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Wastewater-Based Epidemiology to Determine Temporal Trends in Illicit Stimulant Use in Seattle

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Wastewater-Based Epidemiology to Determine Temporal Trends in Illicit Stimulant Use in Seattle.

Wastewater-based epidemiology (WBE) has proven to be a useful tool in estimating illicit drug consumption at the community level.[1–3] WBE provides near real-time drug use without the bias and limitations of traditional measures such as surveys, overdose reporting, police seizures, and emergency room visits, making it a complementary tool in estimating community drug consumption. Wastewater-based epidemiology can be used to estimate drug consumption over many years or around a specific time or event such as a festival, a football game, or a specific holiday.[4–11]

Previous studies have shown that illicit drug use, specifically 3,4-methylenedioxymethamphetamine (MDMA) or ecstasy, is frequent at festivals in the U.S. and on an international scale. In Australia, a study done by the National Center for Biotechnology Information (NCBI) in 2016 found that almost 60% of drug users at festivals were using ecstasy.[12] Additionally, young people, age 18-25 years old, have the highest MDMA use among all other age groups.[13] Chow et al showed that gay or bisexual men and women have higher MDMA use than their heterosexual counterparts in the last 30 days.[14] MDMA, and other illicit drugs found at festivals, have the potential to be laced with more dangerous drugs that can be fatal.[15] To help drug users use more safely, organizers of the Pride Festival in Vancouver, Canada, provided free drug testing stations to help people know what is in their drugs before they take them.[16] The need for drug testing at Vancouver Pride suggests that Pride Festivals may not be different from other festivals when it comes to drug use. MDMA use is common at festivals, however, the prevalence and patterns of use remain unknown. Wastewater-based epidemiology has the potential to provide answers to questions about drug use and trends for a specific population.

Many users of club drugs like MDMA (ecstasy), cocaine, and ketamine will often use more than one drug at a time, this is called *polydrug* use. There are many dangers associated with mixing various illicit drugs. Over 86% of MDMA users combine it with another drug.[17] The most used club drugs are cocaine and MDMA, respectively, and the most common combination is MDMA and cocaine.[17] Therefore, it would be expected the MDMA and cocaine follow a similar trend in use, however, it may be hard to discern considering the mass load of cocaine is considerably larger than that of MDMA and an event in an otherwise large city may not be obvious.

Many ecstasy users refer to the drug as “Molly” which was originally short for “molecular” because it was marketed as pure MDMA, however, there have been increasing levels of adulterants found in Molly, thus decreasing the purity and increasing the risk of fatalities.[18]

MDMA can be laced with drugs such as bath salts (cathinones), fentanyl, and methylone. Methylone, a cathinone and often a component used in bath salts, is particularly dangerous because it has ecstasy-like effects, can be more addictive, and is more lethal than MDMA itself.[19] Wastewater-based epidemiology has the potential to be used to inform officials if MDMA laced with methylone, or fentanyl, is present at a festival or on the rise across festivals within a certain city or region. This type of monitoring would require the proper sampling protocols and either interest in specific adulterants using targeted analysis or interest in finding new compounds that could be identified with non-targeted analysis and high resolution mass spectrometry.

This chapter reports on a four year period with annual 14-day sampling periods, collected as daily, 24-hour composite samples of municipal wastewater. Specific high values of MDMA in Monday composite samples during the last week of June led to a deeper investigation into its source and the recognition of the Seattle Pride festival's effects.

Materials

All analytical standards and internal standards were acquired from Cerilliant Corporation (Round Rock, TX, USA). Methanol was LC-MS grade from JT Baker and water was produced by a Millipore Elix 5 system. Formic acid and ammonium formate were mass spectrometry grade (>99.0%) from Sigma/Fluka (St. Louis, MO, USA).

Wastewater Collection

Samples were obtained from the Westpoint treatment plant in Seattle, WA. This plant services approximately 893,000 people and has a catchment area that closely aligns with the City of Seattle. Twenty-four hour, volume-proportional composites were collected as part of the daily routine at the plant and a 50 ml aliquot from the composite was obtained for each day for drug analysis. In the first year, wastewater samples were collected from March 24th to April 6th, 2015 and subsequent years samples were collected from June 15th to July 2nd in 2016, 2017, 2018. Samples were immediately frozen and shipped to the University of Puget Sound, Tacoma, WA, USA, where they were stored at -20 ℃ until extraction and analysis.

Chemical Analysis

The experimental methods are adapted from Burgard et al. and Chen et al.[20,21] The following drugs and metabolites were extracted, analyzed, and validated: cocaine (cocaine-D_3), benzoylecgonine (benzoylecgonine-D_3), amphetamine (amphetamine-D_6), methamphetamine (methamphetamine-D_9), and MDMA (MDMA-D_5).

Wastewater samples were thawed to room temperature. A 25 g aliquot was obtained from each daily composite sample along with one daily tap water method blank. Each sample and blank were spiked with a deuterared internal standard mixture containing 100 ng of each. A drop of concentrated HCl was added to each sample and blank. The samples were subjected to solid phase extraction (SPE) using mixed-mode reversed-phase cation exchange cartridges (Oasis-MCX, 3 cc, 60 mg, Waters Corp., Milford, MA, USA). The cartridges were conditioned with 1 mL of methanol followed by 2 mL pH 2 ultrapure water. Just prior to SPE, the blanks and samples were filtered (0.2 µm regenerated cellulose Phenex, Phenomenex, Torrance, CA). The cartridges were washed with 1 mL of 85:15 Milli-Q UltraPure water/acetonitrile and dried for approximately 5 min. The compounds of interest were eluted with 2 mL of methanol followed by 3 ml of 5% ammonium hydroxide in methanol. The two eluates were combined and placed under a nitrogen stream until each sample reached complete dryness. The samples were reconstituted in 400 µL 5 mM ammonium formate for LC-MS/MS analysis.

Drug metabolites were analyzed by LC-MS/MS (Agilent 1260 Infinity II LC/Agilent 6420A Triple Quadrupole). A Kinetex C18 column 2.1 x 100 mm 2.6 µm (Phenomenex) was used for separations with a matching C18 3.0 mm SecurityGuard Phenomenex guard column. The LC injection volume was 20.00 µL with a 3.0 s needle wash between injections. A flow rate of 0.300 mL/min was consistent throughout each 9.0 min run. The mobile phases used were 0.1% formic acid (A) and methanol (B) (gradient in Table 1 below).

Table 1. Mobile phase gradient for the LC-MS/MS analysis of drugs and metabolites. Mobile phase A is 0.1% formic acid and mobile phase B methanol.

Time (min)	Solvent Composition % (A)	Solvent Composition % (B)
1.00	95.00	5.00
4.50	72.00	28.00
5.50	5.00	95.00
6.00	5.00	95.00
6.50	95.00	5.00
9.00	95.00	5.00

The mass spectrometer operated in positive ESI mode with the source gas temperature set at 350 ℃ at 50 psi. Gas flow was 10 L/min with capillary set at 2500 V. The chromatograms collected were in MRM mode. The precursor ions, product ions, fragmentation voltage, and collision energy are listed in Table 2. Quantification was achieved with an 11 point calibration curve from 0.12 ng/ml to 240 ng/ml.

Table 2. Extensive list of all analytes quantified in MRM method.

Compound	Precursor Ion (m/z)	Product Ion 1 (m/z)	Product Ion 2 (m/z)	Frag (V)	CE (V)
COC-d3	307.3	185.3		65	20
COC	304.3	182.2	82.2	65	25
BZE-d3	293.3	171.4	--	65	20
BZE	290.3	168.3	105.3	65	15
MDMA-d5	199.3	165	107	65	20
MDMA	194.1	163	105	65	20
MAMP-d9	159.1	125.1	93.1	60	7
MAMP	150.1	119	91	70	17

AMP-d6	142.3	125.1	91	60	4
AMP	136.1	119	91	60	16

Method Validation

The analytical method was internally and externally validated. Internal validation is performed with wastewater samples and spiked wastewater samples (Table 3). Accuracy was achieved with spiked wastewater where concentration was increased by 5 ng/mL of each drug.

External validation is achieved by participating in the Sewage analysis CORe group Europe (SCORE) annual interlab study.[22] Cocaine, its metabolite benzoylecgonine, MDMA, methamphetamine, and amphetamine were quantified in blind spiked methanol and tap water samples. Our lab's results met acceptable criteria for these compounds over the years reported here.

Table 3. Internal validation for precision and accuracy of wastewater samples.

Drug	%RSD	Accuracy
MDMA	1.8	102.6%
Cocaine	0.5	103.7%
Benzoylecgonine	0.8	109.5%
Amphetamine	1.6	103.5%
Methamphetamine	0.4	119.8%

Determining Daily Load of Illicit Drugs

After extraction and analysis, the measured concentration from each 24-h composite sample was multiplied by the 24 h total volume of wastewater through the plant to determine the daily load (g /day).

RESULTS AND DISCUSSION

Scope of Illicit Drugs in Seattle Wastewater

Figure 1 shows the average drug load for amphetamine (AMP), methamphetamine (MAMP), MDMA, cocaine (COC), and benzoylecgonine (BZE) for all week samples which includes two weeks in spring 2015 and the last two weeks in June in 2016-2018. It should be noted that the uncertainty shown here only includes the variability of the daily loads as calculated with the measured concentrations and treatment plant reported 24 h volumes. Uncertainty estimates from population, flow measurement, analytical measurement, and in-sewer degradation can be estimated but were taken to be static across this 4 year period.[2,23,24]

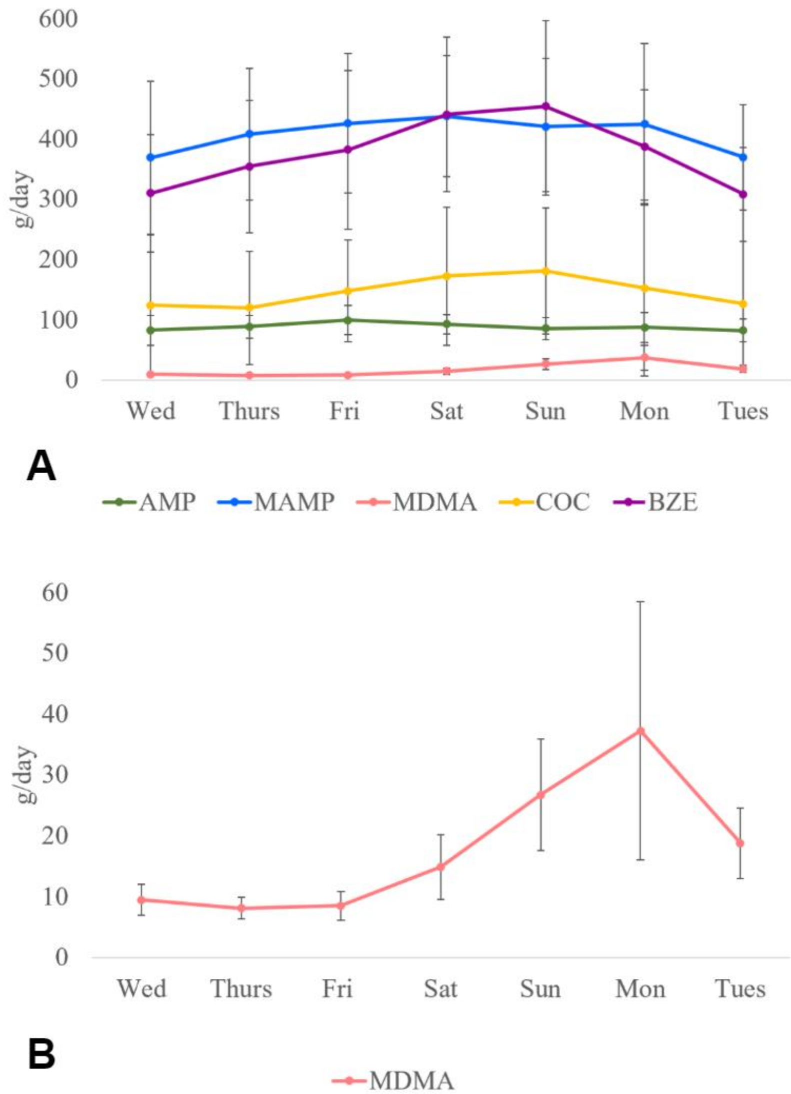

Figure 1. The weekly average of illicit drug loads for all weeks sampled. Mass loads of A) amphetamine (AMP), methamphetamine (MAMP), MDMA, cocaine (COC), and benzoylecgonine (BZE) and B) expanded MDMA for the weekly average of all samples. Each day had between n=8 and n=11 representative samples based on the previously determined sampling method. Uncertainty bars are represented by the 95% confidence interval around the mean day of week loads.

While the uncertainties appear to show no significant trend for all drugs, much of the variability comes from the differences among years and not in the shape of the plots across the week. Cocaine and benzoylecgonine typically have maximum mass loads on weekends, while there is no obvious maximum for amphetamine or methamphetamine. Since cocaine is typically a recreational drug, spikes on Saturday and Sunday are expected for the parent compound and its metabolite in wastewater.[9] MDMA has a clear increase during the weekend, which is also consistent with the club-like nature of this drug.

For MDMA, Figure 1B shows the largest loads and mean uncertainty on Monday in Seattle which is a day later than typically observed with wastewater data. Thus, an investigation was started to try to understand if there were other factors that could have contributed to this day lag or increased Sunday use. Through further investigation, it was observed that the sampling in June had overlapped with a large festival-like event: Seattle Pride Parade. Seattle Pride in late June brings thousands of visitors to Seattle to participate in a variety of events, concerts, and parades celebrating the LBGTQ community. In 2017, over 200,000 people were estimated to have participated in Seattle Pride, however, an exact number of Seattle residents and/or participants from outside the Seattle area, is unclear.[25,26]

The data were reevaluated, separating out the weeks of overlap with the Pride with the other weeks, or "normal" weeks, providing a baseline of MDMA consumption as shown in Figure 2. Notably, in all three years (2016, 2017, 2018), there was a higher

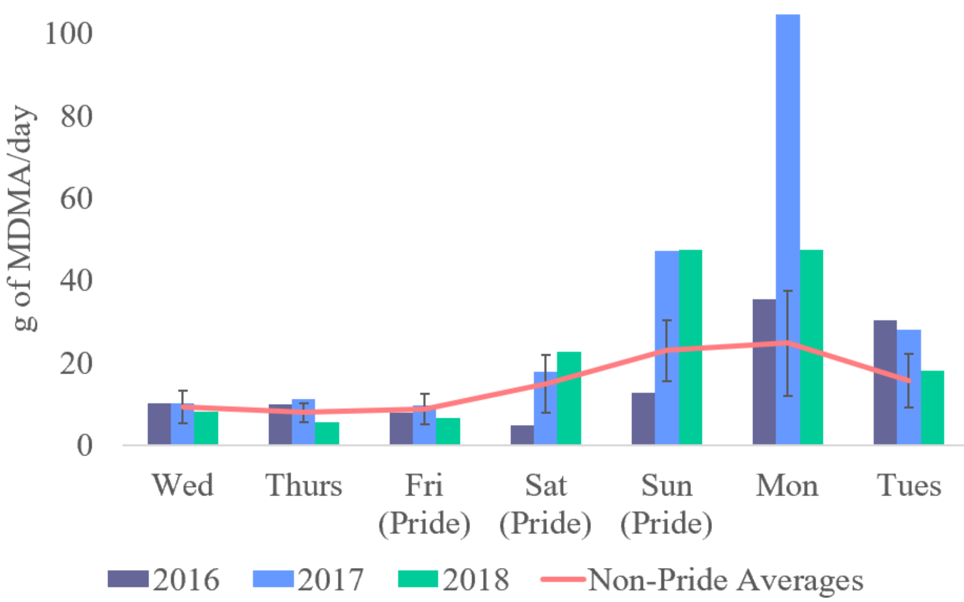

Figure 2. Mass load of MDMA in Seattle wastewater during Pride weekends compared to other (non-Pride) weeks. Uncertainty bars are represented by the 95% confidence.

MDMA mass load on the Monday after Pride weekend with a maximum mass load observed on a Monday in 2017. Samples from Tuesday – Saturday on Pride weeks have similar MDMA loads to samples taken over the two weeks in March of 2015 and the weeks sampled in June that did not correspond to Pride week. In the non-Pride Weekend data, there is an increase in MDMA mass load on weekends, known as the "Weekend Effect".[9] Typically, the majority of MDMA use occurs on Friday and Saturday nights, therefore, observing a maximum sewer mass load on Monday would indicate that people would be using these drugs on Sunday, which would seem atypical.

The increase in MDMA loads is likely due to the nature of the Pride events on Sunday each year. In all three years, a major event, the Pride Parade, was on Sunday.[27,28] The increase in all three years could be due to an increase in MDMA use during the Parade from the festival-like nature of the parade as well as, or in addition to the reported increased use by the groups that would attend this event.[14] However, confounding these results is that in 2016, MDMA loads were not significantly different from non-Pride weeks. In 2017 and 2018 MDMA use during Pride was significantly different from the other weeks.

The 2017 and 2018 significant difference in MDMA use led to investigating a cause for the increase. After looking back at events during those two years it was discovered that in addition to the parade, there was a festival with several concerts on Sunday.[25,26,29] However, no references were found retroactively that indicated a concert on Sunday in the schedule for 2016.[30] This festival setting and general increases in activities seems to likely account for the significant difference in MDMA consumption between 2016 and the following years. Ultimately, due to the nature of WBE, it cannot be determined whether more people were using MDMA on those days, or the same number of people as a typical week were using higher quantities.

The two illicit drugs that are most often combined are cocaine and ecstasy.[17] It would be expected that cocaine use would follow similar trends to MDMA use if the two are often used together, or at similar events, among similar populations. The cocaine trends during the event were less obvious and often not significantly different from the non-Pride weekends, as shown in Figure 3.

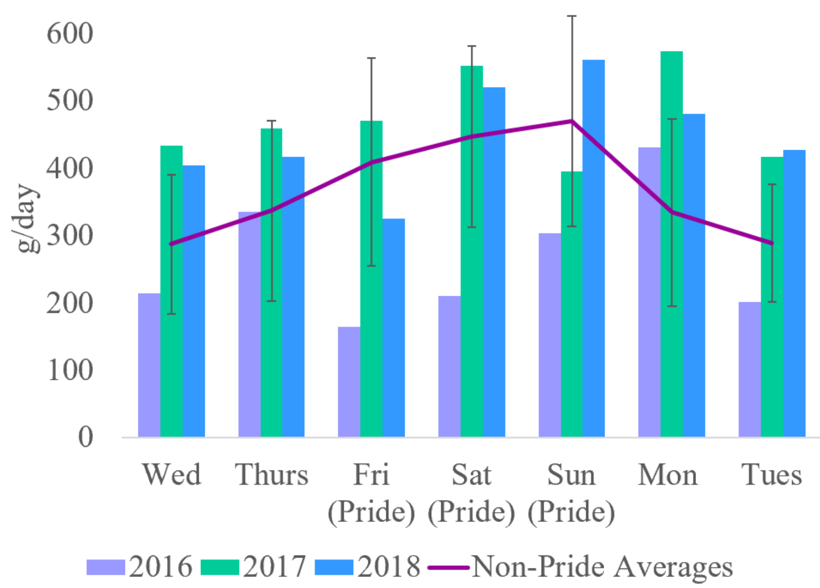

Figure 3. Plot of Benzoylecgonine over Pride Week. The mass load of benzoylecgonine to represent cocaine use during Seattle Pride weekend compared to the mass load of a non-pride weekends (2015,16,17,18). Error bars are represented by the 95% confidence

The trends seen in the non-Pride week have a maximum on Sunday, whereas, there is no consistent maximum across the three years of Pride data for benzoylecgonine. The maximum occurs on Sunday, for 2018, however, the maximum for 2016 and 2017 is on Monday. In 2018 and 2016, the increase on the Monday after Pride is not significantly different. Benzoylecgonine has a half-life of about 12 hours.[31] Therefore, 75% of the drug would be excreted in two half-lives, or 24 hours. The majority of people who use cocaine may also be using on Friday or Saturday nights, indicating that a maximum benzoylecgonine mass load in the wastewater should be seen on Saturday and Sunday, respectively. This trend is observed in the non-Pride week data; however, it is not necessarily obvious in the Pride weekend data. There is an inconsistency in the maximum mass load day in 2017 (a dip on Sunday) that cannot be easily explained based on the Pride weekend events. While literature has shown that polydrug users often use cocaine with MDMA, this data suggests that cocaine use can be independent of MDMA use.

When investigating the MDMA use, potential impurities could also be detected. Methylone is a lethal drug that can be laced in ecstasy tablets. The analytical method used in this study for processing wastewater also quantifies methylone. However, even with an LOD of 2 ng/L, all values were not detectable. While this methylone method could be better optimized, these results show the difficulty of potentially finding drugs that are laced in such a large catchment.

Wastewater-based epidemiology is a useful tool for determining differences in trends in illicit drug consumption and can show anomalies in these drugs trends. Through further investigation, changes in drug use behavior from a specific event, such as Pride weekend, can be observed. WBE is a useful and complimentary method for understanding drug use.

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