Wastewater Surveillance of Illicit Drugs in Southern Nevada: Sucralose Normalization to Translate Data into Public Health Action

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Abstract

The COVID-19 pandemic highlighted the value of wastewater surveillance in providing unbiased assessments of incidence/prevalence for infectious disease targets, ultimately leading to the development of local, state, and national programs across the United States. To address the growing epidemic of drug abuse, there have been calls to extend these programs to illicit drugs and metabolites, while leveraging the experience gained during the pandemic and from ongoing efforts in other countries. This study advances the science of wastewater surveillance for illicit drugs by (1) highlighting analytical and sewer transport considerations; (2) proposing sucralose normalization to adjust for varying human urine/fecal load and confounded population estimates (e.g., high tourism areas); and (3) characterizing temporal and geographic trends in drug use. This one-year study across eight sewersheds in Southern Nevada (208 total samples) monitored concentrations of 17 pharmaceuticals and personal care products (PPCPs) and 22 drugs and metabolites, including natural, semi-synthetic, and synthetic opioids. The data indicated a ~200% increase in heroin and methamphetamine use since 2010, a stark increase in fentanyl consumption beginning in October 2022, and statistically significant differences in drug consumption patterns between sewersheds and on certain dates. Notably, the latter outcome highlights the potential for wastewater surveillance data to be strategically translated into public health action to reduce and/or more rapidly respond to overdoses.

Keywords: Wastewater-based epidemiology (WBE); trace organic compound (TOrC); opioid; biomarker

1.0 Introduction

In the early 2000s, the water industry turned its focus to trace organic compounds (TOrCs), including pharmaceuticals and personal care products (PPCPs), when scientists highlighted their ubiquitous occurrence in surface water (Kolpin et al., 2002). TOrC occurrence in the environment is often linked to effluent discharges from wastewater treatment plants (WWTPs), which are not specifically designed to target these diverse compounds. However, one of the ancillary benefits of robust secondary biological wastewater treatment (Achermann et al., 2018) and some disinfection processes, particularly ozone (Lee et al., 2013), is significant attenuation of some PPCPs and even illicit drugs and metabolites (Gerrity et al., 2011).

Leveraging this direct connection between anthropogenic activity and wastewater, the emergence of SARS-CoV-2 in early 2020 prompted the water industry to shift its focus to untreated wastewater (Bivins et al., 2020). Unprecedented resources were allocated to wastewater surveillance to provide public health officials and policymakers with an unbiased assessment of COVID-19 incidence and SARS-CoV-2 variant characterization (Vo et al., 2022). This ultimately led to the development of the National Wastewater Surveillance System (NWSS) by the United States (U.S.) Centers for Disease Control and Prevention (CDC). Over the next several years, the scope of these wastewater surveillance efforts expanded to address historical targets such as poliovirus (Link-Gelles et al., 2022) and emerging targets such as mpox virus (Wolfe et al., 2023) and *Candida auris* (Babler et al., 2023; Barber et al., 2023; Rossi et al., 2023). As the COVID-19 pandemic subsided, wastewater surveillance efforts returned to another historical application—licit and illicit drugs (Adhikari et al., 2023)—with some studies specifically focusing on natural, semi-synthetic, and synthetic opioids to address the growing epidemic of drug abuse (Murthy, 2016). The CDC estimates that nearly 1 million people died from drug overdoses in the U.S. between 1999 and 2022, including 100,000 deaths in 2020 alone (28.3 deaths per 100,000 people), and ~80% of the recent deaths were linked to synthetic opioids (CDC, 2022).

Because it does not require active participation, wastewater surveillance has the potential to elucidate the true incidence and/or prevalence of public health threats that lack adequate clinical surveillance, including targets with waning urgency (e.g., SARS-CoV-2), potential for stigmatization (e.g., mpox), or legal implications (e.g., illicit drugs). As demonstrated in the literature, this tool has already been implemented to characterize licit and illicit drug use across broad temporal and geographic domains (González-Mariño et al., 2020; Huizer et al., 2021; van Nuijs et al., 2011). In fact, Australia implemented a National Wastewater Drug Monitoring Program in 2016 to assess trends and policy measures following an observed spike in methamphetamine use (Ahmed et al., 2021; Lai et al., 2016). With calls to integrate illicit drug targets into NWSS (Ahmed et al., 2021), the industry should leverage the vast experience gained during the COVID-19 pandemic to improve the science, develop strategies to translate the data into public health action, and consider the ethical and legal ramifications of these potentially sensitive datasets (Coffman et al., 2021).

As such, the goal of this study was to build upon the existing wastewater surveillance literature, specifically for licit and illicit drugs, by (1) highlighting analytical and sewer transport considerations, (2) evaluating the potential benefits of data normalization, and (3) using wastewater to characterize real-time and annual consumption patterns in Southern Nevada. This builds upon prior work from 2010 when PPCPs, drugs, and metabolites were monitored in Southern Nevada’s untreated and treated wastewater during a major sporting event (Gerrity et al., 2011). The current study also describes the implications of data normalization to adjust for human urine/fecal load, but instead of using pepper mild mottle virus (PMMoV) or common water quality parameters, as has been done for SARS-CoV-2 (Maal-Bared et al., 2023), normalization to a high occurrence TOrC such as sucralose is proposed for illicit drug wastewater surveillance. This is intended to correct for hydraulic anomalies or nonrepresentative plugs of wastewater that might occur in smaller collection systems (Gerrity et al., 2022b), or to correct for transient populations (e.g., tourists and commuters) that might confound wastewater-based epidemiology (WBE) efforts (Vo et al., 2023). Finally, the data are used to retrospectively identify outlier events and locations that could have been prioritized for public health action (e.g., targeted interventions) and to develop relative comparisons between local sewersheds and across geographic regions.

2.0 Methodology

2.1 Wastewater Sample Collection

Wastewater surveillance was performed biweekly from May 2022 through April 2023 at eight sampling locations across six different WWTPs in Southern Nevada (N = 26 samples per location and 208 samples in total) (Figure 1). Collectively, these sampling locations capture the vast majority of the population of the Las Vegas metropolitan area, excluding the sparsely populated outlying areas and the ~2% of the population served by septic tanks. The average daily flow at each location ranged from 0.8 million gallons per day (mgd) to 100 mgd, with the corresponding sewersheds serving approximately 16,000 to 872,000 local residents (Figure 1). Populations were determined based on zip code-level data provided by the Southern Nevada Health District (SNHD, 2023), and zip codes were manually allocated to sewersheds based on jurisdictional boundaries.

In addition to serving the largest proportion of the local population, sewershed 1 receives wastewater from the high-density resort corridor known as the ‘Las Vegas Strip’, a large international airport, and a large university. From May 2022 through April 2023, approximately 40.4 million people visited Las Vegas—an average of 777,000 people per week—and the airport served approximately 53 million travelers (LVCVA, 2023; Velotta, 2023). The Las Vegas Strip also employs approximately 75,000 people within the casino resorts (CGR, 2023), some of whom may commute into sewershed 1. Therefore, non-resident contributions have significant confounding effects on WBE in Southern Nevada, as demonstrated previously for COVID-19 in sewershed 1 (Vo et al., 2023).

For this study, half of the sampling locations consisted of refrigerated, 24-hr (spanning Sunday to Monday morning) composites of wastewater influent. Due to practical limitations, the other locations were monitored with grab influent samples collected on Monday mornings at ~8:00 am. For example, sewershed 4 was represented by a 24-hr composite sample, which was assumed to be more representative of the overall service area, but this sewershed was also subdivided into 4A and 4B through grab samples collected from the west and east trunk lines feeding the WWTP. Sewershed 4 (via 4A) also receives all solids from the membrane bioreactor facility in sewershed 2, potentially confounding sewershed-specific loading estimates for more hydrophobic targets. Early in the study, a single grab primary effluent sample was collected from sewershed 1 for method validation and to compare against similar samples collected in 2010 (Gerrity et al., 2011); the primary effluent sampling time (~10:00 am) reflected untreated influent arriving at the WWTP at ~5:00 am due to the hydraulic retention time of the primary clarifier (Gerrity et al., 2021).

Samples were collected in 40-mL amber glass vials supplemented with 50 mg/L of ascorbic acid for oxidant quenching and 1 g/L of sodium azide for biological preservation. Oxidants were not expected in any samples, but inclusion of ascorbic acid is standard practice for the laboratory in this study. The efficacy and implications of sample quenching and preservation are discussed later. All locations were generally sampled on the same day, with the exception of several holidays and special events that are noted in Tables S8-S33. Samples were transported on ice within four hours to the laboratory for short-term storage at 4°C, and samples were generally processed and analyzed within 7 days. For field blanks (n = 3), Milli-Q water was transferred to sample vials at one of the wastewater collection sites; these samples were then processed in the same manner as actual samples. All field and analytical blanks were negative during this study.

2.2 Analysis of Trace Organic Compounds

The TOrC list included a suite of 17 PPCPs and 22 drugs and metabolites, which are listed in Tables S1 and S3, respectively, and concentrations for all compounds across the 26 sample events are summarized in Tables S8-S33. With respect to the PPCPs, this study focuses primarily on caffeine (Senta et al., 2015) and sucralose (Mawhinney et al., 2011) as indicators of human urine/fecal load. For the licit and illicit drugs, the parent compounds of interest included non-opioids, such as cocaine, methamphetamine, 3,4-methylenedioxymethamphetamine (MDMA), and delta-9-tetrahydrocannabinol (THC), in addition to natural (heroin, morphine, and codeine), semi-synthetic (hydrocodone and oxycodone), and synthetic (fentanyl, methadone, and tramadol) opioids. It is important to note that interpretation of some target compounds is confounded by reactivity with quenching agents/preservatives (e.g., heroin), aqueous instability (e.g., cocaine), or the fact that one or more major metabolites can also serve as parent compounds. The latter is best exemplified by the complicated interplay between heroin, acetylmorphine, morphine, and codeine.

Sample processing and analysis for PPCPs involved filtration using baked, pre-rinsed glass microfiber filters, automated solid phase extraction, and analysis of methanol extracts, while licit and illicit drugs and metabolites were analyzed with direct injection of 10-fold diluted aqueous samples. All compounds were analyzed by liquid chromatography tandem mass spectrometry (LC-MS/MS) with isotope dilution according to previously published methods (Gerrity et al., 2022a; Mawhinney et al., 2011; Vanderford & Snyder, 2006). All methods employed a CTC Autosampler (CTC Analytics, Zwingen, Switzerland) and an Agilent 1260 LC Binary Pump (Palo Alto, CA, USA). PPCP analysis was performed with SCIEX API 4000-series mass spectrometers (Redwood City, CA), and data were collected in multiple reaction monitoring (MRM) mode for electrospray ionization (ESI) negative or positive compounds and their isotopically-labeled analogs. For drugs and metabolites, analytes were monitored with positive ESI in MRM mode on a SCIEX 6500 QTRAP mass spectrometer (Redwood City, CA, USA). Additional details for all target compounds, including method reporting limits (MRLs) and MRM transitions, are included in Text S1.

2.3 Laboratory Hold Time Study

Prior to commencing wastewater surveillance, an extended hold time study was performed for the drugs and metabolites to evaluate target compound stability as a function of water matrix and sample quenching/preservation. Based on prior research (Vanderford et al., 2011), all PPCPs were assumed to be stable for at least 14 days (i.e., worst-case scenario for sample storage) when quenched and preserved. Deionized (DI) water, finished drinking water with a free chlorine residual of 0.8 mg/L as Cl_2, and treated wastewater with no residual disinfectant (influent wastewater was not accessible during the hold time study) were spiked with 500-1,000 ng/L of each target compound. Each water matrix was tested in experimental triplicates with and without the addition of 50 mg/L of ascorbic acid and 1 g/L of sodium azide; ambient concentrations prior to spiking were also tested in duplicate. Samples were held at 4°C for 0, 7, 14, 21, 28, and 60 days to simulate a range of laboratory storage times prior to analysis, and mass balances were performed to characterize stability of parent compounds and metabolites.

2.4 Simulated Sewer Transport Study

Simulated sewer transport experiments were also performed to assess PPCP and drug/metabolite stability in influent wastewater, specifically to determine whether sewer transport times might impact observed concentrations. The largest sewershed in this study has estimated travel times of up to 24 hours (T. Newell, personal communication, May 4, 2023) so it is possible that target compounds could degrade or transition prior to reaching the WWTPs. McCall et al. (2016) and Thai et al. (2014) implemented robust sewer transport experiments, specifically incorporating different types of biofilms, while the current study used a simplified approach similar to Lin et al. (2021) to focus on aqueous phase stability. On three different dates (5/1/23, 5/15/23, and 6/12/23), a 1-L grab sample of influent wastewater was collected from sewershed 1 directly into an amber glass bottle with no quenching agent or preservative. The bottle was kept open to the atmosphere at room temperature (24°C) on a shaker operated at 280 rpm. Aliquots were transferred to 40-mL amber glass vials containing 50 mg/L of ascorbic acid and 1 g/L of sodium azide at 0, 1, 2, 4, 8, 24, 48, and 72 hours.

2.5 Consumption Estimates

When estimating consumption, one or more correction factors (CFs) were applied to parent or surrogate compounds to account for metabolism, compound stability, and/or molecular weight equivalence. The only exception was the highly stable compound sucralose, which was assumed to be excreted from the human body unchanged (CF = 1.00) (Roberts et al., 2000). In contrast, caffeine is known to be highly biodegradable, with only 3% excreted as the parent compound (CF = 1/0.03 = 33.3) (Kot & Daniel, 2008).

Estimating illicit consumption of methamphetamine is a more complex problem because of geographical differences in potential sources and the dominant enantiomer (or formulation), which may impact human excretion rates (Gracia-Lor et al., 2016). This study estimated methamphetamine consumption based on occurrence of the parent compound and assuming a racemic mixture with a mean excretion ratio of 23% (CF = 4.4); the same approach and correction factor were also applied to MDMA (Gracia-Lor et al., 2016).

As discussed later, cocaine and its metabolites are highly unstable even when samples are quenched and preserved, although the overall mass balance appears to be conserved when simultaneously accounting for cocaine (CF = 1.00), benzoylecgonine (BZE) (CF = 1.05), ecgonine (ECG) (CF = 1.64), ecgonine methyl ester (EME) (CF = 1.52), and norcocaine (NOR) (CF = 1.05) (Figure S7). Thus, cocaine consumption was estimated based on the mass balance of these compounds after adjusting for cocaine-equivalent mass (see CFs above), albeit with no adjustment for metabolism due to uncertainty in the true metabolic profile. Similar to cocaine, the parent compound THC and one of its major metabolites (THC-OH) also exhibited stability issues (Figure S10). Thus, the more stable metabolite (THC-COOH) was selected as the principal surrogate for estimating consumption. Gracia-Lor et al. (2016) proposed an overall CF of 182 for THC-COOH based on smoking, but the current study adopted urine-derived excretion ratios because the methods in this study were not optimized for recovery from solids (i.e., feces), to which THC and its metabolites preferentially partition (Campos-Mañas et al., 2022). In other words, detection of THC-related compounds was assumed to be derived entirely from urine, which has published THC-COOH excretion ratios of 2.2% for oral ingestion and 0.5% for smoking (CF = 67.6 after averaging and adjusting for molecular weight) (Gracia-Lor et al., 2016).

With respect to opioids, Huddart et al. (2018) reported that 72% of an oxycodone dose is excreted in urine, with 8% of the original dose excreted in urine as the parent compound. After extrapolating the remaining 28% to feces and assuming a similar metabolic profile, a total of 11% of an oxycodone dose may be excreted in urine or feces as the parent compound (CF = 9.00). Hydrocodone and tramadol consumption estimates were also based on occurrence of the parent compounds, with assumed CFs of 15.4 (Dhillon, 2016) and 3.33 (OMP, 2003), respectively. Because methadone was sometimes <MRL in certain sewersheds, its consumption was estimated based on the major metabolite 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP), for which excretion has been estimated at 5-25% of the original methadone dose (CF = 7.97 as the average of that range and after adjusting for molecular weight) (Broussard, 2019). Consumption of fentanyl was based on occurrence of the major metabolite norfentanyl (CF = 3.58), assuming an average of reported excretion ratios (26-55%) (Cummings et al., 2016) and molecular weight adjustment.

Based on the recommendation of Gracia-Lor et al. (2016), morphine was used to develop a more reliable estimate of heroin consumption, as morphine is more abundant than the unique metabolite acetylmorphine. However, it was first necessary to differentiate heroin-derived morphine from therapeutic morphine. Jakobsson et al. (2021) reported median urine concentrations of 3.07 mg/mL for morphine and 0.47 mg/mL for acetylmorphine upon heroin intake, resulting in a morphine:acetylmorphine ratio of 6.5. For the current study, observed acetylmorphine concentrations (or 1/2×MRL) were multiplied by 6.5 to estimate the heroin-derived morphine concentration, and then therapeutic morphine was calculated as the difference between observed and heroin-derived morphine. Using a similar approach, morphine contributions from codeine metabolism were determined to be negligible. To estimate heroin consumption, heroin-derived morphine was multiplied by CF = 3.07 (accounts for both excretion and molecular weight) (Zuccato et al., 2008), and to estimate direct morphine consumption, therapeutic morphine was multiplied by CF = 11.1 (Andersen et al., 2003). Finally, codeine concentrations were multiplied by the average of CF values reported in Gracia-Lor et al. (2016) (CF = 1.50).

2.6 Statistical Analyses

Statistical analyses and boxplot visualizations were conducted in RStudio version 2023.03.1 (R Core Team, 2022). For boxplots, the box displays the interquartile range (IQR), the median is shown as the center line, whiskers extend to the furthest data points within ±1.5×IQR, and outliers are defined as data points exceeding 1.5×IQR. Significance was determined using the non-parametric Pairwise Wilcoxon Rank Sum Test, with a Bonferroni correction to account for multiple comparisons and reduce the likelihood of obtaining falsely rejected hypotheses.

3.0 Results and Discussion

3.1 Laboratory Hold Time Study

The concentration profiles and mass balances from the laboratory hold time study are summarized in Figures S1-S10. With respect to wastewater surveillance applications, the main conclusion is that wastewater samples that have been quenched, preserved, and refrigerated should yield reliable data if processing and analysis occurs within 7 days of sample collection. However, there are data interpretation caveats for cocaine-, THC-, and heroin-related compounds, as discussed below along with observations related to the other water matrices.

All opioid compounds were clearly attenuated in the non-quenched drinking water sample, presumably due to free chlorine exposure; norfentanyl demonstrated slightly greater stability. Among the non-opioids, MDMA, MDA, amphetamine, and, to a slightly lesser extent, methamphetamine were also attenuated in the non-quenched drinking water sample. It was not possible to deduce any reactivity between free chlorine and the cocaine-related compounds because of their general instability in all water matrices.

Cocaine, EME, and NOR decreased in concentration regardless of water matrix and quenching/preservation, and corresponding increases in concentration were observed for BZE and ECG. As noted earlier, the overall mass balance for the cocaine-related compounds was generally conserved across the hold time study, albeit to a lesser extent for the spiked DI samples.

THC and its two major metabolites were <MRL in both drinking water samples. THC and THC-OH exhibited moderate instability in wastewater and minor instability in DI water, and although unstable in drinking water, THC-COOH was quite stable in DI water and quenched/preserved wastewater. Importantly, the THC mass balance was generally conserved within the 7-day timeframe.

Similar to cocaine, heroin was unstable in the various water matrices, but it was unique in that quenching/preservation led to even greater instability. Although not confirmed in the current study, ascorbic acid addition is known to dissolve heroin and promote conversion to active byproducts, including acetylmorphine (Andersen et al., 2021). Thus, biological preservation only (i.e., no ascorbic acid addition) might be advisable when oxidants are not present, particularly when analyzing for heroin. Despite attenuation of the parent compound, the overall heroin mass balance in wastewater was still conserved within 7 days due to corresponding increases in acetylmorphine and/or morphine, with codeine remaining relatively constant.

3.2 Simulated Sewer Transport Study

As noted earlier, the maximum travel time for this wastewater surveillance study was estimated at approximately 24 hours (sewershed 1). For reference, typical travel times are much shorter, with the national median estimated to be approximately 3 hours (Kapo et al., 2017). The results from the simulated sewer transport study (Table S4) indicate that the following target compounds might be consistently and significantly attenuated (e.g., >10%) by the end of a 24-hour travel period: acetaminophen, cocaine, EME, NOR, and THC-COOH. Other problematic compounds identified in the earlier hold time study, including heroin, THC, and THC-OH, were <MRL at time zero so their degradation could not be assessed in these experiments. As expected, certain compounds increased in concentration due to parent compound conversion, including BZE and ECG; other byproducts/metabolites that were expected to increase were likely limited by low concentrations of the parent compound (e.g., acetylmorphine and morphine for heroin). Interestingly, codeine is a parent compound, but it consistently increased in concentration beyond 24 hours. This is potentially due to deconjugation of a major metabolite, as this was not observed when reagent-grade compounds were spiked during the hold time study. Some compounds exhibited significant attenuation after ‘traveling’ for >48 hours, including amphetamine, caffeine, and ibuprofen. Finally, certain compounds (e.g., fluoxetine and triclosan) exhibited artificially large percent changes that appeared to be driven by low occurrence coupled with variability between time points.

Thus, the simulated sewer transport study largely supported the results and conclusions of the hold time study, particularly in relation to the instability of cocaine- and THC-related compounds. It also highlighted the instability of the highly biodegradable PPCPs, including acetaminophen, caffeine, and ibuprofen.

3.3 Trend Analysis – 2010 vs. 2022-2023

A direct comparison between sewershed 1 concentrations observed in 2010 (Gerrity et al., 2011) versus 2022-2023 is provided in Table S34, and all raw data for the current study are illustrated in Figure S14 and summarized in Tables S8-S33. Relative to 2010, the population of Southern Nevada has increased by ~20%, and visitor volume to the Las Vegas Strip has increased by ~10%. However, sewershed 1 flow rates have remained relatively flat at ~100 mgd. Thus, concentrations might be expected to increase by 10-20% simply due to the increase in loading at the same flow rate. Some of the more common indicators of anthropogenic activity in wastewater, including carbamazepine, primidone, sulfamethoxazole, and trimethoprim, had similar concentrations in 2010 and 2022-2023. Interestingly, meprobamate (a licit anxiolytic drug) exhibited an ~80% decrease in concentration since 2010, presumably due to its declining use in recent years (James et al., 2016).

Based on averages of the recent influent and primary effluent samples, illicit drug/metabolite concentrations generally increased since 2010, including acetylmorphine (>199%; <25 ng/L in all 2010 samples), morphine (57%), methamphetamine (225%), amphetamine (172%), and cocaine (43%; based on mass balance). MDMA decreased by 43% relative to those two dates in 2010, but based on the current study, MDMA concentrations are somewhat sporadic in nature and are significantly impacted by special events. Thus, it is unclear whether the recent decrease is indicative of a reliable trend. Norfentanyl and THC-COOH were not included in the 2010 study so consumption of fentanyl and THC could not be compared. THC and THC-OH were <100 ng/L in all 2010 primary effluent samples, and although they were periodically detected in the more recent influent samples, they were <1,000 ng/L in the single 2022 primary effluent sample.

One of the primary conclusions from the 2010 study was that major sporting events influence markers of human behavior in wastewater (e.g., concentrations of the major cocaine metabolite BZE during the Super Bowl relative to a baseline weekend). Using the mass balance approach in the current study, cocaine use peaked for sewershed 1 on 2/13/23—the day after the Super Bowl—and on 3/20/23—during the 'March Madness' college basketball tournament—at cocaine-equivalent concentrations of 8,237 ng/L and 8,609 ng/L, respectively. The average of all other sampling dates was 5,484±927 ng/L.

For the more recent samples, it is also interesting to note that the most biodegradable compounds, including acetaminophen, caffeine, ibuprofen, and naproxen, seemingly decreased in concentration by >80% through the headworks and primary clarifier, which have a hydraulic retention time of ~5 hours (Gerrity et al., 2021). In contrast, the simulated sewer transport study indicated that ≥24 hours were needed to observe significant degradation of these compounds. Importantly, the concentration of the highly stable compound sucralose was also 55% lower in the primary effluent. This instead suggests that the lower concentrations for some compounds in the primary effluent (equivalent to ~5:00 am influent) were likely driven by diurnal variability in loading and to a lesser extent degradation. A similar conclusion was reached for SARS-CoV-2 in an earlier wastewater surveillance study (Gerrity et al., 2021). This was an initial indication of the importance of sucralose for data interpretation, similar to the use of PMMoV for normalization of SARS-CoV-2.

3.4 Normalization Approach and Sewershed Comparisons

Per capita sucralose and metabolism-corrected caffeine loadings for each sewershed are illustrated in Figure 2. Notably, sewersheds 1 and 4A exhibited significantly higher loadings for both compounds relative to all other sewersheds ($p < 0.05$). Assuming the sewersheds with lower loadings were more representative of the general population, average sucralose and caffeine consumption in Southern Nevada was approximately 26 mg/person-day—equivalent to two packets of Splenda (UAB, 2023)—and 923 mg/person-day, respectively. For additional context, Senta et al. (2015) reported a mean observed loading for caffeine (without metabolism correction) of 14±5.2 mg/person-day across 13 Italian cities in 2012. After removing the metabolism correction, observed caffeine loadings in the current study averaged 49±8.2 mg/person-day for sewersheds 1 and 4A, and 28±7.7 mg/person-day across the remaining sewersheds.

Per capita caffeine consumption varies widely by country but is estimated to be 76 mg/person-day in the U.S. (Rodak et al., 2021), which is considerably lower than the wastewater-derived consumption estimate in the current study (i.e., 923 mg/person-day after metabolism correction). It is possible that this discrepancy stems from disposing of caffeine-containing products (e.g., coffee) down drains coupled with human consumption and excretion. Assuming non-ingested caffeine warrants no metabolism correction and that actual consumption equates to wastewater loadings of 2.3 mg/person-day (76 mg/person-day × CF of 0.03), only ~10% of the observed loadings were linked to actual ingestion. After applying the CF only to the ingested fraction, the modified per capita consumption estimate for caffeine, accounting for both ingestion and disposal, was 101 mg/person-day.

The elevated loadings for sewersheds 1 and 4A are likely explained by the confounding effects of visitors, who are not reflected in the reported population, and bypass flow/solids contributions from a neighboring sewershed, respectively. To a lesser degree, inaccuracies in allocating populations to each sewershed might have also contributed to these deviations. These issues are also apparent when comparing per capita wastewater generation rates between sewersheds, with 1 and 4A at ~117 gallons per capita per day (gpcd) and the other sewersheds at 64±16 gpcd. This explains why the per capita loadings but not necessarily the concentrations of the target compounds were elevated in these particular sewersheds. To adjust for these confounding effects, the aforementioned average per capita loadings for sucralose and caffeine were used in conjunction with the sewershed 1 and 4A loadings to estimate their true populations (Figure 2). Because of its greater stability, sucralose was assumed to provide a more reliable estimate, resulting in adjusted populations of approximately 1.7 million and 259,000, respectively. Considering the weekly visitor (~777,000) and air traveler estimates for sewershed 1, this revised population estimate is entirely plausible.

To eliminate these population issues and allow for direct sewershed comparisons over time, target compound concentrations were normalized to their corresponding sucralose concentrations as a surrogate for human urine/fecal load. To assess how this might impact identification of outlier events (e.g., events that might trigger public health action), absolute vs. sucralose-normalized methamphetamine concentrations were compared (Figure 3). Both approaches generally led to identification of the same outliers, but based on further characterization of those outliers, the normalization approach appeared to be slightly more sensitive and discerning (Figure 3).

This sucralose normalization approach was then used to assess opioid occurrence by sewershed (Figures 4 and S11-S13). The data indicated significantly greater use of heroin (based on acetylmorphine) and fentanyl (based on norfentanyl) in sewershed 3 ($p < 0.05$; Figure 4). However, several extreme outliers in sewershed 6 resulted in statistical similarity with the consistently elevated concentrations in sewershed 3. Otherwise, sewershed 6 was not particularly notable for fentanyl nor heroin, but all other opioids, including morphine, methadone, codeine, oxycodone, hydrocodone, and tramadol, were significantly elevated. Importantly, this was not an artifact of normalization (i.e., low sucralose concentrations), as sewershed 6 only had significantly lower sucralose concentrations than sewershed 4B ($p = 0.02$). This suggests that more controlled opioid use was common in this particular community, but there were also concerning spikes in illicit opioid use.

With respect to public health action, these analyses not only yielded relative comparisons between sewersheds, which might be helpful for long-term strategic planning, but they also highlighted specific outlier samples, potentially identifying opportunities for targeted intervention. Specific dates associated with outlier events are summarized in Tables S5-S6. A notable example is sewershed 2, which is characterized as a higher income area with a large retirement-age population (Vo et al., 2022). Sewershed 2 exhibited moderate occurrence of the licit opioids but significantly lower concentrations of acetylmorphine and norfentanyl relative to many of the other sewersheds (Figure 4). The outliers for sewershed 2 were associated with low sucralose-normalized concentrations, but given the seemingly low use of heroin and fentanyl in this area, this approach still highlighted these events as potential targets for public health investigation and/or intervention. However, it is important to consider that although such spikes may be a harbinger of future overdose deaths, they may not capture individuals who succumb to an overdose without excreting the marker to the sewer collection system (K. Pulver, personal communication, May 3, 2023). Thus, wastewater and overdose data may not necessarily align in all situations.

The norfentanyl timeline in the current study was particularly interesting (Figure 5). Prior to October 2022, only 2 of 88 samples (2%) contained norfentanyl above the MRL of 50 ng/L. Both of these early 'hits' were in sewershed 4A, with one occurring on the Memorial Day holiday. After October 2022, 80% of all samples contained norfentanyl above the MRL, with some sewersheds at 100% frequency and sewershed 2 accounting for 50% of the remaining <MRL samples. Norfentanyl was likely present prior to October, albeit censored by the relatively high MRL of 50 ng/L, but the stark change in concentration profile after October points to an increase in consumption in Southern Nevada, particularly since there were no experimental or analytical changes. This change coincided with the Southern Nevada Health District issuing an advisory noting an increase in local fentanyl deaths (SNHD, 2022) and related news coverage of fentanyl seizures (Gutierrez, 2022).

Sucralose-normalized concentrations for non-opioids are summarized in Figures 6 and S13. These analyses highlighted high methamphetamine, cocaine, and MDMA concentrations in sewershed 3, particularly after the 'Life is Beautiful' music festival in September 2022 when the non-normalized MDMA concentration spiked to 1,100 ng/L—10-fold higher than typical concentrations. In fact, this date was an MDMA outlier for 5 of the 8 sewersheds. In general, MDMA occurrence was very sporadic in nature across all sewersheds except sewershed 3 (35% of samples >MRL) and sewershed 1 (92% of samples >MRL), which serves the Las Vegas Strip.

3.5 Consumption Estimates

Consumption of licit and illicit drugs is often described in the literature after normalization to population (e.g., mg/day per 1,000 people), as summarized in Figure 7 for the current study. Sewershed-specific summaries are provided in Table S7. Population-normalized consumption varies widely across studies (Huizer et al., 2021), in part due to real geographic and temporal differences but also due to differing assumptions related to metabolism (i.e., excretion correction factors). In the current study, log_{10}-transformed medians were as follows: 1-2 = codeine, fentanyl, and MDMA; 2-3 = heroin, hydrocodone, methadone, oxycodone, and tramadol; 3-4 = cocaine, methamphetamine, and morphine; 4-5 = sucralose and THC; and 5-6 = caffeine (not adjusted for ingestion vs. disposal). Similar values have been reported for U.S. wastewater for cocaine and MDMA, but the current estimate for methamphetamine exceeds nearly all values from Huizer et al. (2021), although the studies included in that review were all conducted in 2020 or earlier.

Finally, Table 1 summarizes sewershed-specific and overall consumption estimates (kg/year) for Southern Nevada. For the illicit drugs, these values represent quantities that were unable to be seized prior to consumption (or disposal), despite law enforcement efforts (W. Hall, A. Mehrotra, personal communication, May 25, 2023). These values also provide an opportunity to validate the general accuracy of wastewater surveillance, at least for the more regulated compounds. For example, $965 million (USD) in revenue was generated by marijuana sales in Nevada during the 2021-2022 fiscal year (Sauvageau, 2022). Assuming an average price of $10 per gram (OTC, 2023), the 60,485 kg of THC consumption estimated through wastewater surveillance would equate to ~$600 million. Although reasonably close, there is still a discrepancy between reported and estimated revenue; possible reasons include a decrease in sales in the following fiscal year (i.e., during the study period), uncertainty in converting THC-COOH concentrations to THC consumption, degradation of THC-related compounds during sewer transport, or perhaps purchase of THC in Nevada for consumption elsewhere.

4.0 Conclusions

Wastewater surveillance provides robust incidence and prevalence assessments for a wide range of infectious disease targets because it does not require people to seek testing. The passive nature of this tool may prove to be even more valuable for illicit targets with law enforcement implications—scenarios in which users largely prefer to avoid detection. There is a growing abundance of literature demonstrating the value of wastewater surveillance for characterizing illicit drug use throughout the world, but the industry will ultimately have to balance the potentially life-saving public health benefits against the legal/ethical concerns.

As with microbial targets, advancements in the science are needed to ensure actionable interpretation of data. This study further highlighted the growing epidemic of drug abuse, yielded valuable data for method optimization, and demonstrated sucralose normalization as a means of adjusting for confounded population estimates and varying human urine/fecal load. This complements recent efforts to refine metabolism correction factors (Gracia-Lor et al., 2016) and parameterize models for degradation in sewer collection systems (McCall et al., 2016). Even without these advanced considerations, illicit drug wastewater surveillance has the potential to alert public health officials to alarming shifts in consumption (e.g., fentanyl) or identify areas for targeted intervention (e.g., naloxone deployment, public health messaging). As research studies, pilot projects, and full-scale programs continue to demonstrate the utility of wastewater surveillance, it is prudent that policymakers and stakeholders secure long-term funding and broaden implementation of this powerful public health tool.

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Supplementary Information

A supplementary information file with additional method details, 14 figures, and 34 tables is available at XXX.

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