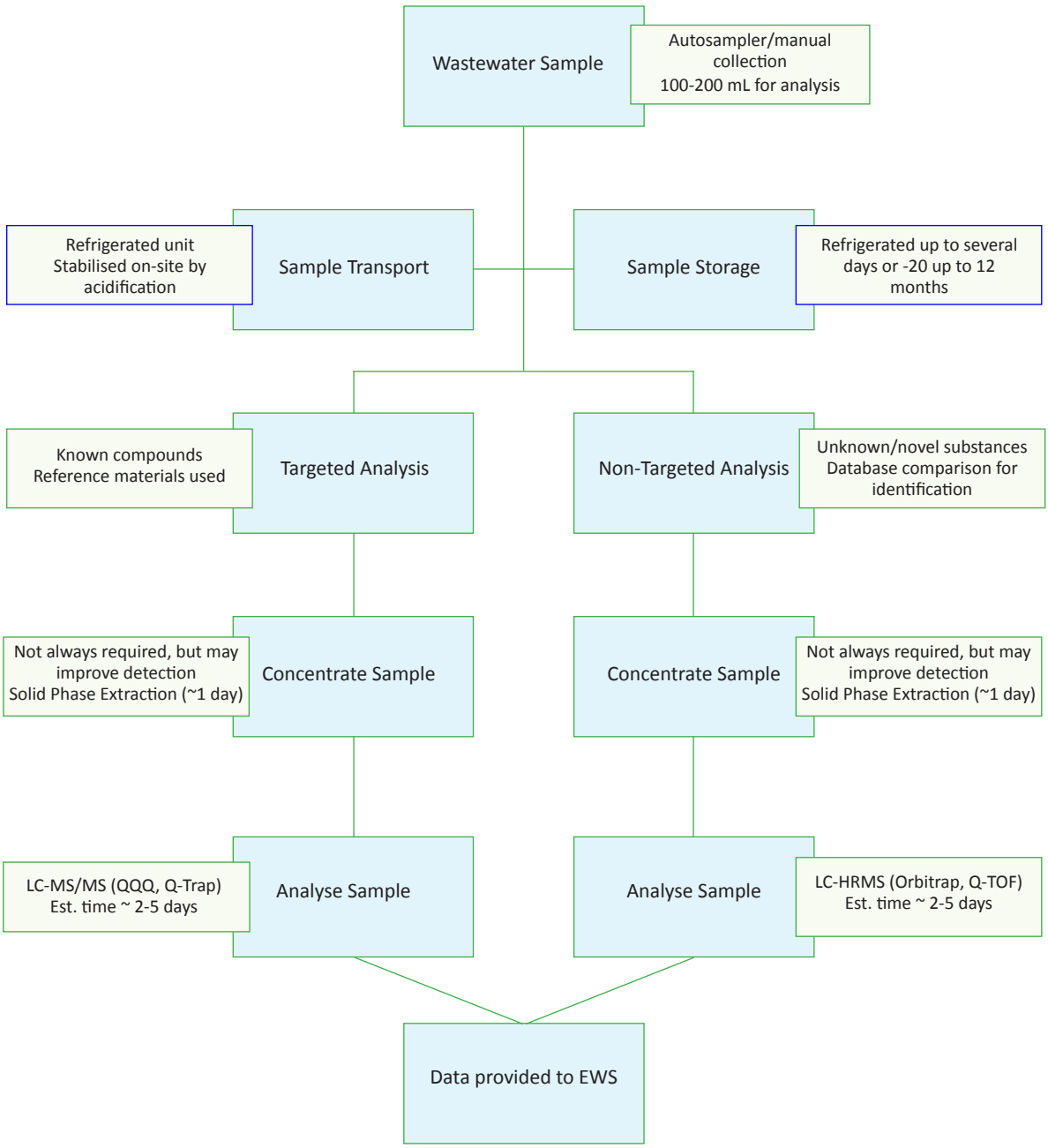

Figure 3. Theoretical workflow outlining a proposed wastewater analysis scheme from collection to reporting.

Table 2: Global EWS frameworks			
Location	**Established**	**Reporting Sources**	**Reporting Methods**
United States (NDEWS)	2014	Sentinel community sites Site visits Social media Toxicology results Urinalysis studies	Website
Australia (SADEWS)	2014	Clandestine drug laboratories Client-based information Community discarded products EDNA (described below) Law enforcement seizures Toxicology & coroner reports Wastewater analysis	Informal discussion Media releases
Australia (EDNA)	2020	Antidrug service (law enforcement) Forensic toxicology research unit Health and care systems Laboratory networks Medicine regulatory authorities Poison centres Tox and analytical labs Universities and research institutes	Data reported to EU EWS (EUDA/ Europol) Network alerts and advisories
UK (FEWS)	2012	Prisons UK border force hubs	Annual report
Scotland (RADAR)	2021	Healthcare sites Laboratory networks Law enforcement Local services and communities Monitoring surveys Prisons RADAR mailbox Reporting forms Toxicology services	Information summaries Quarterly report Risk assessments Warnings/alerts Dashboard with filters
European Union	1997	29 national EWS across Europe European Commission European Medicines Agency European Union Drugs Agency Europol	Periodic publications Searchable database Website
Americas (SATA)	2018	EWS from member states	Bulletins Conferences and training events Social networks
UNODC	2013	Annual report questionnaires Data sharing with partners Global NPS surveys International Collaborative Exercise (ICE) programme (drug analysis labs in 100 countries)	Interactive TOX-PORTAL Periodic publications

Task 4: Analytical Capabilities Search

To identify laboratories in Scotland and the UK with the capability to undertake analyses identified in Task 3, online searches were conducted for companies and research institutes offering these analytical services. Although laboratory accreditation was not specifically noted as a requirement for wastewater testing, the UKAS website was also considered as a potential source of information. However, the sheer number of accredited laboratories (approximately 850 total sites across the ballistics, chemicals, environmental engineering, environment, food, forensic, and medical fields) coupled with a lack of searchable equipment diminished the usability of this site. Therefore, the online search was expanded to include government-funded facilities, private institutions, research consortiums, and university research centres within Scotland. **Table 3** details the provider competencies where available, as well as facility instrumentation. Speed of analysis turnaround was not noted for any providers, nor were lab capacity or staffing capabilities.

Table 3. Scottish laboratory sites with the instrumentation required to test wastewater. Wherever possible this list indicates willingness to participate in external collaborations (as stated on the company website), though that is no guarantee of space, funding, or availability.

Institution	Instrumentation	Application	Collaboration/External Testing Encouraged
Cancer Research UK Scotland Institute	Q-Exactive Orbitrap Atlis QQQ LC-MS/MS	Medical research	Not noted
Eurofins	LC-MS/MS LC-Orbitrap LC-TOF	Water and nutrient concentration	Yes
Glasgow Caledonian University	Not noted	Not noted	Not noted
James Hutton Institute	LC-MS/MS	Environmental	Yes
Moredun Research Institute	Not noted	Livestock	Yes
Scottish Police Authority	Not noted	Forensics	Not noted
St. Andrews University	Sciex 5600+ Triple TOF	Proteomics	Yes
Thurso Environmental Research Institute	LC-QQQ LC-QTof LC-QTrap	Environmental research	Yes
University of Aberdeen Institute of Medical Sciences	Q-Exactive Orbitrap	Proteomics	Yes
University of Aberdeen Marine Biodiscovery Centre	Orbitrap Bruker Maxis II LC-HRMS	Marine chemistry	Not noted
University of Aberdeen Rowett Institute	AB Sciex Q-Trap 4000 AB Sciex API 3200 Agilent 6490 Shimadzu 8060	Nutrition	Yes
University of Dundee	UHPLC-MS/MS	Multidisciplinary	Facilities available for industry use
University of Edinburgh Clinical Research Facility	Sciex QTrap 6500 LC-MS/MS Waters Xevo TQ-s MS/MS Exploris 240 HRMS + Vanquish Duo UHPLC	Medical sciences	Yes
University of Edinburgh Roslin Institute	Q-TOF Ion trap	Veterinary sciences	Not noted
University of Strathclyde	Q-Exactive Orbitrap Shimadzu LC-QQQ 8060 NX Waters Xevo G3 Q-ToF	Chemistry & biomedical sciences	Yes

Task 5: Focus Group

An event was organised as a means of disseminating project findings and as a focus group to identify next steps towards trialling this approach in Scotland. Attendees were stakeholders representing academia (expertise in wastewater monitoring or associated fields), regulation (SEPA), water industry (Scottish Water), public health (Public Health Scotland, NHS) and policy (Scottish Government). There were 29 attendees including the research team and CREW project manager. Following a presentation of the project aims, objectives, approach and findings, attendees were asked to discuss several questions in plenary to build on the evidence synthesised in Tasks 1-4. The discussion was recorded and notes were also taken.

Workshop Summary:

In response to ***'Do you have specific examples of benefits of using wastewater-based monitoring?'***

- Drug data is incomplete. The drug market is unpredictable, and most countries have a limited data source. Scotland does not have drug checking services. Public Health Wales has a UK-wide service, but as it is postal, there is a delay. There is limited data from post-mortem, drug seizures and hospital toxicology results. Therefore, WWBE will be beneficial in synthesising intelligence of changes in the drug market and population including:
    - Impact of seizures on communities – which areas and the resilience of the drug network
    - Size of the problem at catchment level e.g. mass per capita usage
    - Emerging drugs, especially ones that are marketed as a different drug
- WWBE will provide Scotland-specific data, rather than having to rely on data from the EU or US.
- WWBE will be useful in communicating actions to other sectors, for example, if a new drug is found in the wastewater, this could be added to toxicology screening in the clinical setting.

In discussing the benefits of WWBE, several limitations of the approach were discussed.

- It was generally agreed that WWBE will not deliver reductions in drug deaths.
- There will not be correlations with drug deaths.
- There will not be links to small groups of individuals who take the drugs.
- It does not give information on drug potency.
- It will detect the chemical, but not its brand.
- Unless there are enough people consuming the drug, it won't be detected, however, passive sampling may help with concentrating the drug.
- People don't necessarily know what they are taking e.g. it could be cut with a cheaper substance, or it is marketed as something else.
- It is currently still difficult to estimate usage in the population with what is measured in wastewater.
- Given these limitations, if WWBE does not lead to actionable results, then investment in WWBE must be questioned.

In response to ***'What are the next steps towards trialling this approach in Scotland? Target substances to trial'***

- This depends on the data and intelligence relevant to Scotland.
- It should be based on drugs where the science is more robust to remove noise and uncertainty.
- It would be good to create a matrix to understand why certain drugs are being prioritised.

In response to ***'What are the next steps towards trialling this approach in Scotland: Sampling sites'***

- Site should cover all scenarios:
    - Urban and rural
    - Different demographics (e.g. sex, age)
    - Gradients of socio-economic status (considering crime and deprivation)
- WWTW are good for providing greater population coverage
- Portaloos may be good for capturing drug use amongst the homeless. Samples won't suffer from dilution effects.

In response to ***'What are the next steps towards trialling this approach in Scotland: Sampling approach – does current Scottish Water sampling approach fit or what changes?'***

- Need to consider the fact that Scottish Water does not routinely sample at the weekend when most psychoactive drug consumption occurs. If weekend sampling was required, this would be additional to the regular schedule of sampling.
- Approach needs to be adaptive because drug prevalence is constantly evolving. Baseline levels are required so that modifications can be made to sampling approach to respond to emerging issues.
- Small-scale pilot should be done to address specific scientific questions to understand the data better and should consider:
    - Temporal variability
    - Inter-lab variability
    - Grab sampling vs autosampling
- Use the lessons learnt from COVID-19.

In response to ***'What are the next steps towards trialling this approach in Scotland: Who might lead on next steps (which organisations?)'***

- It should be led by Scottish Government based on the intelligence that we already have.

A further theme brought up at the workshop was around sample preservation.

- Stability is impacted by multiple factors – the drug, stabiliser, location, transport and handling, etc.
- Degradation is observed for cocaine, so on-site stabilisation is required prior to freezing.
- Metanitazene did not appear in blood samples in storage. It is often present in low concentrations in fatalities, so this would be an issue to WWBE.
- Nitrobenzodiazepines are not stable.

Conclusions

The findings from this feasibility study have highlighted the importance of wastewater analysis as a supplemental tool for monitoring, and outlined clear benefits related to information availability, public awareness, and active monitoring. The extensive list of target psychoactive substances relevant in Scotland emphasises both individual compounds as well as entire drug classes. While all of the targets would be prioritised in an ideal situation, resource limitations necessitate a streamlined list for initial pilot studies. Sampling infrastructure is in place through Scottish Water; however, investment may be required in additional sampling to ensure data generated are reliable and to coordinate with monitoring across Europe. Reviewed publications included little specific information about timelines for transformation of raw data into format for use in early warning systems or on the resources required to develop statistical pipelines for this purpose – this would be welcomed in future academic studies on WWBE. However, once pipelines are developed, it seems likely that data analysis would be rapid ~ 1 week. Further, there was little information in reviewed literature around reasoning for using a particular sampling design. Appropriate analytical techniques have been successfully implemented using wastewater influents for all target substances, which allows for flexibility and adaptability in future monitoring. Furthermore, a wide array of analytical instrumentation exists at both public and private institutions in Scotland. There should therefore be available laboratory capacity to take on sample analysis.

Recommendations

- Pilot schemes should be trialled in Scotland to establish and develop practical implementation.
- Existing sampling platforms should be built upon to facilitate monitoring of psychoactive substances.
- Monitoring should emphasise identification of both established and novel substances using both targeted and non-targeted analytical screens.
- Given the restricted funding environment, wastewater-based epidemiology (WWBE) trials should focus on a select number of samples/ target substances prior to expansion at a national level.
- Trials should involve the major stakeholders from public health and water industry and would benefit from academic and statistical input into the development of sampling regimes, optimisation of detection methods and development of data analysis pipelines.
- The following could be reasonably trialled in further pilot study; cocaine, diamorphine, methadone, diazepam, and amphetamines through low-resolution LC-MS analysis and synthetic cannabinoids, synthetic opioids, and novel benzodiazepines through high-resolution LC-MS.
- To respond to the focus group outcomes, a matrix of target substances based on reason for inclusion should be produced based on this study and target substances narrowed down for trials at selected locations before up-scaling.

Appendix 1 – Substance Classes

Cathinones:

- 2-MAPB
- 3-methylmethcathinone
- 4 CMC
- 4-methylethcathinone
- 4-FMC
- 4-F-α-PVP
- 4-MEC
- 4-MeO-PV8
- 4-MeO-α-PVP
- 4-methylpentedrone
- 5-EAPB
- 7-OH-mitragynine
- Butylone
- DHM
- Eutylone
- MDPBP
- MDPV
- Mephedrone
- Methedrone
- Methylone
- Mitragynine
- 2-methyl-4'-(methylthio)-2-morpholinopropiophenone
- MPHP
- α-propylaminopentiophenone
- N-ethylhexedrone
- N-ethyl-4'methylpentedrone
- N-ethylpentylone
- Nor-mephedrone
- Pentedrone
- Pentylone
- PV8
- α-Pyrrolidinoisohexaphenone
- α-PVP

Opioids:

- 2-Furanylfentanyl
- 4- fluoroisobutyryl fentanyl
- 4-ANPP
- 4-fluorobutyrfentanyl
- 4-methoxybutyrylfentanyl
- Acetylfentanyl
- Acrylfentanyl
- Benzylfentanyl
- Brorfine
- Butyrylfentanyl
- Carfentanil
- Crotonylfentanyl
- Cyclopropylfentanyl
- Despropionyl fluorofentanyl
- Fentanyl
- Fluorobutyrylfentanyl
- Fluorofentanyl
- Furanylfentanyl
- Isotonitazene
- Methoxyacetylfentanyl
- Metonitazene
- N-methyl U-47931 E
- Norfentanyl
- Ocfentanil
- P-fluorobutyrylfentanyl
- THFF
- U-47700
- U-49900
- Valerylfentanyl
- α-EAPP

Synthetic Cannabinoids:

- 4F-MDMB-BINACA
- 5Cl-AKB-48
- 5F-AB-PINACA
- 5F-ADB
- 5F-AKB-48
- 5F-AMB
- 5F-CUMYL-P7AICA
- 5F-MDMB-PICA
- 5F-PB-22
- AB-CHIMICA
- AB-CHMINACA
- AB-PINACA
- ADB-FUBINACA
- ADB-PINACA
- AMB-FUBINACA
- APP-BINACA
- Cumyl-PEGACLONE
- EMB-FUBINACA
- FUB-AMB
- JWH-022
- MDMB-4en-PINACA
- MDMB-FUBINACA
- Mepirapim
- Methyl ester hydrolysis metabolite of 5F-ADB
- UR-144
- XLR-11

Phencyclidine analogues:

- Diphenidine
- 3-MeO-PCP
- MeO-PCP
- N-ethyldeschloroketamine
- MXE
- 2F-DCK
- 3-MeO-PCE
- Methoxetamine

Phenethylamines:

- 25B-NBOMe
- 25C-NBOMe
- 2-DPMP
- 2-Fluoroamphetamine
- 2-Fluoromethamphetamine
- 3-fluoro-phenmetrazine
- 4-Fluoroamphetamine
- 5-APB
- 6-APB
- MDA
- MDEA
- Methylenedioxymethamphetamine
- Methiopropamine
- PMA
- PMEA
- para-methoxymethamphetamine

Designer Benzodiazepines:

- Delorazepam
- Diclazepam
- Etizolam
- Flualprazolam
- Flubromazepam
- Lormetazepam
- Pyrazolam

Other:

- 3-FPM (Phenmetrazine analogue)
- m-CPP (Piperazine)
- MT-45 (Piperazine);
- Mebroqualone (Methaqualone analogue)
- MDAI (Aminoindane)

Appendix 2 – Detailed review of substance classes

Benzodiazapines (BZD)

Benzodiazepines were reviewed extensively by Brunetti *et al*., (2021) and Al Bahri and Hamnett, (2023) reviewed etizolam in detail. They are used to treat anxiety, panic attacks, sleep disorders and epilepsy and have also been used as muscle relaxants. They are abused at concentrations above the therapeutic dose to enhance the euphoric effects of opioids and to mitigate against post-stimulant crash, and to perpetrate sexual assault (e.g. date-rape) Gautam, Sharratt and Cole, (2014). They act to enhance GABA binding via increasing affinity of the GABAA receptor and they have a high therapeutic index and, taken as prescribed, are considered relatively safe. Approximately 30 designer BZD had been reported to the UNODC EWA by 2021, mostly from European Countries where they are often imported from India or China. The number of DBZD being detected or seized in the US is also rapidly increasing. Seventy percent of new DBZD are introduced into the European area, representing 13 % of seizures globally.

There is a high and increasing number of drug-related deaths involving BZD (such as etizolam) alongside other drugs. For example, in Scotland, there were 678 deaths involving "street benzodiazepines" (mainly etizolam - 4-(2-chlorophenyl)-2-ethyl-9-methyl-6H-thieno[3,2 f][1,2,4]triazolo[4,3-a][1,4] diazepine) in 2023. Etizolam and flubromazolam were the most detected BZD in driving under the influence of drug (DUID) cases in 2021 in the USA and 4870 seizures occurred across 46 states in the USA from 2012-2019. Where etizolam is licenced (Japan, South Korea, Italy, India) it is used to treat common psychiatric symptoms (e.g. anxiety) and as a muscle relaxant. Its use is more prevalent in East Asia, North America and Europe.

Etizolam, often disguised as the licit pharmaceutical products alprazolam and diazepam, is sold illegally throughout the UK and commonly combined with opioids ("benzo-dope") to help alcohol withdrawal symptoms, and with cocaine to decrease the seizure threshold. It was detected in 28 post-mortem cases in England and Wales during a one-year period in 2018-19. Following this, In 2019 the Expert Committee on Drug Dependence (ECDD) made a recommendation to add etizolam to Schedule IV of the on Psychotropic Substances 1971.

It is widely known that dependence and tolerance occur with benzodiazepines. For example, in therapeutic use, dependence arises following relief of anxiety symptoms. Evidence suggests that etizolam may be less likely to induce tolerance. However, there is a paucity of data on dependence. Postmortem cases internationally demonstrate that consumption of multiple psychotropic drugs including etizolam was the main reason for reported etizolam deaths, with etizolam often being reported at therapeutic concentrations Al Bahri and Hamnett, (2023). A Canadian study recently reported that 43% of opioid samples contained etizolam. Despite these figures, it has been suggested that etizolam may have a higher fatal dose than other BZD. However, doses vary in counterfeit etizolam tabs (from 0.7-8.3 mg/tablet).

Al Bahri and Hamnett, (2023) also refer to a number of non-fatal intoxications in the US, Sweden, France and The Netherlands. They cite a study stating that Flumazenil can be used to reverse the effects of etizolam overdose. They also reference a case report stating that Diazepam can reduce withdrawal symptoms of etizolam use. Etizolam still needs more pharmacological and analytical investigations to help toxicologists interpret case results. Etizolam is the street BZD most implicated in drug deaths.

Brunetti et al., (2021) reviewed 31 designer benzodiazepines after consulting the international early warning database. Twelve had sufficient data to be examined further and these were:

- 3-hydroxyphenazepam
- Adinazolam
- Clonazolam
- **Etizolam**
- Deschloroetizola
- Diclazepam
- **Flualprazolam**
- **Flubromazepam**
- Flubromazolam
- Meclonazepam
- **Phenazepam**
- Pyrazolam

Those in bold – etizolam, flualprazolam, flubromazepam and phenazepam were those implicated in most cases of drug offenses, adverse effects and deaths, presenting the greatest public health risk globally among this drug class. Etizolam, flualprazolam and flubromazepam were also most commonly implicated in cases of driving under the influence of drugs. The authors considered Emergency admissions, driving under the influence of drugs (DUID) or fatalities associated with DBZD use. Of 48 studies included, the following DBZD were explicitly implicated in poisoning, driving impairment and death:

3-hydroxyphenazepam, adinazolam, clonazolam, etizolam, deschloroetizolam, diclazepam, flualprazolam, flubromazepam, flubromazolam, meclonazepam, phenazepam and pyrazolam.

The authors provide specific information for each of these substances, collated in **Appendix 3**.

Box 4: Benzodiazepine key points

- Dependence and tolerance occur with benzodiazepines
- Over 800 deaths from "street BZDs" – Scotland 2020
- Globally etizolam, flualprazolam, flubromazepam and phenazepam most implicated in adverse events

Box 4 lists key points and a summary of findings related to benzodiazepines.

Cannabinoids

Over 100 cannabinoids are found naturally in plants, including cannabis and hemp plants and may be extracted directly. Alternatively, cannabinoids can be manufactured semi-synthetically (using a natural plant-based pre-cursor) or entirely synthetically. Synthetic and non-synthetic cannabinoids and their adverse effects were reviewed by Cohen and Weinstein (2018). Caprari et al., (2024) extensively reviewed "designer" THC and de Oliveira et al., (2023b) undertook a systematic review of the toxicity of cannabinoids in K2/Spice.

Cannabis is more widely available and more widely consumed globally than any other psychoactive drug and its therapeutic use is increasing. The main psychoactive substance in cannabis is Δ-9 tetra-hydro-cannabinol (THC). Synthetic cannabinoids mimic the psychotropic effects of cannabis but can have more significant adverse effects (**Table A2.1**). There are two main classes of cannabinoid, non-natural (THC-like) cannabinoids and those found in nature in small quantities. Caprari et al., 2024 suggested the nomenclature adopted in **Figure A2.1** to describe the different types of cannabinoids, based on their derivation and/or synthesis.

Caprari's review reported that up to 4% of the population use synthetic cannabinoids at some time in their lifetime, compared with up to a third

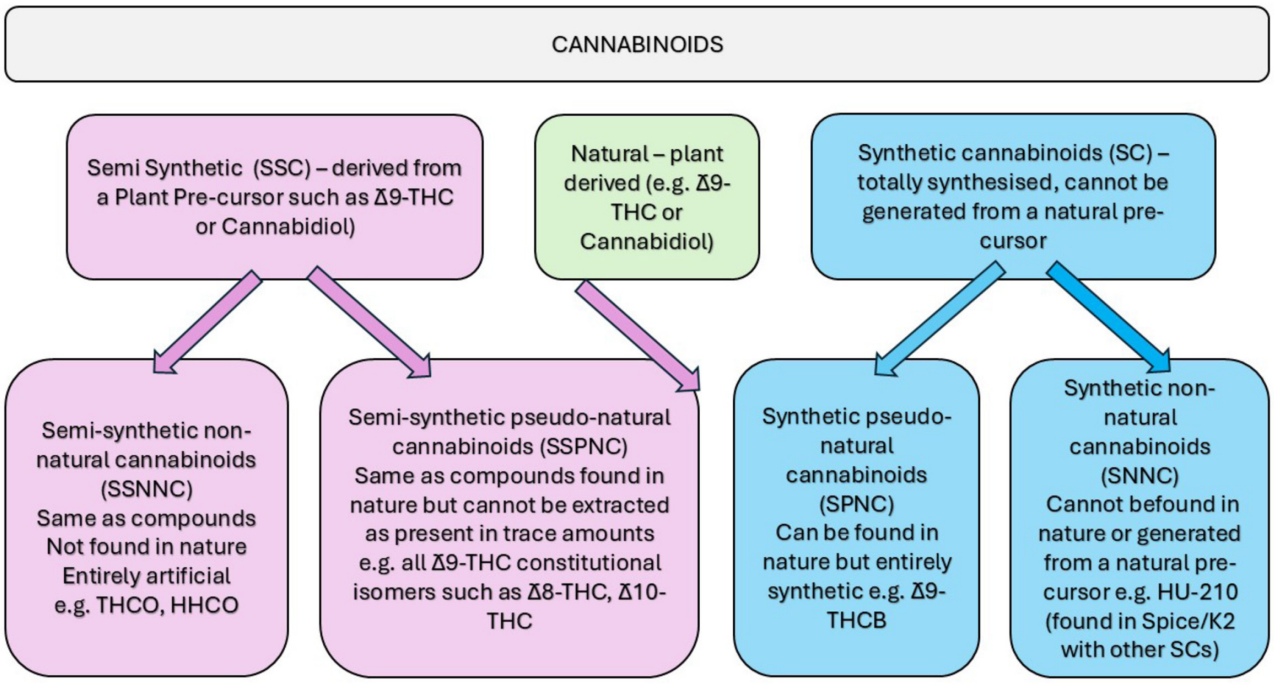

Figure A2.1: Types of cannabinoids based on Caprari et al., (2024).

of the population using natural cannabis. One percent of 14-18 year olds in Europe and up to 17% of US college students were reported to have used SC drugs. Legislation was applied to SCs in the UK in both 2009 and 2013, but novel SCs have been generated that are not legally controlled in the UK and similar manipulation of chemical structures has occurred in other countries, allowing these substances to be sold legally. There are major differences between the effects of cannabis and SCs – SCs show greater toxicity than organic cannabis. Chronic use of SC is of particular concern with respect to the development of serious mental health disorders, especially where use is among young people.

The most extensively studied cannabinoid is Δ^9-tetrahydrocannabinol – the primary psychoactive substance in cannabis which was detected in the 1960s. It activates endocannabinoid receptor 1 (CB1R) in the central nervous system. Novel cannabinoids were originally synthesised in order to improve the pharmacological activity of Δ^9-tetrahydrocannabinol but are emerging as a novel psychoactive substance of concern.

Synthetic cannabinoids are further detailed in **Appendix 4**.

de Oliveira *et al*., (2023b) undertook a systematic review of the toxicity of SCs compounds in Spice/K2 drugs. This included sixty-four articles reporting the effects of synthetic cannabinoids in humans, 10 clinical studies and 64 case reports. The review indicated that health risks associated with SCs are greater than those associated with natural cannabis, with higher toxicity, longer-lasting effects and addiction potential. They may be particularly harmful in people with epilepsy and schizophrenia, having greater potential to trigger a convulsive crisis, reduced consciousness, and impacts on blood flow. More toxicological data is required to better understand harms.

They identified the following synthetic cannabinoids:

AB-CHMINACA	FUB-AMB	5F-PB-22
ADB-CHMINACA	5F-AMB	5F-AKB-48
AB-PINACA	WH-018	PB-22
ADB-FUBINACA	JWH-073	6-APB
ADB-PINACA	JWH-122	EAM-2201
AB-FUBINACA	JWH-022	BB-22
MDMB-FUBINACA	AM-2201	XLR-11
MDMB-CHMICA	AM-694	UR-144
5F-ADB	MMB-2201	

The most frequently identified were AB-CHMINACA, ADB-FUBINACA, and JWH-018, with AB-CHMINACA and ADB-FUBINACA associated most frequently with adverse effects and accounted for 41% of all reported fatalities. SCs can exhibit unexpected effects. Due to the lack of clinical studies, it is impossible to determine fatal doses or harmful effects accurately, especially in new generation SCs such as ADB-FUBINACA, AB-PINACA, AB-CHIMINACA, MDMB-CHMICA, and XLR-11and there is no antidote.

Box 5 lists key points and a summary of findings related to cannabinoids.

Box 5: Cannabinoids key points

- Synthetic cannabinoids can have greater adverse effects than natural cannabis
- Emerging NPS of concern
- Novel synthetic cannabinoids developed to circumvent legislation
- Largest drug class in EU early warning system
- Range of effects and limited data on risks
- Potential toxic contaminants from synthesis

Other synthetic cannabinoids

There are several THC-like cannabinoids and other newly appeared cannabinoids which can be found in substance blends include THC-JD and THC-X. There are few data relating to these. They are summarised as follows:

- THCP-O- reported to give longer lasting and more euphoric effects than Δ9-THCP, but also a later onset (up to 30–45 min). Intense sensations of euphoria, relaxation, intoxication, or drowsiness – 8-hour effects (longer than most cannabinoids). Side effects are short-lived and similar to other psychoactive cannabinoids. Usually consumed as e-cigarette liquids and edible gummy lollies – usually blended with other cannabinoids.
- Hexahydrocannabiphorol acetate (HHCP O) (6aR,9R,10aR)-3-Heptyl-6,6,9-trimethyl-6a,7,8,9,10,10a-hexahydro6H-benzo[c] chromen-1-yl acetate and (6aR,9S,10aR)-3-heptyl-6,6,9-trimethyl-6a,7,8,9,10,10a-hexahydro-6Hbenzo[c]chromen-1-yl acetate (HHCP-O (also known as 9β-hexahydrocannabiphorol acetate, 9β-HHCP acetate, (9R)-HHCP acetate or 9β-HHCP-O))

- Users report similar effect to Δ^9-THCP, very potent, long lasting, slower onset. Desirable effects include relaxation, euphoria, pain relief, warm body vibrations, creativity. Single use can give withdrawal effects. Use includes vape cartridges and dab pens. There is a risk of - e-cigarette or vaping associated lung injury (EVALI) (oral route preferred).
- Tetrahydrocannabinol JD (THC JD) (6aR,10aR)-6,6,9- Trimethyl-3-octyl-6a,7,8,10a-tetrahydro-6H-benzo[c] chromen-1-ol.
- Often not very pure so usually active component is at low concentration, although the compound itself is potent. Reported effects are similar to Δ^9-THC, but with an additional boost, thus enhancing good mood, hunger and sleepiness.
- Tetrahydrocannabinol X (THCX). The compound itself is complex to confirm because it is a blend primarily of Δ8-THCP, Δ8-THC-O, Δ9-THCP, and small amounts of Δ9-THC-O and CBD.
- Positive effects include pain relief, anti-inflammatory effects, and psychoactivity. Effects have described sensations as mellowing, relaxing, euphoriant and energizing. Side effects include anxiety, dry mouth, dry or red eyes, increased appetite, increased heart rate, memory loss, slightly declined cognitive function, and slowed reaction times. Usually consumed as gummies, disposable vape pens and cartridges, tinctures, and flowers.

People who use synthetic cannabinoids such as these are often at risk as they are unaware of the actual composition of the compound because suppliers and producers make false claims about ingredients. Understanding risks to consumers is challenging. Definitive information on pharmacology, psychoactive effects and adverse effects is limited, and substances are likely to evolve rapidly rendering any current review outdated within months. Overall, the market for cannabinoids is different from other illegal drug markets because substances are commonly promoted as legal and as having the health benefits of cannabis and hemp, but even minor chemical modifications can lead to unpredictable health risks.

Synthetic cathinones

An extensive review of synthetic cathinones is given by Kuropka, Zawadzki and Szpot (2023) and Simao et al., (2022). Cathinone occurs naturally in the leaves of khat (Catha edulis), a plant found in East and Southern Africa, the Southwest Arabian Peninsula and Afghanistan. Currently, only one synthetic cathinone is available on the market for therapeutic purposes, namely bupropion, which is used as an antidepressant, coadjutant in smoking cessation therapy, and in the treatment of obesity.

A total of 156 synthetic cathinones are currently monitored by the EU EWS, making it the second largest group of substances under surveillance by this organisation. Numerous cathinone derivatives can be made by modification of the backbone structure. As such, legislation to ban synthetic cathinones is driving the emergence of new derivatives, causing changes in prevalence. For example, in their review, Kuropka, Zawadzki and Szpot (2022) reported the detection of 29 new synthetic cathinones (listed in **Appendix 5**) between 2019 and 2022.

Synthetic cathinones have gained popularity due to wide availability, low prices, good marketing strategies, relatively high purity and popularisation via the internet and smartshops. European studies report an increasing detection of synthetic cathinones in drug checking services (Maghsoudi *et al*., 2022). In the UK, toxicity related to synthetic cathinones consumption increased from 0 to 600 cases from 2009 to 2010. At the same time, the prevalence of consumption in Northern Ireland was about 2%. Street names include “Miaow Miaow”, “M-Cat”, “Msmack”, Drone”, “Fert”, or “Bubbles.” Whether use of these substances is increasing or not globally is unclear as Cohen and Weinstein, (2018) suggest that their use is declining.

Synthetic cathinones are mainly consumed orally, but other methods include nasal insufflation and inhalation (e.g. e-cigarettes), gingival and sublingual mucosal routes, intravenous, intramuscular, and subcutaneous injections, and rectal administration. They can be consumed pure, or in combination with other substances. In particular, people using these drugs may combine them with prescription drugs to reduce negative side effects of cathinones taken in isolation.

Desirable effects include euphoria, stimulation, hallucinations, altered mental status, delusions and increased libido. However, it can also cause hypertension, palpitations, tachycardia, vasoconstriction, cardiac arrest, aggression, anxiety, paranoia, psychosis, confusion, psychomotor agitation, insomnia, impaired vision and speech, dilated pupils, acute kidney failure and renal failure, hyperthermia, acidosis, mydriasis rhabdomyolysis and seizures. Fatalities have been reported in connection with the use of synthetic cathinones.

The repeated use of these substances at high doses may cause craving, dependence, tolerance, and withdrawal syndrome. There is a lack of appropriate clinical support due to a lack of information regarding pharmacological features and toxicity. Consumers do not always know precisely what they are ingesting because these substances are synthesised and sold in illicit markets, which can lead to unwanted effects, overdoses and death.

Synthetic cathinones were not highlighted specifically as psychoactive substances of immediate concern in Scotland but ones that could be investigated further if desired. **Box 6** lists key points and a summary of findings related to synthetic cathinones.

Box 6: Synthetic cathinones key points

- 156 synthetic cathinones monitored by EU EWS
- Legislation drives emergence of new derivatives
- Increasingly detected in drug checking services
- Global trend unclear

Synthetic opioids

Synthetic opioids have been extensively reviewed (Shafi *et al*., 2022). Opioids have been used medicinally for years as an analgesic and sedative, and in the management of chronic and severe pain, as well as palliative care. But the feelings of relaxation, euphoria and well-being have led to non-medicinal and problematic use.

Synthetic opioids include fentanyl and its analogues. In 1959, fentanyl was used medicinally as an analgesic and anaesthetic agent and was a popular choice due to its high potency, quick absorption time and shorter onset time for effects. Over the last decade, new synthetic opioids such as carfentil and ocfentanil have been implicated in an international opioid crisis. For example, in North America, illegally manufactured fentanyl and other synthetic opioids significantly contributed to the 'opioid overdose crisis.' Meanwhile, the UK Advisory Council on the Misuse of Drugs (ACMD) highlighted in 2016 that the rate of drug-related deaths had steadily increased, and that those related to novel synthetic opioids were likely to be under-represented due to the lack of available forensic analyses. However, UK deaths were reported to be much lower than in North America.

Synthetic opioids include fentanyl, sufentanil, alfentanil, remifentanil, carfentanil, ocfentanil, acetylfentanyl and furanylfentanyl. Carfentanil is approved for veterinary medicine only as a general anaesthetic or tranquilising agent for large animals. It has been mis-sold as other drugs or used as a substitute, leading to opioid overdoses, many fatal. Ocfentanil was not developed for medical use and was detected on the drug market after 2010.

Synthetic opioids are manufactured as a powder, tablet, transdermal patch and liquid forms. They can be consumed by swallowing, nasal insufflation, smoking, injecting, transdermal application, or application sublingually, vaginally or rectally. Novel methods include inhalation with electronic devices (vaping). Absorption from oral administration of transdermal patches can be increased by chewing prior to swallowing. Since transdermal patches contain a large amount of drug, it can be extracted and used via alternative routes such as injection and nasal insufflation. Nasal burn or nasal drip after insufflation and a bitter taste after oral ingestion have been reported.

Undesirable effects of synthetic opioids include alterations in muscle tone, chest wall rigidity, seizure-like activity, confusion, affective changes, cough suppression, orthostatic hypotension, urinary urgency or retention, folliculitis and dermatitis with hair loss, dry eyes, elevated liver enzymes and delayed bilateral hearing loss. The "opioid overdose triad" symptoms include miosis (pupil restriction), respiratory depression and decreased level of consciousness or coma. Vomiting during reduced consciousness can risk suffocation. Fatalities associated with severe opioid toxicity have been reported.

A new generation of synthetic opioids structurally different to fentanyl have come on the market since 2010 (extensively reviewed by Zawilska et al., (2023)). Their chemical structures belong to benzamide (e.g. U-47700, U-48800 or AH-7921), acetamide (e.g. U-50488, U-51754) or piperazine (e.g. MT-45) classes of compounds.

MT-45(1-cyclohexyl-4-(1,2-diphenylethyl) piperazine), also known as IC-6, CDEP and NSC299236, was originally developed in the 1970s as a potential analgesic substitute for morphine. MT-45 caused 28 deaths in Sweden within a nine-month period between November 2013 and July 2014, and in 24 cases, other psychoactive substances were also used.

AP-237 (1-butyryl-4-cinnamylpiperazine, also known as bucinnazine), 2-methyl AP-237 and AP-238 appeared in the European and American market in 2019-2020. AP-237 and AP-238 have been used as analgesics in cancer patients in China. Unpleasant sensations have been reported with certain routes of administration. In the US, 2-methyl AP-237 was implicated in at least 17 confirmed cases of fatalities, with several reports of non-fatal intoxications.

AH-7921 (3,4-dichloro-N-(1-(dimethylamino) cyclohexylmethyl) benzamide), known also as 'doxylam', 'doxylan' and 'CN 2924 29 98', was developed in mid-1970s as a potent opioid analgesic agent. It is sold as capsules and tablets. European Early Warning System reported 15 deaths with AH-7921 within a 10-month period between December 2012 and September 2013, and in most cases, other psychoactive substances were also present.

U-compounds or U-drugs consist of two major groups: U-47700 series M compounds such as U-47700, N-ethyl-U-47700, isopropyl-U-47700, 3,4-difluoro-U-47700, U-47109, U-77891, U-47931A (bromadoline), N-methyl-U-47931E, U-48520 and U-49900; and U-50488 series K compounds such as U-504884, U-51574, U-48800, U-69593 and U-62066 (spiradoline). U-47700 (3,4-dichloro-N-[2-(dimethyloamino) cyclohexyl]-N-methylbenzamide) was the first of the U-compounds that appeared on the recreational drug market in 2014, steadily increasing in 2015 and 2016. Street names include "U4," "Fake morphine," "synthetic cocaine" or "Pink," "Pinky" or "pink heroine" due to its pink appearance from impurities during production. U-47700 was identified as a constituent of an opioid cocktail "gray death." It is sold as a powder, tablet or liquid for use in nasal sprays, inhalers and herbal incense. Its low price and ease of access make it attractive to users. It has been reported in seized materials in the US, UK, Sweden, Belgium, Italy, Czech Republic, Finland, Brazil and Australia. There have been many reports of intoxications, including fatalities. In 2016, U-47700 scheduling in the US saw the rise in the use of U-49900 (3,4-dichloro-N-[2-(diethylamino) cyclohexyl]-N-methylbenzamide), which is the diethyl analog of U-47700.

2-benzylbenzimidazoles (nitazenes*) are the most recent to proliferate on the drug market. There is currently no medicinal use of this class of drugs. The appearance of isotonitazene in 2019 triggered a rapid expansion of 2-benzylbenzimidazole opioids on the illicitdrug market and dominated in the first half of 2020. Following its international scheduling in 2021, its popularity has decreased.

Etonitazene or etodesnitazene (2-{2-[(4-ethoxyphenyl) methyl]− 5-nitro-1H-benzimidazol-1-yl}-N,N-diethylethan-1-amine) was the first compound from the nitazene group encountered on the psychoactive substance market. Isotonitazene (N,N-diethyl-2-(2-(4-isopropoxybenzyl)− 5-nitro-1H-benzo[d]imidazol-1-yl)ethan-1-amine) is a structural analogue of etonitazene. Street names include "Iso", "Nitazene" and "Toni". Deaths from isotonitazene intoxication have been reported in the US. Canada, Germany and the UK.

Metonitazene(N,N-diethyl-2-(2-(4-methoxybenzyl)− 5-nitro-1H-benzo[d]imidazol-1- yl)ethan-1-amine) is one of the newest non-fentanyl synthetic opioids, first appearing in the mid-2020s. Street names include "NIH 7606". Protonitazene (N,N-diethyl-2-(5-nitro-2-(4-propoxybenzyl)− 1H-benzo[d]imidazol-1-yl) ethan-1-amine) was first detected in Canada and the US in 2020, with 9 confirmed fatal cases in the US. Brorphine (1-[1-[1-(4-bromophenyl) ethyl]-piperidin-4-yl]− 1,3-dihydro-2H-benzo[d]imidazol-2-one) was first synthesized in 2018 and first reported to the European Monitoring Centre for Drugs and Drug Addiction in June 2020. Street names include "purple heroine" and has been found in falsified oxycodone "A/215" tablets. Its increase in popularity is likely driven by the scheduling of isotonitazene.

Box 7 lists key points and a summary of findings related to synthetic opioids.

Box 7: Synthetic opioids key points

- Include Fentanyl and analogues
- Contribute to opioid overdose crisis
- Steady increase in opioid related deaths in UK
- Veterinary medicines – carfentanyl - mis-sold

Phenethylamines

Phenethylamines have been reviewed in Simao et al., (2022) and Lukic et al., (2021). Phenethylamines were synthesised in the early part of the 20th century, but in the last two decades of the 20th century, other derivatives were made. It consists of an aromatic ring and two carbon side chains, so derivatives are made by substitutions to either of these chemical structures, and features compounds from the 2C and D series. Several phenethylamines are yet to fall under international control. Drug checking services have seen a proliferation of phenethylamines (Maghsoudi *et al.*, 2022) and are

the third-most reported group of new psychoactive substances worldwide (437 cases), after synthetic cathinones and synthetic cannabinoids (684 and 665 cases, respectively).

Therapeutic uses of phenethylamines include appetite suppressants, vasoconstrictors, bronchodilators, or calcium channel blockers, and can be sold as stimulants, anti-depressants, anorectics, hormones, neurotransmitters, or bronchodilators. This is because they act on several systems, such as the serotoninergic, dopaminergic and noradrenergic.

Phenethylamines are consumed differently depending on the specific type of phenethylamine. Ingestion is the most common method, but they can also be insufflated or taken as pills or capsules. Substances in the 2C series can be found in other forms such as powders, liquids and tablets. The NBOMe series compounds are often sold as LSD but have much higher toxicity.

Undesirable effects are also dependent on the substance type. The D series compounds have been reported to induce tachycardia, seizures, hallucinations and kidney failure. The NBOMe series compounds can cause cardiovascular problems, seizures, metabolic acidosis, and organ failure. The 2C series compounds can cause serotonin toxicity or sympathomimetic syndrome. Serotoninergic syndrome is one of the main dangers of phenylethylamines consumption.

Phenethylamines are relatives of MDMA. MDMA is scheduled in the UK by the Misuse of Drugs Act 1971 (MDA) and the Misuse of Drugs Regulations 2001 (MDR). However, it is currently being investigated for therapeutic and medicinal use for patients with chronic, treatment-resistant posttraumatic stress disorder. MDMA is the main constituent of the drug "ecstasy". MDMA and its derivatives promote arousal, euphoria, increased sociability, enhanced mood, and heightened perceptions. However, undesirable effects include headache, nausea, bruxism, tachycardia, trismus, feelings of distress and anxiety, emotional disturbances, unpleasant hallucinations, tachycardia and hypertension, frequent agitation, tremors, and seizures.

"Bromo-dragonfly" (also called "3C-Bromo-Dragonfly" and "DOB-Dragonfly") has been linked to deaths in Scandinavia. Compounds from the 2C series have been linked to 3 fatalities where phenethylamines were ingested within a drug cocktail. PMA and PMMA are the most common substances associated with fatalities.

Xylazine

Xylazine is being increasingly detected in scotland (Public Health Scotland, 2014). Zawilska et al., (2023) mention xylazine as a substance "used in a differing manner from its originally intended use." Ayub et al., (2023) address xylazine through a review of case reports. A veterinary drug, xylazine is commonly added as an adulterant to opioids, including heroin, cocaine and fentanyl, to increase or prolong their euphoric and sedative effects. It is also known as "tranq" and "zombi drug". It acts as an α2-adrenergic receptor agonist producing sedative, analgesic, muscle relaxant effects and has also been detected in combination with amphetamines, cannabinoids and benzodiazepines. Xylazine was first detected as a street drug in South America and its use is rapidly spreading across other states in the USA where it is found in up to 50% of fatal opioid overdoses.

Little is known about its pharmacology or toxicology, except that it has a rapid onset of effects, is rapidly metabolised and eliminated and there is no known safe dose for humans as even trace amounts in blood have been associated with fatality. Adverse effects include severe cardiovascular deterioration, psychiatric and neurological symptoms, respiratory depression, hyperglycaemia, osteomyelitis and severe skin ulcers which can be necrotic.

Xylazine is widely administered intravenously and this, its use with other drugs and fatalities associated with its use all add to its potential for harm. It was classified as a Class C drug in January 2025 under the Misuse of Drugs Act 1971 in the UK.

Tryptamines

Tryptamines have been reviewed in Simao et al., (2022). They occur naturally in plants, fungi and animals. Some have been consumed as part of traditional cultural practices. Importantly, there is no evidence that consumption poses a life-threatening risk. Some examples are outlined below.

Ayahuasca is a hallucinogenic drink made from the plant *Banisteriospsis caapi* or an association with *Psychotria viridis*. It is consumed by tribes of the Amazon as part of religious rituals, and can be used to treat depression, anxiety and addiction.

The fungus *Psilocibo* spp. has been used by indigenous people of South America, India, Mexico and Australia for sacred and therapeutic rituals. They contain psilocybin and psilocin, which have similar properties to LSD. As such, they have

become known as “magic mushrooms”. Psilocybin can be used to treat anxiety, resistant depression, alcohol dependence and for the cessation of tobacco smoking.

5-hydroxy-N,N-dimethyltryptaline(5-OH-DMT), is a positional isomer of psilocin and is the main psychoactive element in the venom of the American desert toad *Bufo Alvarius*.

Lysergic acid amine or (8β)-9,10-didehydro-6-methyl-ergoline-8-carboxamide is also similar to LSD and occurs in the seeds of *Argyreia nervosa* and *Ipomoea violacea*.

These natural compounds have attracted interest by drug developers to make synthetic tryptamines. A growing market has emerged due to ease of access via the internet, nightclubs and raves. They can be taken orally, intramuscularly, intravenously or smoked depending on the substance and the user’s preference. Tryptamines have a short duration time, leading to repeated consumption, potentially leading to dependence. The 2019 Global Drug Survey report noted that tryptamines are increasing in use and that 40% of drug users consume tryptamines. The EMCDDA reported that LSD and hallucinogenic mushrooms are among those which have become established in particular localities or populations (EMCDDA 2024).

Plant-based psychoactive substances

Plant-based psychoactive substances have been reviewed in Simao et al., (2022). They mostly comprise of alkaloids, which can induce hallucinogenic effects and sometimes relaxation. Traditionally, they have been used in traditional medicine and rituals. Three are monitored by the UNODC, specifically, *Catha edulis, Salvia divinorum* and *Mitragyna speciosa*.

Catha edulis, also known as khat, is an indigenous plant from East Africa and the southern Arabian Peninsula (El-Menyar *et al.*, 2015). It is consumed by chewing the leaves, ingestion or smoking. It is frequently used by synthetic cathinone users because it has a lower health risk. Undesirable effects include psychosis, aggressive behaviour, irregular blood pressure, tachycardia, urine retention, constipation and insomnia. There is no evidence of deaths caused by *C. edulis*.

Salvia divinorum is a hallucinogenic, traditionally used in northeastern Mexico. It is consumed by chewing the leaves, making into tea or is smoked. Short-term effects include extracorporeal experiences, relaxation, visions and loss of consciousness. It contains the hallucinogen, salvinorin A, which has been used to treat drug addiction, pain, neurological and gastrointestinal diseases and as an anti-inflammatory agent. There is no evidence of deaths caused by *S. divinorum*.

Mitragyna speciosa, also known as krakom, is a plant native to Southeast Asia. It is used for pain relief, to treat withdrawal symptoms in people who use opioids, hypertension, diarrhoea and coughing. It is consumed by chewing the leaves, making tea or smoking. Undesirable effects include irritability, anxiety and aggressiveness. The main psychoactive substance is mitragynine, which is not yet listed in the schedule of the UN Convention on drugs, though *M. speciosa* is classified as a narcotic in several countries. A number of fatalities have been reported with *M. speciosa*, but this was likely due to consumption in combination with other substances.

Aminoindanes

Aminoindanes have been reviewed in Simao et al., (2022). Aminoindanes stimulate the central nervous system, mediating action of serotonin, noradrenaline and dopamine. Street names include “MDAI gold” or “Pink Champagnes.” In 2010, aminoindanes emerged in the UK’s psychoactive substance market as a new class of synthetic aminoindanes due to being alternatives to legislated compounds.

Synthetic aminoindanes are found as powders and crystals, and are consumed through ingestion, snorting, or rectal application. Desirable effects include improved social connectedness and mood, increased responsiveness, and emotional sensitivity. Undesirable effects include anxiety, depression, tachycardia. However, the lack of information on pharmacological effects makes legislation of these compounds challenging. Fatalities have predominantly been reported in cases related to MDAI, 5-Iodo-2-aminoindane (5-IAI), and 2-AI.

Other substances

As noted, this search predominantly highlighted NPS over substances that have been the longstanding in the drug market and patterns of use, including cocaine, diamorphine, methadone, and amphetamine-type stimulants such as methamphetamine or amphetamine, due to the inclusion of “psychoactive” in the search parameters. Regardless, polysubstance use remains a key factor in drug use deaths in Scotland. While some emerging synthetic drugs cause harm due

alone to their toxicities, longstanding psychoactive substances such as opioids, benzodiazepines, and cocaine remain the highest contributors to harms and drug use deaths, and it is often these substances which are adulterated. Cocaine is a naturally occurring alkaloid of *erythroxylum coca* plant and elicits strong stimulant effects. While its powdered hydrochloride form has legitimate, albeit limited, medical use as an anaesthetic, cocaine remains a Class A substance in the United Kingdom and a Schedule I substance according to the UNODC. "Crack" cocaine, or the free-base form, is often smoked due to its lower boiling point compared to the hydrochloride form. Acute cocaine toxicity can lead to a variety of symptoms, most notably tachycardia, hypertension, epistaxis and confusion, and more severely stroke, respiratory failure, and subsequent death. Scotland has seen a rise in cocaine overdoses with it being a contributing factor to 41% of drug-related deaths in 2023.

Amphetamine and methamphetamine, also central nervous system stimulants, are structurally similar synthetic substances and members of the phenethylamine family. As described above, effects of both boost confidence and energy by stimulating noradrenaline and dopamine systems in the short term, followed by restlessness, lethargy, anxiety and depression; acute overdose may lead to tachycardia and hypertension. While fatalities directly attributed to amphetamine use is rare, chronic use can lead to severe neurological deficits.

While not explicitly noted in many of the search results, diamorphine (heroin) and methadone remain critical contributors to drug use deaths in Scotland (Public Health Scotland, 2024). Polysubstance use continues to drive the majority of harms, with high-risk combinations frequently identified involve cocaine, gabapentinoids, benzodiazepines (notably diazepam and bromazolam) and opioids. At this point it is not possible to distinguish heroin and morphine post-mortem; however, opioids and/or opiates, including heroin, morphine, and/or methadone, were implicated in over 900 drug use deaths in 2023. Heroin is a crude preparation resulting from the acetylation of morphine with acetic anhydride. It can be smoked or injected, with production occurring worldwide. As an analgesic, it produces similar effects to other opioids with tolerance and dependence occurring on repeated use. An estimated 1.7 million people in the European Union receive substitution treatment for opioid dependence, with approximately 55% of those in treatment receiving methadone (EMCDDA, 2024); methadone treatment has reduced mortality rates compared to those who use opioids out of treatment (Sordo *et al.*, 2017).

Box 8 lists key points and a summary of findings related to these additional substances

Effects and risks

While the original intention was to rank substances according to the level of potential harm based on prevalence and toxicological effects, it rapidly became apparent that this task is extremely complex to undertake and, pertinently, the information is frequently unavailable or insufficient. Further, the nature of the analytical techniques emerging was such that the focus would emphasise substance groups rather than individual substances for subsequent tasks, which negated the requirement to compare and rank individual substances.

Table A2.1 summarises the main effects of the major drug classes of concern.

Box 8: Other substances key points

- Phenethylamines – 3rd most reported NPS group globally; related to MDMA, linked to polysubstance use fatalities
- Xylazine - veterinary drug, commonly added to opioids and other stimulants – no known safe dose for humans, intravenous administration
- Tryptamines – naturally occurring - no evidence of threat to life, can lead to dependence
- Plant based psychoactive substances - often hallucinogenic, some fatalities
- Aminoindanes – fatalities have been reported

Table A2.1. Drug classes and effects outlined from the literature search				
Drug Class	**Example Compound**	**Adverse Effects/Toxicity**	**Reference**	**Type of evidence**
Benzodiazepines	General	Drowsiness, dizziness, fatigue, dysarthria, loss of coordination, headache and amnesia. Long term use leads to tolerance and dependence Increased risk of death arising from high doses taken with CNS depressants (including opioids). Designer BZD (NPS) – stronger sedation than classical BZD	(Brunetti *et al.*, 2021)	Scoping Review on Designer Benzodiazepines based on 42 reports from emergency dept., driving under influence of drugs reports and postmortem reports.
Benzodiazepines	Etizolam	Common: drowsiness, muscle weakness, paradoxical excitation. Withdrawal symptoms include jerking, confusion, palpitations, impaired sleep, agitation, tremors. Also reported: Catatonia Therapeutic in some countries (not licensed in the UK), toxic and fatal blood-plasma concentrations of etizolam are around 8–18, 30 and 260 ng/mL, respectively, but there is overlap between them. Etizolam may be less lethal than other BZD requiring higher fatal doses. However, doses vary in counterfeit etizolam tabs (from 0.7-8.3 mg/tablet). Mild to moderate impairment of driving (may be worse in older subjects)	(Al Bahri and Hamnett, 2023)	Short Review (narrative) on Etizolam and its major metabolites.
Synthetic Cannabinoids	General	Negative mood, panic attacks, manic behaviour depression and suicidal ideation Severe psychotic symptoms including; agitation aggression, catatonia, paranoia, auditory and visual hallucinations, perceptual alterations, and persistent psychosis episode Long term: Chronic use may increase the risk for developing psychotic disorders Depression irritability and persistent anxiety Cognitive Acute: Severe cognitive impairments including memory alteration, attention difficulties, and amnesia Long term: Executive function deficits of working memory and attention Cardiovascular Acute: Tachycardia, hypertension, myocardial infraction, arrhythmias, chest pain, and palpitations Long term: Prolong use may increase risk of cardiovascular disease Neurologic Acute: Dizziness, somnolence, seizures, hypertonicity, hyperflexion, hyperextension, sensation changes, and fasciculations Long term: Preliminary evidence for structural and functional central nervous system alterations Gastro-intestinal Acute: Nausea, emesis, and appetite change	(Cohen and Weinstein, 2018)	Mini-review (narrative) on synthetic and non-synthetic cannabinoids

Table A2.1. Drug classes and effects outlined from the literature search				
Drug Class	**Example Compound**	**Adverse Effects/Toxicity**	**Reference**	**Type of evidence**
		Long term: Severe weight loss after prolonged use Other Acute kidney injury, abdominal pain, miosis, mydriasis, xerostomia, hyperthermia, fatigue, rhabdomyolysis, cough. deficits of driving ability Long term: kidney diseases, insomnia, nightmares, dependency, tolerance, and withdrawal		
Cannabis		Neuropsychiatric acute: Perceptual alterations including; hallucinations and distortion of spatial perception are typical edects. Paranoia, aggressiveness, and prolonged psychosis were observed in vulnerable users and are doserelated Anxiety and panic attacks; especially in naïve users Long-term An increased risk of psychotic disorders in vulnerable individuals and naïve users An increased risk for developing anxiety and mood disorders Cognitive Acute Wide range of dose-related cognitive deficits including; attention, working-memory, cognitive inhibition, and psychomotor function. Long-term Impairments of set-shifting, verbal learning, attention, short-term memory and psychomotor functions. Cardiovascular Acute An increase of cardiovascular activity, increase heart rate, and decrease blood pressure. Long-term An increased risk of cardiovascular disease after prolonged use. Neurologic Acute Dizziness, somnolence, and muscle tension Long-term Structural and functional abnormalities in a range of brain areas including the hippocampus and amygdala. Gastrointestinal Acute Hyperemesis, and increase appetite. Long-term Low body weight among regular users Other Acute Bronchodilation, impairments of driving ability	(Cohen and Weinstein, 2018)	Mini-review (narrative) on synthetic and non-synthetic cannabinoids

Table A2.1. Drug classes and effects outlined from the literature search				
Drug Class	**Example Compound**	**Adverse Effects/Toxicity**	**Reference**	**Type of evidence**
		Long-term Kidney diseases, insomnia, nightmares, dependency, tolerance, and withdrawal. An increased risk of obstructive lung disease including lung-cancer, an increased risk of cancers of the oral cavity, pharynx and oesophagus, cannabis addiction, tolerance, and withdrawal.		
Synthetic cathinones	General	Hypertension, palpitations, tachycardia, vasoconstriction, cardiac arrest, aggression, anxiety, paranoia, psychosis, confusion, psychomotor agitation, insomnia, impaired vision and speech, dilated pupils, acute kidney failure and renal failure, hyperthermia, acidosis, mydriasis rhabdomyolysis and seizures, death	(Simao *et al.*, 2022)	Review (narrative and update) on NPS in public health
Synthetic opioids	General	Alterations in muscle tone, chest wall rigidity, seizure-like activity, confusion, affective changes, cough suppression, orthostatic hypotension, urinary urgency or retention, folliculitis and dermatitis with hair loss, dry eyes, elevated liver enzymes and delayed bilateral hearing loss. The "opioid overdose triad" symptoms include miosis (pupil restriction), respiratory depression and decreased level of consciousness or coma. Vomiting during reduced consciousness can risk suffocation.	(Shafi *et al.*, 2022)	Review (narrative) and Clinical Update on synthetic opioids.
Phenethylamines	D series compounds	Tachycardia, seizures, hallucinations and kidney failure.	(Simao *et al.*, 2022) and (Lukic *et al.*, 2021)	Review (narrative and update) on NPS in public health Review (narrative) of NPS
Phenethylamines	NBOMe series compounds	Cardiovascular problems, seizures, metabolic acidosis, and organ failure.	(Simao *et al.*, 2022) and (Lukic *et al.*, 2021)	Review (narrative and update) on NPS in public health Review (narrative) of NPS
Phenethylamines	2C series compounds	Serotonin toxicity or sympathomimetic syndrome	(Simao *et al.*, 2022) and (Lukic *et al.*, 2021)	Review (narrative and update) on NPS in public health Review (narrative) of NPS
Plant-based psychoactive substances	*Catha edulis*, also known as khat	Psychosis, aggressive behaviour, irregular blood pressure, tachycardia, urine retention, constipation and insomnia.	(Simao *et al.*, 2022)	Review (narrative and update) on NPS in public health
Aminoindanes	General	Anxiety, depression tachycardia	(Simao *et al.*, 2022)	Review (narrative and update) on NPS in public health
Xylazine	General	Deterioration, psychiatric and neurological symptoms, respiratory depression, hyperglycaemia, and severe festering skin ulcers of the limbs with subcutaneous tissue necrosis and osteomyelitis	(Zawilska *et al.*, 2023)	Review (systematic) of non-fentanyl synthetic opioids

Appendix 3 – Benzodiazepines of interest

Adinazolam

- 1-(8-chloro-6-phenyl-4*H*-[1,2,4]triazolo[4,3-a][1,4]benzodiazepin-1-yl)-*N,N*-dimethylmethanamine
- Short-acting anxiolytic, antidepressant, anti-convulsant, sedative; amnestic and psycho-motor effects at higher doses
- Illegal designer drug (emerged 2015)
- US – 3 deaths in year 2020-21 -in combination with etizolam, fentanyl and flualprazolam, not quantified/listed as cause of death

Clonazolam

- 6-(2-Chlorophenyl)-1-methyl-8-nitro-4*H*-[1,2,4]triazolo[4,3-a][1,4]benzodiazepine
- Extremely powerful – strong sedation and amnesia at low doses
- Emerged 2014
- Main adverse effect – CNS depression

Deschloroetizolam

- 2-Ethyl-9-methyl-4-phenyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepine
- Short-acting
- Reported by UK Focal Point 2014
- Few data available
- Death of young male (France?) – in combination with other BZD

Diclazepam (Ro5-3448; 2-Chlorodiazepam)

- Emerged Germany 2013
- In the reports in this study – 1 death (flubromazolam, opioids, stimulants also present); 13 drivers impaired, four drivers not impaired, 1 patient admitted with anxiety but discharged same day – diclazepam and 2-aminoindane present.

Etizolam

- 4-(2-chlorophenyl)-2-ethyl-9-methyl-6*H*-thieno[3,2-*f*][1,2,4]triazolo[4,3-α][1,4]diazepine
- Short-acting; approved India, Italy, Japan and Korea short-term treatment of insomnia, anxiety and panic attacks
- Reported UK 2011
- 3 children – drowsy, wobbly (etizolam in one's urine)
- 1 patient unconscious (syringe of heroine with him – unclear if used)
- 3 patients needing detox after tolerance and withdrawal symptoms
- 6 DUID cases – motor and functional impairment; 1 driver unclear whether impaired
- 34 deaths reported; 33 – polydrug related.

Flualprazolam

- (8-chloro-6-(2-fluorophenyl)-1-methyl-4*H*-[1,2,4]triazolo[4,3-a][1,4]benzodiazepine
- Reported 2018 , Sweden
- Patients have exhibited sedation, verbal impairment, CNS depression, and been asymptomatic. 13 DUID cases reported – motor/functional skills impairment. 38 deaths reported – all multiple drugs, most listed as accidental o/d multiple drugs but 2 cases intentional flualprazolam poisonings. 28 further deaths where flualprazolam wasn't reported as cause of death (Finland, Sweden, US).
- An emerging NPS that may be encountered more often in the future (Zawilska and Wojcieszak, 2019).
- Flumenazil may be a safe paediatric antidote (Zawilska and Wojcieszak, 2019).

Flubromazepam

- 7-Bromo-5-(2-fluorophenyl)-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one
- Reported Germany 2013
- Patients have shown agitation and delirium, rigidity, CNS depression; 1 case DUID – mildly impaired, another driver, flubromazepam in blood, no impairment on his CTI. 1 fatal case – Flubromazolam and U-47700 listed as the cause of death.

Flubromazolam

- Reported Sweden 2014
- Strong and long-lasting depressive effect on the CNS
- 18 cases showed severe CNS depression/ functional/motor impairment. Flubromazolam only drug detected in 16/18 cases.
- 11 DUID cases – all showed motor & functional impairment & contributory to death – 4 cases
- Slow elimination

Meclonazapam

- Reported Sweden 2014; 1 ED admission – patient not lucid.
- Phenazepam and 3-Hydroxyphenazepam ("Bonsai", "Zannie" or "Supersleep)
- Long acting BZD
- Used in USSR as amylolytic, hypnotic & to treat alcohol withdrawal.
- Phenazepam reported Germany & UK 2011
- 3-Hydroxy reported Denmark 2916
- 4 patients admitted to ED – motor functional impairment/depressant
- Moderate – considerable motor and functional impairments were evident in 19 DUID drivers
- Slow elimination
- Case of 1 driver unimpaired
- 6 deaths – phenazepam alone cause of death in 2 cases, others polydrug

Pyrazolam

- Finland 2012
- 1 death – polydrug use

The EU market is dominated by clonazolam, diclazepam, etizolam, flualprazolam, flubromazolam and phenazepam. DBZD come in the form of blotters, liquids, pills, powders and tablets. They are sold at low prices and etizolam and phenazepam are illegally imported from countries where they are licensed. Both pharmacokinetics and the role of DBZD in deaths are poorly understood, yet they are linked to significant social harms such as criminal activity, violence, risk-taking behaviour, suicide attempts and concurrent substance use disorders. Clinicians are often unaware of DBZD and may attribute an incorrect cause of death. This is exacerbated by the fact polydrug use with DBZD is the rule rather than the exception.

Appendix 4 – Synthetic cannabinoids of interest

PNCs

Non-natural and synthetic cannabinoids are the largest drug class represented in the EU early warning system. Over two hundred substances were reported over a ten-year period up to 2019. The number of NNCs has grown and evolved to circumvent legislation and they have different pharmacokinetics as well as a different profile of metabolites making detection challenging. Pseudo-natural cannabinoids (PNCs) strongly resemble Δ^9-THC. For example, the regioisomer Δ^9-THC has comparable cannabimetric activity and is a controlled substance USA. The hydrogenated versions – (9R)-hexahydrocannabinol ((9R)- HHC) and (9S)-HHC evade legislation. There has been a shift in the market towards the acetylated version – HHC-acetate (AcO-HHC) and recently discovered PNCs include Δ9 -tetrahydrocannabutol (Δ9 -THCB), Δ9-tetrahydrocannabiphorol (Δ9-THCP) and Δ9-tetrahydrocannabihexol (Δ9-THCH) with THCP being sold as tinctures, gummies, distillates and vape cartridges. A hexahydrocannabiphorol (HHCP) has also appeared for online sales. Adverse effects are unknown due to the rapidity with which new formulations are introduced to market. Recreational effects include energising/creative/uplifting effects and increased cognitive function, mental clarity, spacey feeling, quick onset, accelerated heartrate and racing thoughts, sedation/calming, psychosis, tremors, more reactive to liver metabolism than TCH, longer or shorter lasting effects than THC, panic attacks, chronic depressive mood afterwards. The range and relatively sparse understanding of effects makes it difficult to rank these substances in terms of impact on users.

Semi synthetic pseudo-natural cannabinoids (SSPNCs)

These compounds can be found in nature but in small quantities so are chemically synthesised. Heath concerns arise around the potential for toxic contaminants and illegal concentrations Δ^9-THC in the substance.

They are sold as a range of product types including distillate cartridges and syringes, vape cartridges, tinctures, oils, sauces, waxes, concentrates, gummies and edibles, beverages, and flowers, disposable pens, dabbing syringes, chocolate bars, lollipops, pre-rolls.

- Δ^8-Tetrahydrocannabinol (Δ^8-THC) (6a*R*,10a*R*)-6,6,9-Trimethyl-3-pentyl-6a,7,10,10a-tetrahydrobenzo[*c*]chromen-1-ol. Effects are commonly reported as milder than Δ^9-THC.
- Δ^{10}-Tetrahydrocannabinol (Δ^{10}-THC)
- (6a*R*,9*R*)-6,6,6a,9-Tetramethyl-3-pentyl-6a,7,8,9-tetrahydro-6*H*-benzo[*c*]chromen-1-ol and(6a*R*,9*S*)-6,6,6a,9-tetramethyl-3-pentyl-6a,7,8,9-tetrahydro-6*H*-benzo[*c*]chromen-1-ol. Effects are commonly reported as similar to Δ8-THC but less potent.
- $\Delta^{6a,10a}$-Tetrahydrocannabinol ($\Delta^{6a,10a}$-THC)
- (*S*)-6,6,9-Trimethyl-3-pentyl-7,8,9,10-tetrahydro-6*H*-benzo[*c*]chromen-1-ol and (*R*)-6,6,9-trimethyl-3-pentyl-7,8,9,10-tetrahydro-6H-benzo[*c*]chromen-1-ol.The latter is reported as new on the market. Reported to have similar effects to Δ^{10}-THC therefore milder than Δ^9-THC.
- 10-Oxo-$\Delta^{6a,10a}$-Tetrahydrocannabinol (10-Oxo-$\Delta^{6a,10a}$-THC)
- (*R*)-1-Hydroxy-6,6,9-trimethyl-3-pentyl-8,9-dihydro-6H-benzo[*c*]chromen-10(7*H*)-one and (*S*)-1-hydroxy-6,6,9-trimethyl-3-pentyl-8,9-dihydro-6*H*-benzo[*c*]chromen-10(7*H*)-one. Not widely used recreationally. Advertised as having similar effects to Δ^9-THC.
- $\Delta^{9,11}$-Tetrahydrocannabinol ($\Delta^{9,11}$-THC)
- (6a*R*,10a*R*)-6a,7,8,9,10,10a-Hexahydro-6,6-dimethyl-9-methylene-3-pentyl-6*H*-dibenzo[*b,d*]pyran-1-ol. Reported to be less psychoactive than Δ^9-THC. Complex mixture of cannabinoids in product so unclear which the effects can be ascribed to.
- 11-Hydroxy-tetrahydrocannabinol (11-OH-THC)
- (6a*R*,10a*R*)-9-(Hydroxymethyl)-6,6-dimethyl-3-pentyl-6a,7,8,10a-tetrahydro-6*H*-benzo[*c*]chromen-1-ol. A metabolite arising following oral consumption or inhalation of Δ^9-THC or THC-containing products. Higher potency than Δ9-THC with rapid onset of effects. Complex mixture of cannabinoids as above. Frequently found to be fake.
- Hexahydrocannabinol (HHC)

- (6a*R*,9*R*,10a*R*)-6,6,9-trimethyl-3-pentyl-6a,7,8,9,10,10a-hexahydrobenzo[c]chromen-1-ol and (6a*R*,9*S*,10a*R*)-6,6,9-trimethyl-3-pentyl-6a,7,8,9,10,10a-hexahydrobenzo[*c*]chromen-1-ol. Uncertain composition. Freely sold online as a legal cheaper alternative to THC/cannabis. Concern over contamination due to synthetic pathway as with all commercialised semi-synthetics cannabinoids. Effects comparable to Δ^9-THC.
- 10-Hydroxy-hexahydrocannabinol (10-OH-HHC)
- (6a*R*,9*R*,10*S*,10a*R*)-6,6,9-Trimethyl-3-pentyl-6a,7,8,9,10,10a-hexahydro-6*H*-benzo[c]chromene-1,10-diol and (6a*R*,9*S*,10*R*,10a*R*)-6,6,9-trimethyl-3-pentyl-6a,7,8,9,10,10a-hexahydro-6*H*-benzo[*c*]chromene-1,10-diol. Effects similar to HHC
- Hexahydrocannabinolic acid (HHCA)
- (6a*R*,9*R*,10a*R*)-1-Hydroxy-6,6,9-trimethyl-3-pentyl-6a,7,8,9,10,10a-hexahydro-6H-benzo[*c*]chromene-2-carboxylic acid and (6a*R*,9*S*,10a*R*)-1-hydroxy-6,6,9-trimethyl-3-pentyl-6a,7,8,9,10,10a-hexahydro-6*H*-benzo[*c*]chromene-2-carboxylic acid. Limited information available – more readily metabolised in liver than Δ9-THC.

Synthetic Pseudo – Natural Cannabinoids (SPNCs)

SPNCs are sold as a range of products, those reported include tinctures, vape cartridges, gummies, cookies, sprayed herbs. They are entirely chemically synthesised because they are found in nature only in trace amounts. All have been discovered recently and include:

- Δ^9-Tetrahydrocannabutol (χ^9-THCB)
- (6a*R*,10a*R*)-3-Butyl-6,6,9-trimethyl-6a,7,8,10a-tetrahydro-6*H*-benzo[*c*]chromen-1-ol.
- Unclear whether pure or a mixture of Δ^9-THCB or Δ^8-THCB. Available as pure (or described as pure) isolate (for sale to other businesses) as well as usual formulations. Comparable or higher potency to Δ^9-THC, rapid onset, short duration of effects, tolerance to effects
- Δ^9-Tetrahydrocannabihexol (Δ^9-THCH)
- (6a*R*,10a*R*)-3-Hexyl-6,6,9-trimethyl-6a,7,8,10a-tetrahydro-6*H*-benzo[*c*]chromen-1-ol.
- No/little data on psychoactive effects Δ^9-THCH may be the only active compound or it may be a mixture with other cannabinoids. Psychoactive effect often reported to last longer than Δ^9-THC
- Δ^9-Tetrahydrocannabiphorol (Δ^9-THCP)
- (6a*R*,10a*R*)-3-Heptyl-6,6,9-trimethyl-6a,7,8,10a-tetrahydro-6*H*-benzo[c]chromen-1-ol.
- Similar cannabimetric activity to Δ^9-THC (based on mouse studies). Effects thought to last up to 24 hours but unclear which active compound this relates to.
- Hexahydrocannabutol (HHCB)
- (6a*R*,9*R*,10a*R*)-3-Butyl-6,6,9-trimethyl-6a,7,8,9,10,10a-hexahydro-6*H*-benzo[*c*]chromen-1-ol and (6a*R*,9*S*,10a*R*)-3-Butyl-6,6,9-trimethyl-6a,7,8,9,10,10a-hexahydro-6H-benzo[*c*]chromen-1-ol.
- Reports suggest rapid onset of effects, rapid decline in effects, fewer bad side effects the following day
- Hexahydrocannabihexol (HHCH)
- (6a*R*,9*R*,10a*R*)-3-Hexyl-6,6,9-trimethyl-6a,7,8,9,10,10a-hexahydro-6H-benzo[*c*]chromen-1-ol and (6aR,9S,10aR)-3-Hexyl-6,6,9-trimethyl-6a,7,8,9,10,10a-hexahydro-6*H*-benzo[*c*]chromen-1-ol.
- Reported high psychoactive effects
- Hexahydrocannabiphorol (HHCP)
- (6a*R*,9*R*,10a*R*)-3-Heptyl-6,6,9-trimethyl-6a,7,8,9,10,10a-hexahydro-6*H*-benzo[*c*]chromen-1-ol and (6a*R*,9*S*,10a*R*)-3-Heptyl-6,6,9-trimethyl-6a,7,8,9,10,10a-hexahydro-6*H*-benzo[*c*]chromen-1-ol.
- Limited information on effects, but some intense effects reported (uplifting) – often not a mixture of cannabinoids.

Non-natural cannabinoids

Semi synthetic non-natural (SSNNCs)

These substances are based on Δ^9-THC or its legal alternative CBD as precursors but are non-natural compounds. Those identified include:

- Tetrahydrocannabinol acetate (THC-O)
- (6a*R*,10a*R*)-6,6,9-Trimethyl-3-pentyl-6a,7,8,10a-tetrahydro-6H-benzo[*c*]chromen-1-yl acetate. Potency depends on whether it is based on Δ^9-THC or Δ^8-THC. Vaping has been linked with EVALI. May have psychedelic effects, no effects, typical THC-like effects. Composition of the product varies – may explain mixed effects
- Hexahydrocannabinol acetate (HHC-O)

- (6a*R*,9*R*,10a*R*)-6,6,9-Trimethyl-3-pentyl-6a,7,8,9,10,10a-hexahydro-6H-benzo[*c*]chromen-1-yl acetate and (6a*R*,9*S*,10a*R*)-6,6,9-Trimethyl-3-pentyl-6a,7,8,9,10,10a-hexahydro-6H-benzo[*c*]chromen-1-yl acetate. Inhalation may cause EVALI. May be more potent than HHC. Effects include sedation/relaxation and relief of anxiety, depression, sleep improvements, a sense of bliss and openness. Negative effects include panic attacks, chronic depressive mood (for several weeks).
- 8-Hydroxy-hexahydrocannabinol (8-OH-HHC)
- (6a*R*,8*R*,9*S*,10a*R*)-6,6,9-Trimethyl-3-pentyl-6a,7,8,9,10,10a-hexahydro-6*H*-benzo[c]chromene-1,8-diol *and* (6a*R*,8*S*,9*R*,10a*R*)-6,6,9-Trimethyl-3-pentyl-6a,7,8,9,10,10a-hexahydro-6H-benzo[*c*]chromene-1,8-diol. Quicker onset of effects compared to HHC but similar physical and mental experience.

Synthetic non-natural cannabinoids (SNNCs)

These substances are not found in nature and can't be synthesised from a natural product. They include:

HU-210

(6a*R*,10a*R*)-9-(Hydroxymethyl)-6,6-dimethyl-3-(2-methyloctan-2-yl)-6a,7,10,10a-tetrahydro-6H-benzo[*c*]chromen-1-ol. First seized in USA 2009. Found in K2, Spice, Spice gold/silver/diamond, Bliss Black Mamba", "Bombay Blue", "Fake Weed", "Genie," and "Yucatan Fire with HU-211. Significantly higher potency than Δ^9-THC. Animal studies indicate marked sedation, both depressant and stimulatory effects. Cannabimetric activity occurred at lower dose than with Δ^9-THC – studies suggested heightened emotional states/fear occur. 100-500 x more potential to induce analgesia/hypothermia in rats than Δ^9-THC. Often present in "Spice" which is a psychoactive herbal mix commonly smoked. Users described a higher potency and safety than Δ^9-THC – lower dose needed. High doses were linked to dissociative/psychedelic effects, medium doses with analgesia. Effects described as long lasting.

The stereoisomer HU-211 is also mentioned.

Appendix 5 – Novel synthetic cathinones detected 2019-2022

As reviewed by Kuropka, Zawadzki and Szpot (2023)

1. N-Butylhexedrone (Molecular Weight (MW): 247.4)
2. N-Butylpentylone (MW: 277.4)
3. N-Ethylheptedrone (MW: 233.3)
4. 4-Et-α-PVP (MW: 259.4)
5. N,N-Diethylhexedrone (MW: 247.4)
6. α-PCYP (MW: 271.4)
7. Isohexedrone (MW: 205.3)
8. Hexylone (MW: 249.3)
9. Isohexylone (MW: 205.3)
10. N,N-Diethylpentylone (MW: 277.4)
11. 4-Methylehexedrone (MW: 219.3)
12. 3F-α-PiHP (MW: 263.4)
13. MDPV8 (MW: 303.4)
14. N-Ethylheptylone (MW: 277.4)
15. 3F-α-PHP (MW: 263.4)
16. MFPVP (MW: 263.4)
17. MDPiHP (MW: 289.4)
18. 3F-N-ethylhexedrone (MW: 237.3)
19. α-D2PV (MW: 265.3)
20. α-PipBP (MW: 231.3)
21. MFPHP (MW: 277.4)
22. 2-Me-α-PVP (MW: 231.3)
23. 3F-NEB (MW: 209.3)
24. 3-Methyl-N-propylcathinone (MW: 205.3)
25. Dipentylone (MW: 249.3)
26. 4Cl-3-MMC (MW: 211.7)
27. N-Methyl-N-cyclohexylmethylone (MW: 275.3)
28. N-Cyclohexylmethylone (MW: 275.3)
29. N-Cyclohexylbutylone (MW:289.4)

Appendix 6 – Evidence mapping search terms

The final search terms applied in Web of Scholar were as follows:

"(**wastewater** and **analy***) and (**cocaine** or **amphetamine** or **opioid** or **ketamine** or **cannab*** or **benzodiazepine** or **gabapentin** or **pregabalin** or **methadone** or **buprenorphine** or **nitazene**)"

Document type: **Any**
Years selected: **2020, 2021, 2022, 2023, 2024**
No geographical constraints were applied.

This search yielded **251** results which were imported into Mendeley for review. All abstracts were scanned. Articles related to the following were excluded from further consideration:

- Mining
- Heavy metal pollution
- Water treatment processes
- Airborne detection
- Degradation processes
- Ecological effects
- Population predictions
- Papers not in English

The refined list contained **86** results.

A second refinement was conducted and articles pertaining to the following were excluded from this aspect of the review but saved for future consideration:

- Duplicate analytical techniques for the same target substance(s)
- Novel experimental techniques not in mainstream, peer-reviewed use (e.g. nano-particles)
- Case studies of single-event festival or holiday drug use

Appendix 7 – Evidence mapping details

Appendix 7 is a separate excel spreadsheet which can be found on the CREW publication page.

Appendix 8 – Early Warning System literature search

"[Early warning system]'" and "drug"

Document type: **Any**
Years selected: **2020, 2021, 2022, 2023, 2024**
No geographical constraints were applied.

This search yielded **166** results which were imported into Mendeley for review. All abstracts were scanned. Articles related to the following were excluded from further consideration:

- Pandemic/virology monitoring
- Vaccine vigilance systems
- Evolution of synthetic cannabinoids
- Cardiovascular function and effects
- Cannabinoid effects in animal models
- *In vivo* and *in vitro* toxicological effects
- Sentiment regarding drug use
- Adulterants
- Quality assurance testing
- Genotoxicity
- NPS nomenclature systems
- Artificial intelligence in healthcare delivery

References

Acosta, T., Chavez, V., Fernandez, N., Perry, E., Good, K. and Concheiro, M. (2024). The Impact of Combined Sewer Overflows on Pharmaceutical and Illicit Drug Levels in New York/New Jersey Waterways. *Environmental toxicology and chemistry*.

Adhikari, S., Kumar, R., Driver, E. M., Bowes, D. A., Ng, K. T., Sosa-Hernandez, J. E., Oyervides-Munoz, M. A., Melchor-Martinez, E. M., Martinez-Ruiz, M., Coronado-Apodaca, K. G., Smith, T., Bhatnagar, A., Piper, B. J., McCall, K. L., Parra-Saldivar, R., Barron, L. P. and Halden, R. U. (2023). Occurrence of Z-drugs, benzodiazepines, and ketamine in wastewater in the United States and Mexico during the Covid-19 pandemic. *Science of the total environment*, pp. 857.

Al Bahri, A. A. and Hamnett, H. J. (2023). Etizolam and Its Major Metabolites: A Short Review. *Journal of analytical toxicology*, 47, 216-226.

Artigiani, E. E. and Wish, E. D. (2020). Introducing the National Drug Early Warning System. *Current opinion in psychiatry*, 33, pp. 319-325.

Ayub, S., Parnia, S., Poddar, K., Bachu, A. K., Sullivan, A., Khan, A. M., Ahmed, S. and Jain, L. (2023). Xylazine in the Opioid Epidemic: A Systematic Review of Case Reports and Clinical Implications. *Cureus*.

Bade, R., Abdelaziz, A., Nguyen, L., Pandopulos, A. J., White, J. M. and Gerber, C. (2020a). Determination of 21 synthetic cathinones, phenethylamines, amphetamines and opioids in influent wastewater using liquid chromatography coupled to tandem mass spectrometry. *Talanta*, 208.

Bade, R., Eaglesham, G., Shimko, K. M. and Mueller, J. (2023). Quantification of new psychoactive substances in Australian wastewater utilising direct injection liquid chromatography coupled to tandem mass spectrometry. *Talanta*, 251.

Bade, R., Ghetia, M., White, J. M. and Gerber, C. (2020b). Determination of prescribed and designer benzodiazepines and metabolites in influent wastewater. *Analytical methods*, 12, pp. 3637-3644.

Bade, R., Nadarajan, D., Driver, E. M., Halden, R. U., Gerber, C., Krotulski, A., Hall, W. and Mueller, J. F. (2024a). Wastewater-based monitoring of the nitazene analogues: First detection of protonitazene in wastewater. *Science of the total environment*, 920.

Bade, R., van Herwerden, D., Rousis, N., Adhikari, S., Allen, D., Baduel, C., Bijlsma, L., Boogaerts, T., Burgard, D., Chappell, A., Driver, E. M., Sodre, F. F., Fatta-Kassinos, D., Gracia-Lor, E., Gracia-Marín, E., Halden, R. U., Heath, E., Jaunay, E., Krotulski, A., Lai, F. Y., Löve, A. S. C., O'Brien, J. W., Oh, J. E., Pasin, D., Castro, M. P., Psichoudaki, M., Salgueiro-Gonzalez, N., Gomes, C. S., Subedi, B., Thomas, K. V., Thomaidis, N., Wang, D. G., Yargeau, V., Samanipour, S. and Mueller, J. (2024b). Workflow to facilitate the detection of new psychoactive substances and drugs of abuse in influent urban wastewater. *Journal of hazardous materials*, 469.

Bijlsma, L., Simpson, B., Gerber, C., van Nuijs, A. L. N. and Burgard, D. (2024). Making waves: Wastewater-based surveillance of cannabis use. *Water research*, 255.

Boden, M. and Day, E. (2023). Illicit drug use in university students in the UK and Ireland: a PRISMA-guided scoping review. *Substance abuse treatment prevention and policy*, 18.

Boogaerts, T., Quireyns, M., Covaci, A., De Loof, H. and van Nuijs, A. L. N. (2021). Analytical method for the simultaneous determination of a broad range of opioids in influent wastewater: Optimization, validation and applicability to monitor consumption patterns. *Talanta*, 232.

Brunetti, P., Giorgetti, R., Tagliabracci, A., Huestis, M. A. and Busardo, F. P. (2021). Designer Benzodiazepines: A Review of Toxicology and Public Health Risks. *Pharmaceuticals*, 14.

Camilleri, A., Alfred, S., Gerber, C., Lymb, S., Painter, B., Rathjen, A. and Stockham, P. (2021). Delivering harm reduction to the community and frontline medical practitioners through the South Australian Drug Early Warning System (SADEWS). *Forensic science medicine and pathology*, 17, pp. 388-394.

Campo, J., Vitale, D., Sadutto, D., Vera-Herrera, L. and Picó, Y. (2023). Estimation of legal and illegal drugs consumption in Valencia City (Spain): 10 years of monitoring. *Water research*, 240.

Caprari, C., Ferri, E., Vandelli, M. A., Citti, C. and Cannazza, G. (2024). An emerging trend in Novel Psychoactive Substances (NPSs): designer THC. *Journal of cannabis research*, 6.

Castiglioni, S., Bijlsma, L., Covaci, A., Emke, E., Hernández, F., Reid, M., Ort, C., Thomas, K. V., Van Nuijs, A. L. N., De Voogt, P. and Zuccato, E. (2013). Evaluation of uncertainties associated with the determination of community drug use through the measurement of sewage drug biomarkers. *Environmental Science and Technology*, 47, pp. 1452-1460.

Chen, J. H., Zang, Y. S., Chen, M. Y., Shi, C., Zhang, M. L., Di, B. and Jiang, C. (2023). Sensitive determination of 11-nor-9-carboxy-Δ9-tetrahydrocannabinol in wastewater by solid-phase extraction with pre-column derivatization and liquid chromatography-tandem mass spectrometry. *Analytical methods*, 15, pp. 2693-2699.

Cohen, K. and Weinstein, A. M. (2018). Synthetic and Non-synthetic Cannabinoid Drugs and Their Adverse Effects-A Review From Public Health Prospective. *Frontiers in Public Health*.

de Morais, J., Evans-Brown, M., Gallegos, A., Christie, R., Jorge, R., Planchuelo, G., Berkowicz, K. and Sedefov, R. (2024). Update from the EU Early Warning System on new psychoactive substances. *Clinical toxicology*, 62, pp. 3-3.

de Morais, J., Evans-Brown, M., Gallegos, A., Christie, R., Jorge, R., Planchuelo, G. and Sedefov, R. (2023). The EU early warning system on new psychoactive substances: 25 years of early warning and response in Europe. *Clinical toxicology*, 61, pp. 42-43.

de Oliveira, A. F. B., Vieira, A. D. and Santos, J. M. (2023a). Trends and challenges in analytical chemistry for multi-analysis of illicit drugs employing wastewater-based epidemiology. *Analytical and bioanalytical chemistry*, 415, pp. 3749-3758.

de Oliveira, M. C., Vides, M. C., Lassi, D. L. S., Torales, J., Ventriglio, A., Bombana, H. S., Leyton, V., Perico, C. d. A.-M., Negrao, A. B., Malbergier, A. and Castaldelli-Maia, J. M. (2023b). Toxicity of Synthetic Cannabinoids in K2/Spice: A Systematic Review. *Brain sciences*, 13.

El-Menyar, A., Mekkodathil, A., Al-Thani, H. and Al-Motarreb, A. (2015). Khat use: History and heart failure. *Oman Medical Journal*, 30.

Ferrari Jr, E., Leite, B. H. M., Gomes, E. B., Vieira, T. M., Sepulveda, P. and Caldas, E. D. (2022). Fatal cases involving new psychoactive substances and trends in analytical techniques. *Frontiers in toxicology*, 4.

Fitzgerald, N. D., Cottler, L. B. and Palamar, J. J. (2024). Public health surveillance of new psychoactive substances: recent developments. *Current opinion in psychiatry*, 37, pp. 270-276.

Frankenfeld, F., Wagmann, L., Jacobs, C. M. and Meyer, M. R. (2023). Quantitative analysis of drugs of abuse and cognitive enhancers in influent wastewater by means of two chromatographic methods. *Drug testing and analysis*.

Gautam, L., Sharratt, S. D. and Cole, M. D. (2014). Drug facilitated sexual assault: Detection and stability of benzodiazepines in spiked drinks using gas chromatography-mass spectrometry. *PLoS One*, 9.

Gent, L. and Paul, R. (2021). The detection of new psychoactive substances in wastewater. A comprehensive review of analytical approaches and global trends. *Science of the total environment*, 776.

Gracia-Marín, E., Hernández, F., Ibáñez, M. and Bijlsma, L. (2024). Dilute-and-shoot approach for the high-throughput LC-MS/MS determination of illicit drugs in the field of wastewater-based epidemiology. *Water research*, 259.

Grafinger, K. E., Bernhard, W. and Weinmann, W. (2019). Scheduling of new psychoactive substance the Swiss way: A review and critical analysis. *Science and Justice*.

Graziano, S., Zaami, S., Tittarelli, R., Pichini, S., Sciotti, M., Berretta, P., Busardo, F. P., Marinelli, E., Tini, A., Scaravaggi, G., Buscaglia, E., Brambilla, E., Cavallo, M., Locatelli, C. A., Pacifici, R. and Varí, M. R. (2021). The complex and constantly evolving public health threat of new psychoactive substances in Italy: addressing the main functions of a national observatory of drugs. *Annali dell istituto superiore di sanita*, 57, pp. 144-150.

Grp, E. P. R., Syrjanen, R., Schumann, J. L., Lyons, T., McKinnon, G., Hodgson, S. E., Abouchedid, R., Gerostamoulos, D., Koutsogiannis, Z., Fitzgerald, J. and Greene, S. L. (2023). A risk-based approach to community illicit drug toxicosurveillance: operationalisation of the Emerging Drugs Network of Australia - Victoria (EDNAV) project. *International journal of drug policy*, 122.

Public Health Scotland. (2024a). Estimated Prevalence of Opioid Dependence in Scotland.

Public Health Scotland. (2024b). Evaluating the public health utility of wastewater-based surveillance of SARS-CoV-2 in Scotland: Technical Report A Management information release for Scotland.

Hehet, P., Koeke, N., Zahn, D., Froemel, T., Roessler, T., Knepper, T. P. and Puetz, M. (2021). Synthetic cannabinoid receptor agonists and their human metabolites in sewage water: Stability assessment and identification of transformation products. *Drug testing and analysis*, 13, pp. 1758-1767.

Hernández, F., Castiglioni, S., Covaci, A., de Voogt, P., Emke, E., Kasprzyk-Hordern, B., Ort, C., Reid, M., Sancho, J. V., Thomas, K. V., van Nuijs, A. L. N., Zuccato, E. and Bijlsma, L. (2018). Mass spectrometric strategies for the investigation of biomarkers of illicit drug use in wastewater. *Mass Spectrometry Reviews*. John Wiley and Sons Inc.

Huizer, M., ter Laak, T. L., de Voogt, P. and van Wezel, A. P. (2021). Wastewater-based epidemiology for illicit drugs: A critical review on global data. *Water research*, 207.

Jaunay, E. L., Simpson, B. S., White, J. M. and Gerber, C. (2023). Using wastewater-based epidemiology to evaluate the relative scale of use of opioids. *Science of the total environment*, 897.

Katz, D. L., Williams, A. L., Girard, C., Goodman, J., Comerford, B., Behrman, A. and Bracken, M. B. (2003). The evidence base for complementary and alternative medicine: Methods of evidence mapping with application to CAM. *Alternative Therapies in Health and Medicine*, 9.

Khangura, S., Konnyu, K., Cushman, R., Grimshaw, J. and Moher, D. (2012). Evidence summaries: The evolution of a rapid review approach. *Systematic Reviews*, 1.

Kuropka, P., Zawadzki, M. and Szpot, P. (2023). A review of synthetic cathinones emerging in recent years (2019-2022). *Forensic toxicology*, 41, pp. 25-46.

Lukic, V., Micic, R., Arsic, B., Nedovic, B. and Radosavljevic, Z. (2021). Overview of the major classes of new psychoactive substances, psychoactive effects, analytical determination and conformational analysis of selected illegal drugs. *Open chemistry*, 19, pp. 60-106.

Maghsoudi, N., Tanguay, J., Scarfone, K., Rammohan, I., Ziegler, C., Werb, D. and Scheim, A. (2022). Drug checking services for people who use drugs: a systematic review. *Addiction*, 117, pp. 532-544.

O'Rourke, C. E. and Subedi, B. (2020). Occurrence and Mass Loading of Synthetic Opioids, Synthetic Cathinones, and Synthetic Cannabinoids in Wastewater Treatment Plants in Four US Communities. *Environmental science & technology*, 54, pp. 6661-6670.

Salgueiro-Gonzalez, N., Béen, F., Bijlsma, L., Boogaerts, T., Covaci, A., Baz-Lomba, J. A., Kasprzyk-Hordern, B., Matias, J., Ort, C., Bodik, I., Heath, E., Styszko, K., Emke, E., Hernández, F., van Nuijs, A. L. N. and Castiglioni, S. (2024). Influent wastewater analysis to investigate emerging trends of new psychoactive substances use in Europe. *Water research*, 254.

Salgueiro-González, N., Castiglioni, S., Gracia-Lor, E., Bijlsma, L., Celma, A., Bagnati, R., Hernández, F. and Zuccato, E. (2019). Flexible high resolution-mass spectrometry approach for screening new psychoactive substances in urban wastewater. *Science of the Total Environment*, 689.

Saran, A. and White, H. (2018). Evidence and gap maps: a comparison of different approaches. *Campbell Systematic Reviews*, 14.

Shafi, A., Berry, A. J., Sumnall, H., Wood, D. M. and Tracy, D. K. (2022). Synthetic opioids: a review and clinical update. *Therapeutic advances in psychopharmacology*, 12.

Simao, A. Y., Antunes, M., Cabral, E., Oliveira, P., Rosendo, L. M., Brinca, A. T., Alves, E., Marques, H., Rosado, T., Passarinha, L. A., Andraus, M., Barroso, M. and Gallardo, E. (2022). An Update on the Implications of New Psychoactive Substances in Public Health. *International journal of environmental research and public health*, 19.

Sims, N. and Kasprzyk-Hordern, B. (2020). Future perspectives of wastewater-based epidemiology: Monitoring infectious disease spread and resistance to the community level. *Environment international*, 139.

Sordo, L., Barrio, G., Bravo, M. J., Indave, B. I., Degenhardt, L., Wiessing, L., Ferri, M. and Pastor-Barriuso, R. (2017). Mortality risk during and after opioid substitution treatment: systematic review and meta-analysis of cohort studies. *BMJ (Clinical research ed.)*, 357.

Steenbeek, R., Emke, E., Vughs, D., Matias, J., Boogaerts, T., Castiglioni, S., Campos-Mañas, M., Covaci, A., de Voogt, P., ter Laak, T., Hernández, F., Salgueiro-González, N., Meijer, W. G., Dias, M. J., Simoes, S., van Nuijs, A. L. N., Bijlsma, L. and Béen, F. (2022). Spatial and temporal assessment of crack cocaine use in 13 European cities through wastewater-based epidemiology. *Science of the total environment*, 847.

Syrjanen, R., Schumann, J., Fitzgerald, J., Gerostamoulos, D., Abouchedid, R., Rotella, J. A., Knott, J., Maplesden, J., Hollerer, H., Hannon, L., Bourke, E., Hodgson, S. E. and Greene, S. L. (2023). The Emerging Drugs Network of Australia - Victoria Clinical Registry: A state-wide illicit substance surveillance and alert network. *Emergency medicine Australasia*, 35, pp. 82-88.

Verovsek, T., Krizman-Matasic, I., Heath, D. and Heath, E. (2020). Site- and event-specific wastewater-based epidemiology: Current status and future perspectives. *Trends in environmental analytical chemistry*, 28.

Zarei, S., Salimi, Y., Repo, E., Daglioglu, N., Safaei, Z., Güzel, E. and Asadi, A. (2020). A global systematic review and meta-analysis on illicit drug consumption rate through wastewater-based epidemiology. *Environmental science and pollution research*, 27, pp. 36037-36051.

Zawilska, J. B., Adamowicz, P., Kurpeta, M. and Wojcieszak, J. (2023). Non-fentanyl new synthetic opioids-An update. *Forensic science international*, 349.

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