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Wastewater analysis for psychoactive substances at music festivals across New South Wales, Australia in 2019–2020

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ABSTRACT

Introduction: Implementation of wastewater surveillance at music festivals has been limited to date. We aimed to use wastewater analysis and a self-report survey to determine the range of psychoactive substances being used during a music festival season in New South Wales, Australia.

Methods: We sampled six single-day music festivals requiring a music festival license in New South Wales from March 2019 to March 2020; between 15% and 100% of portaloos (temporary, un-fixed toilet facilities) were sampled at each festival. Samples were screened for 98 psychoactive substances and/or their metabolites with results qualitatively expressed as detection frequencies for each substance at each festival and across all festivals. We compared these data with the results of surveys of self-reported drug use at four of the six festivals.

Results: Festival attendance ranged from 6200 to 14,975 people. Amphetamine, cocaine, ketamine, methylone, MDMA, MDA, alprazolam, diazepam, etizolam, oxazepam and temazepam were found in almost all samples from all festivals. Ethylone, mephedrone and methcathinone were also found in over 50% of festivals. A norfentanyl (a fentanyl metabolite) and n-ethylpentylone were found at 2/6 and 1/6 festivals. No festival survey participant reported intentionally taking cathinones.

Discussion: The detection frequency for cathinones was higher than expected relative to recent other data sources and this may represent adulteration or substitution. Similarly, the appearance of etizolam may be related to the use of counterfeit alprazolam. The detection of highly toxic substances such as N-ethylpentylone and norfentanyl may warrant public health alerts.

Conclusion: If provided close to real time, wastewater analysis at festivals could be complemented with information sources such as drug checking, on-site surveys, medical presentations and intelligence from peer networks to feed into early warning systems, public health alerts and peer-based harm reduction education during the festival season.

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Introduction

The use of wastewater surveillance for monitoring prevalence and trends in illicit drug use is becoming well established and part of routine practice across a number of countries [1–5]. Wastewater surveillance has multiple applications, including longitudinal surveillance of relative volumes of drug use in geographically defined areas of wastewater collection, and for signal detection of novel psychoactive substances (NPS) that may be of high or unknown clinical toxicity [6]. The most widespread site of wastewater analysis to date has been at wastewater treatment plants. However, there is emerging interest in wastewater surveillance at sites that represent a higher prevalence of drug consumption relative to the general population and increased risk of toxicity, such as music festivals [7–12].

There is utility in tracking the range of psychoactive substances consumed at festivals and particularly in signal detection for NPS which can be translated into public health warnings and local harm reduction advice. However, there are few contemporary data in the festival setting, most of which are based on surveys and some of which are combined with individual-level drug or biological sample testing [8,9,13]. These methods rely on self-report and limited sampling strategies, usually capturing less than 5% of festival attendees [9]. While NPS may be intentionally consumed due to cost, availability or curiosity [14,15], adulteration or substitution (mis-selling) [16] of conventional illicit drugs such as MDMA and cocaine with other NPS has also been reported [13,17]. Combinations of stimulants or lack of awareness of drug constituents can lead to significant and unexpected

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Supplemental data for this article can be accessed here.

toxicity. As a direct measurement of a larger sample of festival attendees, wastewater surveillance can overcome some of these limitations. However, the optimal approach to wastewater sampling at festivals and translation into useful public health and harm reduction messaging remains unclear.

Past efforts at wastewater sampling at festivals have used a range of sampling strategies, from sampling individual urinals or "portaloos" (temporary, un-fixed toilet facilities), to sampling from pooled wastewater collection tanks [10,12,18]. Analytical approaches have also varied from the use of targeted mass spectroscopy (MS) based techniques with standards, but may also include MS techniques cross-referenced with spectrographic libraries [19]. There have also been a number of approaches to expressing results, including detection frequencies (i.e., number of positive samples) to efforts at quantification of compounds in wastewater by back calculation based on known excretion rates and population size estimates [20].

The aim of this study was to determine the range of psychoactive substances being used during a festival season between March 2019 and March 2020 across New South Wales, Australia by qualitative analysis of wastewater at music festivals compared with self-reported drug use from an on-site festival survey.

Methods

NSW music festivals were preferentially selected on the basis of requiring an approved Safety Management Plan under the 2019 Music Festivals Act [21]. Requiring a Safety Management Plan indicates that the festival was deemed higher-risk of drug-related harm by the Independent Liquor and Gaming Authority [22]. Permission was obtained from event organisers as well as logistics companies responsible for sanitation prior to each event. Festivals were recruited between March 2019 and March 2020. We recorded the number of attendees and duration of each festival as well as the predominant music genre and the utilisation of medical services during the festival.

Wastewater sampling

Signs were posted on all toilets that could be sampled alerting patrons that sampling would be occurring with information pointing participants to a Facebook page that was created to provide further information. A pragmatic convenience sampling approach was taken to sample wastewater at each festival. Approximately 50 mL of wastewater was collected using a pipette either directly from individual portaloos or from centralized tanks with efforts to avoid collection of solid materials at the close of the festival. Attempts were made to either sample all individual portaloos or pooled collecting tanks at the festival. If this was not feasible because of the number of portaloos and time window available for sampling relative to study personnel, a selection of portaloos and pooled tanks were sampled; for instance, every second or third portaloo and/or samples from two to five individual portaloos were pooled.

We recorded the total number of portaloos and centralised tanks at each festival; the number sampled; their location within the festival; the identity of any chemical antiseptic present; and, where applicable, whether they were intended for male or female use. We also recorded whether other toilets were present that could not be sampled; for instance, fixed flushing toilets draining wastewater directly into the sewage system.

Analytical methods

Samples were stored at −20 °C immediately after collection and up to the day of analysis. These samples were treated with two different analytical methods. A 1 mL volume of portaloo sample was diluted 100-fold prior to Solid-Phase Extraction (SPE) based on a method developed for wastewater [23,24], except for ketamine which were quantitated using a direct injection method. SPE was used to remove potential interferences and to recover the target compounds. These samples were then injected onto an Ultra High-Performance Liquid Chromatography (UPLC) Exion system to achieve the separation of compounds and detected using a triple quadrupole mass spectrometer (Sciex 6500 plus). These analytical methods had the capacity to detect up to 98 psychoactive substances and/or their metabolites (Supplementary Table). The substances and metabolites tested for were based on impurities commonly found in police seizures [17] as well as common drugs associated with festivals, identified in identified in UN Office on Drugs and Crime [25] and European Monitoring Centre for Drugs and Drug Addiction [26] but was practically restricted to those currently available due to analytical constraints.

Detection frequencies

Qualitative results were obtained for each sample analysed, indicating the presence or absence of compounds detected. Where possible, results were stratified by toilet gender.

Festival detection frequency for each substance was calculated as number of positive samples for the given substance at a given festival as a proportion of total number of samples taken at that festival.

Study detection frequency for each substance was calculated as the number of festivals in which that substance was detected divided by the total number of festivals sampled.

Festival survey

At four of the festivals in which wastewater collection was performed, festivalgoers were also approached to self-complete an anonymous survey on prior and intended drug use. This survey was performed independently of wastewater collection and so does not necessarily reflect festivalgoers using portaloos sampled. Methods for this survey are reported elsewhere (Healey et al., "Correlates of higher risk drug-related behaviours at music festivals in New South

Wales, Australia" 2021 under review). Of note for the present analysis, not all psychoactive substances tested for in the present wastewater analysis were directly asked about in the survey, but respondents were asked if they had used or intended to use any other psychoactive substances not listed. A free text box was provided to record any other drugs taken/planned. For each festival, we compared self-reported drug use from survey data with wastewater analyses by reporting the number of people surveyed using each drug as a proportion of all people surveyed.

Ethics approval for this study was granted by The University of Sydney Human Research Ethics Committee (ref: 2019/091).

Results

Between March 2019 and March 2020, we performed wastewater collections at five different festivals; collections were performed at one of these festivals on two consecutive years (2019 and 2020). Festivals varied in size, ranging from 6200 to 14,975 attendees (total 66,939, median 10,880). All festivals sampled were from within the Greater Sydney Area, all required a festival license, electronic dance music was the predominant music genre at all festivals and all were single-day events. On-site surveys were performed at four of these six festivals; between 144 and 387 attendees were surveyed at each festival (total 948, median 209). Of those surveyed, 42.8% aged 18 to 20 years, 41.6% 21–25 years and 15.6% were 26 years or older, 49.5% were female, 46.6% were male and 3.9% identified as "other" gender, 79.2% identified as heterosexual and 20.8% identified as a sexuality other than heterosexual. Presentations for medical assistance varied from 40 to 200 per 10,000 attendees (total 656 attendees across all festivals, median 100/10,000) and transports to hospital from 2 to 7 per 10,000 attendees (total 31 attendees across all festivals, median 5/10,000).

For all but one of the festivals, individual portaloos were sampled, for the remaining, samples were taken from a central collecting tank. Where portaloos were available, between 15% and 100% of those at each festival were sampled (Table 1). Two of the festivals (festival 002 and 003) in which portaloos were sampled provided separate toileting areas for men and women which was enforced by security guards. Only the festival in which collection occurred over two consecutive years (festivals 001a and 001 b) had other toilets available that drained into the public sewage system and so could not be sampled. For all but the first festival sampled (festival 001a), extended screening for benzodiazepines and their metabolites and other pharmaceuticals with abuse potential were performed, as assays were not available at the time of the first festival.

Detection frequencies

Of the approximately 74 non-benzodiazepine compounds that were screened for, 31 were detected in the samples collected. Some compounds were detected at all festivals and found in more than 80% of samples at each festival including: amphetamine; cocaine; ketamine; MDMA and MDA (Table 2). Other compounds were detected at all festivals but had substantial variability in the proportion of samples testing positive at each festival including: codeine (40–100%); methamphetamine (18–100%); and methylone (53–100%). Finally, there were some compounds that were not detected at all festivals; ethylone, methadone, methcathinone were detected at 4/6 (67%) festivals sampled and buprenorphine, mephedrone, morphine, n-ethylpentylone, and noroxycodone, an oxycodone metabolite at less than half of festivals. For festivals where mephedrone was detected (3/6), it was detected in between 68% and 100% of samples from those festivals. Norfentanyl, a fentanyl metabolite, was detected in one sample from each of two festivals. There were no significant differences in detection frequencies between male and female toilets and so stratified results were not reported here.

Due to the large volume of wastewater required for benzodiazepine analyses, samples for each festival were pooled, meaning only the detection frequency across all festivals could be measured. Of the 25 benzodiazepine-related compounds and their metabolites screened for, alprazolam, alpha-hydroxy-alprazolam, diazepam, etizolam, oxazepam and temazepam were found at all festivals sampled. Clonazepam and lorazepam were detected at 4 (80%) and 3 (60%) festivals respectively and 7-amino clonazepam, 7-amino nimetazepam, diclazepam at 2 (40%), 1 (20%) and 1 (20%) festival respectively (Table 2). Although not a benzodiazepine, quetiapine was also detected in all pooled samples from all festivals.

Survey comparisons

According to surveys collected from each festival, MDMA was identified as the most frequently consumed substance

Table 1. Festival details.

Festival	001a	001b	002	003	004	005
Year	2019	2020	2019	2020	2020	2020
Attendees	11,250	14,000	10,003	6200	10,511	14,975
medical/10,000 attendees	99	118	40	97	199	47
Portaloos available	20	19	60 × male, 60 × female	50 × male, 50 × female	8 × 16 pan units, 4 × urinal blocks	180unisex, 10 × urinals
Portaloos sampled *n* (%)	20 (100%)	19 (100%)	63 (52.5%)	15 (15%)	2 (25%)	55 (30.6%)
Other toilets	Yes	Yes	No	No	No	No
Pooling of samples	No	No	2/sample	No	Yes	5/sample
Number samples	20	19	33	15	1	11
Number of people surveyed	NA	NA	223	114	387	194

NA: not available.

Table 2. Festival and study detection frequencies for drugs and metabolites measured – number and percentages of samples positive for drugs detected at each festival and over the entire study for all festivals.

	Number samples at each festival present (%)						Number festivals present (%)
Drug/Festival	001a	001b	002	003	004	005	
Amphetamine#	20 (100%)	18 (95%)	32 (97%)	13 (87%)	1	11 (100%)	6 (100%)
Benzoylecgonine*	20 (100%)	19 (100%)	33 (100%)	15 (100%)	1	11 (100%)	6 (100%)
Buprenorphine	0	0	1 (3%)	0	0	0	1 (17%)
Cocaine	20 (100%)	19 (100%)	33 (100%)	15 (100%)	1	11 (100%)	6 (100%)
Codeine	18 (90%)	18 (95%)	30 (91%)	6 (40%)	1	11 (100%)	6 (100%)
Ethylone	6 (30%)	0	9 (27%)	6 (40%)	1	0	4 (67%)
Ethyl sulphate*	NA	19 (100%)	33 (100%)	15 (100%)	1	11 (100%)	5 (100%)
Ketamine	NA	19 (100%)	32 (97%)	15 (100%)	1	11 (100%)	5 (100%)
MDA#	20 (100%)	18 (95%)	33 (100%)	15 (100%)	1	11 (100%)	6 (100%)
MDMA	20 (100%)	19 (100%)	33 (100%)	15 (100%)	1	11 (100%)	6 (100%)
Mephedrone	18 (90%)	13 (68%)	0	0	0	11 (100%)	3 (50%)
Methadone	0	1 (5%)	8 (24%)	0	1	2 (18%)	4 (67%)
Methamphetamine	20 (100%)	14 (74%)	18 (55%)	5 (33%)	1	2 (18%)	6 (100%)
Methcathinone	4 (20%)	0	4 (12%)	1 (7%)	1	0	4 (67%)
Methylone	20 (100%)	10 (53%)	33 (100%)	13 (87%)	1	10 (91%)	6 (100%)
Morphine	12 (60%)	0	9 (27%)	0	1	0	3 (50%)
N-Ethyl pentylone	1 (5%)	0	0	0	0	0	1 (17%)
Norfentanyl*	NA	1 (5%)	0	0	0	1 (9%)	2 (40%)
Noroxycodone*	NA	0	10 (30%)	1 (7%)	1	0	2 (40%)
7-Amino Clonazepam*	NA	detectable	0	0	0	detectable	2 (40%)
7-Amino Nimetazepam*	NA	0	detectable	0	0	0	1 (20%)
Alpha-OH Alprazolam*	NA	detectable	detectable	detectable	detectable	detectable	5 (100%)
Alprazolam	NA	detectable	detectable	detectable	detectable	detectable	5 (100%)
Clonazepam	NA	detectable	detectable	detectable	0	detectable	4 (80%)
Diazepam	NA	detectable	detectable	detectable	detectable	detectable	5 (100%)
Diclazepam	NA	0	detectable	0	0	0	1 (20%)
Etizolam	NA	detectable	detectable	detectable	detectable	detectable	5 (100%)
Lorazepam	NA	detectable	0	0	detectable	detectable	3 (60%)
Oxazepam#	NA	detectable	detectable	detectable	detectable	detectable	5 (100%)
Temazepam#	NA	detectable	detectable	detectable	detectable	detectable	5 (100%)
Quetiapine	NA	detectable	detectable	detectable	detectable	detectable	5 (100%)
Total n samples	20	19	33	15	1	11	

#Metabolites or parent compounds; *metabolites.
NA: not available.

Table 3. Of those surveyed at each festival, number and percentage of people reporting that they had used or planned on using a given drug.

Drug/festival	002	003	004	005
Cocaine	11 (4.9%)	12 (8.3%)	14 (3.6%)	6 (3.1%)
Ketamine	4 (1.8%)	6 (4.2%)	10 (2.6%)	1 (0.5%)
MDMA	64 (28.7%)	42 (29.2%)	64 (16.5%)	38 (19.6%)
Methamphetamine	2 (0.9%)	1 (0.7%)	0 (0%)	2 (1%)
Total n surveyed	223	144	387	194

(16.6–29.2% of those surveyed), followed by cocaine (3.1–8.3%) and then ketamine (0.5–4.2%) (Table 3). There were no reports of consumption of cathinones or opioids or any other NPS according to the survey.

Discussion

Here, we demonstrate the feasibility of wastewater screening at music festivals in NSW, Australia. This is the most contemporary festival wastewater analysis worldwide and the only study in Australia to sample individual portaloos across multiple different festivals; the only prior study of festival wastewater screening in Australia sampled from an onsite wastewater treatment plant inlet and only tested for 13 different compounds, only three of which were NPS [10].

The detection frequency within individual festivals, study detection frequency (amongst all festivals) and adjusted concentrations for traditional illicit drugs was consistent with prior surveys of illicit drug use at similar single day festivals playing electronic dance music (EDM) music [8,9]. Specifically, MDMA, MDA, ketamine and cocaine along with its metabolite benzoylecgonine all had high detection frequencies and adjusted concentrations, corresponding to the prevalence of self-reported use at festivals from surveys here and other studies.

Despite no reports of NPS consumption at festivals surveyed, we detected a range of NPS, albeit at lower detection frequencies and concentrations. The commonest of these was methylone, followed by ethylone and methcathinone. N-ethylpentylone was only found in one portaloo at one festival (001a) in which all portaloos were sampled, a particularly toxic cathinone that may have gone undetected had all portaloos not been sampled [27]. In an Australian survey sample, intentional use of synthetic cathinones amongst a group of people from the Ecstasy and Related Drugs Reporting System (EDRS) was as low as 1% in 2020, and listings on the darknet continue to drop [28]. While some of this cathinone use may be intentional and not captured in festival surveys, the implication is that cathinones may either be adulterating more traditional illicit drugs or mis-sold. Supporting this, a recent study of police seizures of illicit substances found that MDMA was adulterated with ethylone and less frequently methylone [17]. Drug checking services invite members of the public to anonymously submit psychoactive drug samples for forensic analysis and then

provide individualised feedback of results and counselling as appropriate [29]. Formally approved drug checking services have only been provided twice in Australia, at the same music festival in Canberra in 2018 and 2019 [2]. In 2019, this service identified seven samples containing N-ethylpentylone that were reportedly sold as MDMA [30]. A similar UK study found that up to one in five substances was not as sold or acquired and 3.5% of samples contained cathinones [13]. Adulteration or substitution with cathinones has implications as some of these compounds are known to be highly toxic, particularly in combination with other stimulants such as MDMA. Despite screening for a wide range of other NPS compounds, few were identified. This may be because few to none were consumed, despite being found in police seizures in a prior Queensland based study [17], or that they were not detected for sampling or analytical reasons. The detection of norfentanyl may be related to therapeutic use of fentanyl, recreational use or adulteration or substitution of other drugs; however, there have been recent alerts of fentanyl-contaminated cocaine [31].

Prescription drugs detected here such as alprazolam, diazepam, oxazepam and quetiapine could have been the participants prescribed medications and not used recreationally. However, etizolam, a benzodiazepine not registered with the Australian Therapeutic Goods Administration (TGA) or available on prescription in Australia, was also detected across all festivals. While this is a known drug of abuse, no participants in the EDRS sample reported intentional etizolam use in 2019, despite being specifically asked [28]. A NSW Health Alert was posted in December 2019 about counterfeit alprazolam containing predominantly etizolam [32], which is likely to explain the presence of etizolam given that 26% of the 2019 EDRS sample did report alprazolam use in the six months prior to being surveyed [28]. Information from festival wastewater analysis may be an important supplement to other data sources available in Australia such as police seizures [17], toxicological reports [7] and surveys [9,28] in publishing public health alerts given the limitations of all of these methods in detecting actual or intended drug use at festivals. Police seizures at festivals only capture a small proportion of drugs used at festivals and these seizures are not routinely analysed, nor are results publicly available. Toxicological reports only capture those with the most severe toxicity, which is often a complex interaction between the drug taken, the festival environment and the physiological response of the individual and surveys may fail to identify drug substitution or adulteration. Drug checking is not routinely available in Australia for legislative reasons and not everyone will use this service even if it were available. Wastewater analyses at festivals have the capacity to provide broad capture of drugs taken and any unexpected results, particularly the detection of highly toxic substances, can be fed into public health alerts.

While this study provides potentially valuable insights into the range of drugs used at the festivals sampled, there are some important limitations. Not all drugs taken during the festival may appear in wastewater; detectability depends both on participants using the portaloos, the timing of drug consumption relative to passing urine and the elimination half-life of the substance. Very recent use might have led to a substance not being detected in this case, whilst drugs may have been taken in the preceding days with ongoing excretion. The use of stimulants and dehydration from vigorous activities such as dancing may reduce urine output. Not all portaloo units were sampled and some festivals had toilets draining directly into the sewage system (festivals 001a and 001 b). It is challenging to draw any conclusions regarding the impact of this given other potential confounders such as unmeasured differences in festival attendee demographics as these festivals were not surveyed. The laboratory assays used were not validated for portaloo liquid and we were unable to test for cannabinoids and GHB at the time of the study. Quantitative results were not available due to complexities associated with interpretation given variable excretions and volumes of dilution in portaloos. These could be overcome by adjusting for fractions excreted and expressing concentrations as ratios relative to creatinine concentrations. Creatinine has limited stability in such samples [33], but this could be overcome by analysing samples immediately after collection or freezing immediately at minus 20 degrees. We did not specifically ask survey respondents about their use of benzodiazepines, including etizolam, cathinones or NPS directly, even though there was an "other drug" response option. For wastewater sampling at festivals to be truly useful and scalable as a method of signal detection, appropriate funding would be required for staffing to allow for sufficient sampling and to make results available close to real time. This could also improve peer education approaches at subsequent festivals. Previous survey-based studies of illicit drug use at festivals have demonstrated disparities between single and multiple day events [8,9] and so performing wastewater analysis at multi-day events would be an important extension to this study.

Conclusion

Wastewater sampling at festivals is both feasible and potentially useful as a method of determining the range of psychoactive substances use at festivals. This information, if provided close to real time, could be triangulated with complementary data sources currently available in Australia such as police seizures, surveys, medical presentations, information from peer-based harm reduction services and toxicological analyses to feed into public health alerts. Each of these sources provide unique insights into dynamic drug markets but each have their own limitations. Wastewater has the potential advantage of sampling an entire population, is non-invasive and can be performed overtly but yields little information about individual drug use. All of this information can be used inform peer-based harm reduction approaches during the festival season.

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