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Wastewater-based epidemiology pilot study to examine drug use in the Western United States

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HIGHLIGHTS

• Drug use assessed using wastewater-based epidemiology in western United States
• Comparison of rural community drug consumption rates with urban areas
• Highlighting the need for standardization in WBE testing

ABSTRACT

The extent of prescription and illicit drug abuse in geographically isolated rural and micropolitan communities in the intermountain western United States (US) has not been well tracked. The goal of this pilot study was to accurately measure drug dose consumption rates (DCR) between two select populations, normalize the data and compare the DCRs to similar communities. To learn about patterns of drug abuse between the two disparate communities, we used the emergent field of wastewater-based epidemiology (WBE). A rapid, quantitative and systematic process for the determination of multiple classes of prescribed and illicit drugs was applied to influent wastewater samples. Influent samples were collected over the course of three months (April to June 2019) at two wastewater treatment plants representing a small urban and a rural community. Collection of sewage influent included 24-h composite samples and the use of polar organic chemical integrative samplers (POCIS), time-weighted samplers. Using the results from the composite sampling data, DCRs per 1000 population could be calculated from the concentration data and the use of excretion correction factors. The following 18 compounds: amphetamine, methamphetamine, MDA, MDMA, morphine, 6-acetylmorphine, methadone, EDDP, codeine, benzoylecgonine, hydrocodone, hydromorphone, oxycodone, noroxycodone, ketamine, fluoxetine, tramadol, and ritalinic acid; represent a subset of the targeted analytes that were consistently measured at detectable concentration levels, and present at both sites. Following normalization of the drug measurements to influent flow rates and per capita, the small urban community demonstrated greater collective excretion rates (CER) than the rural community, with the exceptions of amphetamine and methamphetamine.
1. Introduction

The extent of prescription and illicit drug abuse in isolated rural and small urban (micropolitan) communities in the intermountain western United States (US) is information that is difficult to track. Databases, such as the Automated Reports and Consolidated Ordering System (ARCONS), in which controlled substances transactions are reported to the US Drug Enforcement Administration (USDEA), exist (USDEA, 2020). However, these reports have limitations, for example, in 2018 and 2019 the USDEA decided not to report data on methamphetamine, methylphenidate, and oxycodone. Consequently, gauging the prevalence of drugs flowing through a community is not always readily, or easily, available. The lack of records can leave state and community level officials, social workers, crisis counselors, and first responders, without enough knowledge to respond appropriately to their community’s focused health needs. Consequently, there is a need for rapid tools that can estimate community drug usage, effectiveness of drug interventions, and emerging drug threats. Wastewater testing is therefore an opportunity to establish individual usage anonymity, while informing public and environmental health community efforts with a faster turnaround time. Wastewater-based sewage epidemiology (WBE) has emerged as a non-intrusive response for estimating drug dose consumption rates (DCR) for communities, and to ground-truth with evidenced-based data (Banta-Green et al., 2009; Banta-Green et al., 2009; Baz-Lomba et al., 2017; Boles and Wells, 2014; Castiglioni et al., 2013; Gracia-Lor et al., 2017; Lin et al., 2019; Mastroianni et al., 2017; Mercan et al., 2019; Prichard et al., 2014; Thai et al., 2019; Zuccato et al., 2008). The methodology used in this study built upon procedures implemented in previous published sampling studies to determine drug concentrations and drug use in communities (Banta-Green et al., 2009; Boles and Wells, 2014; Jones-Lepp et al., 2004; Zuccato et al., 2008). This approach considered that the active parent compounds, or metabolic residues, of drugs ingested in the human body are excreted with urine and faeces into the sewer networks, and end up at a single-point, namely wastewater treatment plants (WWTPs) (Banta-Green et al., 2009; Zuccato et al., 2008). Several of these WBE studies have shown a good correlation with the occurrence of drugs in wastewater and community-use data (Banta-Green et al., 2009; Baz-Lomba et al., 2016; van Nuijs et al., 2011; Van Nuijs et al., 2009).

The goal of this pilot study was to accurately measure DCRs between two select populations, normalize the data and compare the DCRs to similar communities. A rapid, quantitative and systematic process for the determination of multiple classes of prescribed and illicit drugs (44 different compounds and their isotopic analogues), including opioids, benzodiazepines, and amphetamines, in influent wastewaters, was developed in order to generate the WBE calculations.

2. Materials and methods

2.1. Chemicals

All target compounds (SI Table 1), as well as the corresponding isotopically labeled analogues used as internal and surrogate standards (SI Table 2), were purchased from Cerilliant (Round Rock, TX). The high purity (>98%) solutions were provided in either methanol or acetonitrile at concentrations of 1000 μg mL⁻¹, or 100 μg mL⁻¹. Separate working solutions, containing a mixture of all target analytes at 0.1 ng μL⁻¹, and all internal standards at 0.25 ng μL⁻¹, were prepared in LCMS grade (Optima™) methanol (MeOH) (Fisher Scientific; Hampton, NH) and stored in the dark, in amber vials, at −20 °C. LCMS grade water, formic acid (FA, 99.9%), ammonium formate (10.0 M), and ammonium acetate (NH₄Ac, 5.0 M) were purchased from Fisher Scientific (Hampton, NH). Ammonium hydroxide (NH₄OH, 28%), toluene, dichlorodimethylsilane (DCDMS, ~99%), and hydrochloric acid (HCl, 1.0 M) were all high purity grade and purchased from Fisher Scientific (Hampton, NH).

2.2. Site description and sampling design

A total of 20 influent samples were collected from two WWTPs, Site A and Site B, located in an intermountain western US state. The first WWTP was located in a small micropolitan community (population ~ 45,000, Site A), while the second WWTP was located in a small, geographically isolated rural community (population ~ 4000, Site B) (USDA, 2020). Both WWTPs separate their domestic sewer flows from their stormwater flows. Flow rates were averaged over the course of the sample collection period, April 2019 to June 2019, Site A averaged 27 megaliters per day (MLD), while Site B averaged 2 MLD. Both sites had influent headworks with grit removal. Site A had both primary and secondary clarifiers, while Site B used only secondary clarifiers. Site A used a 5 Stage Bardenpho aeration basin for activated sludge, and Site B used an oxidation ditch with surface agitator aeration. Both sites used ultra-violet (UV) radiation for disinfection as the final step of their treatment process before release into the nearby surface waters.

2.2.1. Composite sampling

At Site A, 24-h composite samples were obtained, beginning on Monday morning through Tuesday morning, using a permanently installed automatic composite sampler. Samples were then transferred into low-density polyethylene (LDPE) bottles. Site B samples were collected beginning on Tuesday morning through Wednesday morning using an Aquacell P2 Coolbox (Lower 48 Instruments; Dayton, OH) in a time-dependent manner (100 mL hr⁻¹). Samples were transferred from collection containers through a 0.2 μm Nalgene Rapidflow Sterile Disposable Bottle Top Filter into 250 mL glass bottles, or 250 mL Nalgene™ bottles. All media bottles were wrapped in Parafilm for transportation and storage. Approximately 150 mL of sample were sent to a partner clinical laboratory (Assurity Laboratory, Las Vegas, NV) for extraction and analyses. Samples were kept on ice during transportation to the laboratory, where they were refrigerated at 4 °C until extraction, usually within one to two days upon receipt of the samples. Field blanks consisted of laboratory DI water, stored in the same collection bottles used for wastewater samples. The field blanks were transported into the field and exposed to the same environment as the samples at every sample collection and processing step.

2.2.2. Passive sampling

The polar organic chemical integrative samplers (POCIS) are passive samplers that were used as a complementary sampling technique. The POCIS sample consists of dissolved organic chemicals with moderate to high hydrophilicity in water (Alvarez et al., 2004), this occurs through a diffusion process where chemicals permeate a microporous polyethersulfone membrane and become trapped on a solid sorbent (Oasis HLB). A deployment canister, containing four POCIS devices, were deployed for a 30-day time period in the influent wastewaters at Site A. After 30-days the samplers were removed from the field and were sent to the United States Geological Survey (USGS) laboratory (Columbia, MO), and stored at ≤−20 °C until extraction of the devices. The sorbed chemicals have been shown to be stable on the POCIS devices when frozen over long periods of time until extraction (Alvarez et al., 2004).

2.3. Analytical methodology

2.3.1. SPE extraction methodology

A unique solid-phase extraction (SPE) method was developed in order to sequester various classes (amphetamines, opioids, benzodiazepines, etc.) of drugs, their metabolites, and isotopic analogues, from influent wastewaters. Briefly, a 100-ml aliquot of wastewater was removed from the initial sample collected and acidified to pH ~2 to preserve samples by inhibiting microbial degradation and transformation and to ensure full recovery of the analytes of interest during the SPE process. Internal standards (30 μL of 0.25 ng μL⁻¹) and 0.5 g of sodium bicarbonate (NH₄Ac, 5.0 M) were purchased from Fisher Scientific (Hampton, NH).
chloride (to enhance analyte recoveries) were added to all samples. The extractions were carried out using a Promochrom (Vancouver, BC, Canada) automated SPE-03 unit, using Oasis MCX (Mixed-mode Cation eXchange; 6 cc, 150 mg, Waters Corp., Milford, MA) cartridges. Extracts were evaporated under a gentle stream of nitrogen at ambient temperature to near dryness and then reconstituted in 100 μL 95% water/5% MeOH (0.1% FA). More details can be found in the Supplemental information (SI) file.

2.3.2. POCIS extraction process

The POCIS were removed from the deployment canisters, gently cleaned under deionized water to remove any surficial particles, and allowed to air dry prior to extraction. Two POCIS from each canister were extracted with methanol and those extracts were combined to create a composite sample to increase the analyte mass available for detection (Alvarez et al., 2004; Jones-Lepp et al., 2004). The remaining POCIS in the deployment canisters were archived for future analyses. Following extraction, the extracts were concentrated to a 1-mL volume, flame sealed in amber ampoules and shipped to our partnering laboratory (Las Vegas, NV) for LC-MS/MS analyses. Procedural detail can be found in the SI.

2.3.3. Liquid chromatography - tandem mass spectrometry analysis

A liquid chromatography-tandem mass spectrometry (LC-MS/MS) method was employed to analyze low (ppt to ppb) drug levels in extracted wastewater influent samples. A complete list of all targeted compounds is located in SI Tables 1 and 2. All LC-MS/MS data were collected using a Shimadzu 8050 triple quadrupole mass spectrometer, operating in the positive electrospray mode (ESI+), coupled to a Nexera HPLC (Shimadzu; Kyoto, Japan). Analyte separations were achieved using a Phenomenex (Torrance, CA) Kinetex Phenyl-Hexyl reversed phase HPLC column (50 × 4.6 mm, 2.6 μm), and corresponding guard column. The HPLC column, and guard column, were kept at 40 °C throughout all chromatographic runs. Mobile phase A was 0.1% ammonium formate in water and mobile phase B was 0.1% FA in MeOH. An injection volume of 15 μL and total flow rate of 0.700 mL min⁻¹ were used. Retention times, precursor ions, and product ions monitored for the non-labeled compounds are listed in SI Table 1, and the labeled compounds are listed in SI Table 2. More details, on the LC-MS/MS method can be found in the SI file.

2.4. Quality control and quality assurance (QA/QC)

Every sampling event had a field blank (consisting of clean laboratory DI water) accompany the samples through the process flow—from collection in the field, to the extraction and analysis in the laboratory. The POCIS included both a laboratory (fabrication) and field blank. For quality control purposes, every sample batch had one spike and one duplicate, extracted and analyzed alongside the wastewater samples. All samples were spiked with the same level of internal standards to: (1) compensate for matrix effects during LC-MS/MS analyses; (2) to accurately quantitate the compounds; and (3) to compensate for any potential loss of the analytes during the extraction process. The results from the spikes, blanks, and duplicates, are located in SI Tables 3 and 4.

For calibration and quantification of the LC-MS/MS prior to each analytical run, a set of seven calibration standards were prepared from the working standards in concentrations ranging from 1 to 1000 ng mL⁻¹. All calibrators were prepared in the same diluent as was used for the reconstitution of the extracts. The limit-of-quantitation (LOQ) for the analytes in this study was determined at 10 ng/L (10 parts per trillion). These LOQ values had previously been determined, from repeated measurements which could be reliably determined at 10 times the signal-to-noise on the Shimadzu LC-MS/MS (Loftberg, 2019, personal communication).

3. Results and discussion

The goal of this pilot study was to optimize and accurately measure drug consumption rates such that WBE data could be compared between two pre-selected populations, a western urban and rural region. Results are discussed below, while specific method optimization with quality control and assurance practices are further discussed in the SI file.

3.1. Wastewater-based epidemiology

Wastewater-based sewage epidemiology was used to provide a snapshot of drug use in two communities, one micropolitan (Site A), and one rural (Site B). The data generated allowed consideration of drug use trends over a three-month timeframe, and generated WBE results for the following 18 compounds: amphetamine, methamphetamine, MDA, MDMA, morphine, 6-acetylmorphine, methadone, EDDP, codeine, benzoylecgonine, hydrocodone, hydromorphone, oxycodone, noroxycodone, ketamine, fluoxetine, tramadol, and ritalinic acid. These 18 compounds represent a subset of the targeted analytes that showed up consistently, with detectable concentration levels, and identified at both sites. Raw concentration levels of the targeted drugs ranged from non-detect (ND) to ppb (μg/L) levels at both sites, see Table 1.

3.1.1. Measured concentrations converted to collective excretion rates

The Collective Excretion Rate (CER) is the daily amount of a targeted drug reaching the influent into a WWTP. In order to correctly calculate the doses consumed by a community, the concentration (ng/L) of the target drug A must first be multiplied by the flow rate (L per day) of the influent wastewater. Assuming that there are no leaks in the sewer system, the CER, calculated as grams per day (g/day), should give a sensible value upon which to base the consumption rates.

The CERs were calculated using the following equation:

\[
A \text{ (ng/L)} \times \text{Influent flow rate (L/day)} \times \frac{1}{10^6} \text{g/} \text{g} \times \text{day} = \text{CER (g/day)} \tag{1}
\]

Raw concentrations of some of the targeted drugs at Site B were higher compared to Site A. However, when the raw drug concentration data was normalized against the inflow flow rates; Site A, showed greater CERs than Site B, with the exceptions of amphetamine and methamphetamine.

3.1.2. Collective excretion rates extrapolated to dose consumption rates

The dose consumption rate (DCR) is the amount of excreted targeted drug consumed by the population served by the WWTP. In order to extrapolate the DCR from the CER, there needs to be an accounting of what fraction of the targeted drug is actually excreted and then a correction factor (CF) applied to the concentration in order to take into account the percentage of excretion. See Table 2 for a list of CFs. Some targeted drugs had a range of excretion rates; therefore, both the high and low CFs are reported in Table 2.

The DCRs were calculated using the following equation:

\[
\text{CER (g/day)} \times \text{CF} = \text{DCR (g/day)} \tag{2}
\]

For some compounds it is difficult to interpret their DCRs, due to the complexity of their excretion patterns. For example, 50–70% of codeine is primarily metabolized to codeine-6-glucuronide (Cone et al., 1991b). However, bacterial degradation reverts the Phase II codeine metabolite back into the parent compound, codeine. This is not unusual chemical behavior, as others have demonstrated this occurrence with the Phase II pharmaceutical metabolite acetaminophen glucuronide in wastewater (Sunkara and Wells, 2010). Another compound, morphine forms two major conjugated metabolites, designated as M3G and M6G, which comprise 57.3% and 10.4% of the dose excreted as residue.
was 100% representative of the amount of cocaine present.

Before the CER calculation was based on the premise that benzoylecgonine (Warner and Norman, 2000) There are no CF values for cocaine nor benzoylecgonine; there-

of the parent molecule forming benzoylecgonine (Warner and Norman,

occurs rapidly (within 1.5–2 h), both enzymatically and spontaneously of the parent molecule forming benzoylecgonine (Warner and Norman,

respectively (Cone et al., 1991b). As with codeine, bacterial degradation in the sewer can revert the two morphine metabolites back into the parent compound, morphine (van Nuijs et al., 2011). Therefore, the CF calculations for codeine and morphine were based on the percentage of dose excreted as residue rates, see Table 2. For this study heroin was not monitored, but instead 6-acetylmorphine, the main metabolite of heroin, was monitored (Cone et al., 1991a). Heroin is nearly 100% ex-

pected to normalize the data so that the data in this study could be com-

pared to other communities globally. While the numeric data presented

means to normalize the data so that the data in this study could be com-

per 1000 population (pop) (Eq (3b)). The latter calculation provided a

approach was to take the DCR (g/day) divided by the actual num-

ber of the community. The data were calculated two different ways (Eqs.(3a) and (3b)), one method was to take the DCR (g/day) and divide it by the actual pop-

ulation of the community, divided by 1000, to get dose consumed in grams per day per 1000 population (pop) (Eq. (3b)). The latter calculation provided a

means to normalize the data so that the data in this study could be com-

pared to other communities globally. While the numeric data presented remains the same value, it should be noted that the units are different.

3.1.3. Extrapolating DCR to dose consumed per population

The final part of estimating a community’s drug usage is to extrapolate from the DCR (g/day) to the dose consumed by the population of the community. The data were calculated two different ways (Eqs. (3a) and (3b)), one method was to take the DCR (g/day) and divide it by the actual population of the community to get a per capita dose (Eq. (3a)). The other approach was to take the DCR (g/day) divided by the population of the community, divided by 1000, to get dose consumed in grams per day per 1000 population (pop) (Eq. (3b)). The latter calculation provided a means to normalize the data so that the data in this study could be compared to other communities globally. While the numeric data presented remains the same value, it should be noted that the units are different.

These calculations are as follows:

\[
(DCR \text{ (g/day)/population)} \times 10^{3} \text{ mg/g} = \text{Dose consumed mg/day per capita (3a)}
\]

\[
(DCR \text{ (g/day)/population)} / 10^{3} = \text{Dose consumed g/day per 1000 pop (3b)}
\]

Table 1

| Compounds          | Average (ng/L) | Site A influent | Average (nmol/L) | Detection frequency | Average (ng/L) | Site B influent | Average (nmol/L) | Detection frequency |
|--------------------|----------------|----------------|------------------|---------------------|----------------|----------------|------------------|---------------------|
|                    |               | April–May–June 2019 |                  |                     |               | April–May–June 2019 |                  |                     |
|                    | (n = 10) Min–Max |                  |                  | %                  | (n = 10) Min–Max  |                  |                  | %                  |
| 6-Acetylmorphine    | ND             |                 | 0.395            | 100                 | ND             |                 | 193              | 100                 |
| Codeine             | 178            | 193             | 100              | 0                   |
| Morphine            | ND–529         | 193             | 100              | 0                   |
| Hydrocodone         | 41             | 103             | 100              | 0                   |
| Norhydrocodone      | ND–36.2        | 103             | 100              | 0                   |
| Hydromorphone       | 38             | 108             | 100              | 0                   |
| Oxycodone           | 47             | 86              | 100              | 0                   |
| Noroxycodone        | 54             | 1.284           | 100              | 0                   |
| Oxymorphone         | 193            | 100             | 100              | 0                   |
| Tramadol            | 674            | 1357            | 100              | 0                   |
| Buprenorphine       | ND–15.2        | 0.133           | 100              | 0                   |
| Naloxone            | 0              | 0.050           | 100              | 0                   |
| Alprazolam          | 0              | 0.014           | 100              | 0                   |
| Ahydroxylpazolam     | 0              | 0.081           | 100              | 0                   |
| Dazepam             | 0              | 0.126           | 100              | 0                   |
| Nor Diazepam        | 0              | 0.080           | 100              | 0                   |
| Temazepam           | ND–34.1        | 0.113           | 100              | 0                   |
| Oxazepam            | ND–25.3        | 0.088           | 100              | 0                   |
| Cyclobenzaprine     | ND–15.6        | 0.097           | 100              | 0                   |
| Methadone           | 19             | 0.061           | 100              | 0                   |
| EDDP                | 54             | 0.064           | 100              | 0                   |
| Meperidine          | 0              | 0.064           | 100              | 0                   |
| Tapentadol          | ND–63          | 0.046           | 100              | 0                   |
| Norpropylone        | ND–12          | 0.024           | 100              | 0                   |
| Naltrexone          | ND             | 0.165           | 100              | 0                   |
| Ritalinic Acid      | 235            | 243             | 100              | 0                   |
| Amphetamine         | 530            | 147–340         | 100              | 0                   |
| Methamphetamine    | 750            | 763–1339        | 100              | 0                   |
| Benzoylecgonine     | 818            | 5421            | 100              | 0                   |
| MDA                 | ND–53          | 203             | 100              | 0                   |
| MDMA                | 73             | 41–121          | 100              | 0                   |
| MDEA                | ND             | 51–84           | 100              | 0                   |
| PCP                 | ND             | 48              | 100              | 0                   |
| Ketamine            | 36             | 0.151           | 100              | 0                   |
| Nor Ketamine        | ND             | 0.067           | 100              | 0                   |
| Butyline            | ND             | 0.268           | 100              | 0                   |
| MDPV                | ND             | 0.440           | 100              | 0                   |
| Methyline           | ND             | 0.380           | 100              | 0                   |
| Methylene           | ND             | 0.268           | 100              | 0                   |
| Ethylone            | ND             | 0.440           | 100              | 0                   |
| Fentanyl            | ND             | 0.380           | 100              | 0                   |
| Zolpidem tartrate   | ND             | 0.268           | 100              | 0                   |
| Fluoxetine          | ND–114         | 0.360           | 100              | 0                   |
| Norfluoxetine       | ND–35          | 0.119           | 100              | 0                   |
3.2. Pilot study limitations

3.2.1. Sample collection and holding times

Preanalytical measurement error may occur during sample collection and sample processing. The logistics of the community sampling events required a 6-hour round trip drive, 1–2 days post-collection, packing and overnight shipping to the laboratory for processing. This sample holding time-frame is consistent with other peer-reviewed studies. For example, Zhao and Metcalf, state a one-week sample holding time at ~4 °C (Zhao and Metcalf, 2008); Kasprzyk-Hordern et al. report a one-week sample holding time at ~4 °C (Kasprzyk-Hordern et al., 2007); and in Jiang et al. they describe two weeks of refrigerated storage before extracting (Jiang et al., 2015). While we earnestly attempted to minimize preanalytical error due to storage time from collection to extraction, travel logistics to collection sites contributed on average 3 to 4 days between sample collection and sample processing. Based on the previous publications, mentioned earlier, we did not anticipate a substantial change in drug measurements under these storage conditions. Furthermore, all samples were filtered upon collection, and prior to shipment, with a 0.2 µm Nalgene Rapidflow Sterile Disposable Bottle Top Filter. This was done according to our university’s safety sample collection protocols. It is acknowledged that the process of filtering may remove a fraction of some drug analytes, especially if they are more readily adsorbed onto any particulate matter. However, due to the hydrophilicity characteristics of most of these measured drugs (log D<sub>7,4</sub>s between −1 and +1), this was not considered a primary source of measurement error in this study (Wells, 2006).

3.2.2. POCIS sampling

Due to budget constraints the study could only employ one POCIS sampler at the influent entry Site A WWTP.

3.3. Comparing POCIS vs composite sampling for WBE study

A POCIS collection device was deployed in the Site A influent waste stream to study the versatility of POCIS vs. 24-h composite sampling. There are both advantages and limitations to each type of sampling methodology. For example, it is known that 24-h composite sampling on select days may miss a spike event. Over long holiday weekends it has been demonstrated that illicit drug use can rise and then revert to a more “normal” daily distribution (Gerrity et al., 2011). The POCIS, when deployed for 30-days, could potentially account for spike drug usage during the 30-day sampling deployment period. Conversely, if the intent is to better understand human drug abuse behavior over weekends vs daily use, then a 24-h composite sampling effort after the weekend would be more useful to show the rise and fall of drug consumption on a specific day.

A comparison between the time-weighted average water concentrations, estimated from chemical residues measured in the POCIS, with concentrations, estimated from chemical residues measured in the POCIS, with
described in Table 4. Overall, the POCIS underestimated the influent concentrations when compared to the median composite values, with approximately 48% of the values within 3-fold of each other. When taking into consideration the range of values reported in Table 1, the differences between the POCIS estimates and the 24-hour composite samples may be less than what they appear. Overall, the agreement between the two sampling types is better than may have been expected. One potential limitation contributing to a general decrease in drug detection may have been due to occlusion of the POCIS membrane over the deployment period by the buildup of solids on the sampler surface.

3.4. Comparison of WBE data from pilot study to other WBE data in the literature

In the last few years, there has been an exponential increase in WBE studies reported in the literature. Many of these studies emphasize applications of WBE in urban areas while this study highlights the utility of WBE for small, rural communities. A remarkable observation measured in this study is the level of methamphetamine flow through two representative western U.S. rural communities at rates up to 12-fold higher than reported for major metropolitan cities. At Site A and Site B, dose consumption rates for methamphetamine are 0.984 and 6.51 g/day per 1000 pop, respectively. This is in contrast to a methamphetamine dose consumption rate of 0.22 g/day per 1000 pop reported for Ho Chi Minh City, Vietnam (Nguyen et al., 2018), 0.45 g/day per 1000 pop in Istanbul, Turkey (Mercan et al., 2019), and 0.157 g/day per 1000 pop in Barcelona, Spain (Mastroianni et al., 2017). Dose consumption rates for methamphetamine for a few Australian cities were comparable to Site A, although none reached the levels detected from Site B (Bannwarth et al., 2019). The abuse of methamphetamines is corroborated in rural communities. However, it is important to note at least two potential sources of measurement error. Firstly, wastewater infrastructure serving large metropolitan communities is certainly more expansive than that of a small rural community, therefore, it is important to learn if the extended drug residence time in city wastewater infrastructures may lead to deterioration of the drug prior to collection at the WWTP influent site. Secondly, cities typically do not account for the transient influx of tourists and their contribution to wastewater, this factor may dilute the measurement of the drug delivered to the wastewater treatment plant.

Measured dose consumption rates for MDMA were similar from the small urban community when compared to some larger cities. In 2017, Mastroianni et al. reported WBE data from a 5-year monitoring study of drugs of abuse from a WWTP in Barcelona, Spain (Mastroianni et al., 2017). They reported on average MDMA dose consumption rates between 0.041 and 0.372 g/day per 1000 pop, which at the lower end was comparable with the level of Site A (0.066 g/day per 1000 pop). Sydney, Australia was comparable to Site A, with a dose consumption rate of 0.06 g/day per 1000 pop (Bannwarth et al., 2019).

Finally, a paper by Gushgari et al. (2018) has reported dose consumption rate data from a US Southwestern urban area (pop <600,000) (Gushgari et al., 2018). Their data focused on wastewater collection at a main sewer lateral from a local university that discharged to the local WWTP. As Site A includes a university population, some comparisons can be made. The cocaine consumption rate was similar, with approximately 0.6 g/day per 1000 pop, while Site A was 0.5 g/day per 1000 pop. Additionally, Site A had a MDMA consumption rate that was nearly 3-fold lower, while the codeine consumption rate was 10-fold higher, when compared to the Southwestern urban area (Gushgari et al., 2018). Understanding these trends in communities, including those with university populations, increases awareness of which drugs are most consumed, ultimately driving better drug intervention strategies.

4. Conclusions

The intersection of the COVID pandemic has exacerbated the opioid epidemic (Alexander et al., 2020). Not only can community wastewater screening serve as a drug test for the community, but SARS-CoV-2 sewage viral load can be monitored for early detection of rising infection rates (Ahmed et al., 2020; Becker and Fiellin, 2020; Daughton, 2020; Medema et al., 2020). By using wastewater to monitor viral and drug loads in a community, more effective intervention strategies may be implemented to collectively address virus spread including asymptomatic, social distancing and vaccine interventions in tandem with drug campaigns (Alexander et al., 2020; Daughton, 2020; Jenkins et al., 2020). As testing in municipal wastewater sites expands to monitor for infectious disease rates in communities, the need for standardization of testing becomes even more critical. As we compare the results of this study to other publications, we are cautioned when comparing rural sites to other populated locations. Advances in Europe, with the development of the Sewage Analysis Core Group (SCORE), lead the way in standardization of wastewater testing methods with over 6 years of data in interlaboratory split sample analysis and with the collaboration of 37 laboratories from 25 countries. Despite the rapid increase of the use of WBE in the U.S. in the last few years, we fall short of standardizing wastewater testing here. It is critical for this discipline to describe all the potential confounders in the collection and testing process to continue to advance the utility of WBE.

In summary, WBE continues to demonstrate utility in monitoring the prevalence of drugs flowing through WWTWs. Its promise of serving as a public health data tool will be driven by investigators’ efforts to ensure quality and standardization of testing methods. As we learn more regarding variables from collection to technical analyses to extrapolation, WBE will serve as a dependable resource for health professionals and policy makers in communities and states.

CRediT authorship contribution statement

Nicholas Bishop: Investigation, Formal analysis, Methodology. Tammy Jones-Lepp: Investigation, Formal analysis, Validation, Methodology, Supervision, Writing - original draft, Writing - review & editing. Miranda Margetts: Conceptualization, Writing - review & editing, Validation. Jordan Sykes: Investigation, Supervision. David Alvarez: Investigation, Formal analysis, Methodology. Deborah E. Keil: Conceptualization, Project administration, Funding acquisition, Resources.
Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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References

Ahmed, W., Angel, N., Bibby, K., Rivins, A.; O’Brien, J.W., et al., 2020. First confirmed detection of SARS-CoV-2 in untreated wastewater in Australia: a proof of concept for the wastewater surveillance of COVID-19 in the community. Sci. Total Environ. 738, 138764.
Alexander, C.C., Stoller, KB., Hajfaje, R.L., Saloner, R., 2020. An epidemic in the midst of a pandemic: opioid use disorder and COVID-19. Ann. Intern. Med. 173 (1), 57–58.
Alvarez, D.A., Petty, J.D., Huckins, J.N.; Jones-Lepp, T.L., Getting, D.T., Goddard, J.P., et al., 2004. Development of a passive, in situ, integrative sampler for hydrophilic organic contaminants in aquatic environments. Environ. Toxicol. Chem. 23, 1640–1648.
Bannwarth, A., Morelato, M., Benaglia, I., Been, F., Esseiva, P., Delemont, O., et al., 2019. The use of wastewater analysis in forensic intelligence: drug consumption comparison between Sydney and different European cities. Forensic Sciences Research 4, 141–151.
Banta-Green, C.J., Field, J.A., Chiaia, A.C., Sudakin, D.L., Power, L., De Montigny, L., 2009. The spatial epidemiology of cocaine, methamphetamine and 3,4-methylenedioxymethamphetamine (MDMA) use: a demonstration using a population measure of community drug load derived from municipal wastewater. Addiction 104, 1874–1880.
Baz-Lomb, J.A., Salvador, S., Gracia-Lor, E., Bade, R., Castiglioni, S., Castrignano, E., et al., 2016. Comparison of pharmaceutical, illicit drug, alcohol, nicotine and caffeine levels in wastewater with sale, seizure and consumption data for 8 European cities. BMC Public Health 16, 1035.
Baz-Lomb, J.A., Harman, C., Reid, M., Thomas, K.V., 2017. Passive sampling of wastewater as a tool for the long-term monitoring of community exposure: illicit and prescription drug trends as a proof of concept. Water Res. 121, 221–230.
Becker, W.C., Fiellin, D.A., 2020. When epidemics collide: coronavirus disease 2019 (COVID-19) and the opioid crisis. Ann. Intern. Med. 173 (1), 59–60.
Boles, T.H., Wells, M.J.M., 2014. Pilot survey of methamphetamine in sewers using a polar organic chemical integrative sampler. Sci. Total Environ. 472, 9–12.
Castiglioni, S., Bigiilsa, L., Covaci, A., Emke, E., Hernández, F., Reid, M., et al., 2013. Evaluation of uncertainties associated with the determination of community drug use through the measurement of sewage drug biomarkers. Environmental Science & Technology 47, 1452–1460.
Cone, E.J., Welch, P., Mitchell, J.M., Paul, B.D., 1991a. Forensic drug testing for opiates: I. Detection of 6-acetylmorphine in urine as an indicator of recent heroin exposure; drug and assay considerations and detection times. J. Anal. Toxicol. 15, 1–7.
Cone, E.J., Welch, P., Paul, B.D., Mitchell, J.M., 1991b. Forensic drug testing for opiates. III. Urinary excretion rates of morphine and codeine following codeine administration. J. Anal. Toxicol. 15, 161–166.
Daughton, C., 2020. The international imperative to rapidly and inexpensively monitor community-wide Covid-19 infection status and trends. Sci. Total Environ. 726, 138–149.
Gerrity, D., Trenholm, R.A., Snyder, S.A., 2011. Temporal variability of pharmaceuticals and illicit drugs in wastewater and the effects of a major sporting event. Water Res. 45, 5399–5411.
Jiang, J.-J., Lee, C.-L., Fang, M.-D., Tu, B.-W., Liang, Y.-J., 2015. Impacts of emerging contaminants on surrounding aquatic environment from a youth festival. Environmental Science & Technology 49, 792–799.
Kasprzyk-Hordern, B., Dinsdale, R.M., Cussy, A.J., 2007. Multi-residue method for the determination of basic/neutral pharmaceuticals and illicit drugs in surface water by solid-phase extraction and ultra performance liquid chromatography–positive electrospray ionization tandem mass spectrometry. J. Chromatogr. A 1161, 132–145.
Lin, W., Zhang, X., Tan, Y., Li, P., Ren, Y., 2019. Can water quality indicators and biomarkers be used to estimate real-time population? Sci. Total Environ. 660, 603–610.
Loftberg E. 2019, (personal communication).
Mercan, S., Kologlu, M., Tekin, S., Turkmen, D., Dogru, A.O., et al., 2019. Wastewater-based monitoring of illicit drug consumption patterns and trends. Sci. Total Environ. 609, 916–926.
Nguyen, H.T., Thai, P.K., Kaserzon, S.L., O’Brien, J.W., Eaglesham, G., Mueller, J.F., 2018. Assessments of drugs and personal care products biomarkers in the influent and effluent of two wastewater treatment plants in Ho Chi Minh City, Vietnam. Sci. Total Environ. 622, 671–680.
Prichard, J., Hall, W., de Voogt, P., Zuccato, E., 2014. Sewage epidemiology and illicit drug abuse by wastewater analysis. Environ. Health Perspect. 116, 1027–1031.
Sunkara, M., Wells, M.J.M., 2010. Phase II pharmaceutical metabolites acetonaphthone gluconide and acetonaphthone sulfate in wastewater. Environ. Chem. 7, 111–122.
Thai, P.K., O’Brien, J.W., Banks, A.P.W., Jiang, G., Cai, J., Choi, P.M., et al., 2019. Evaluating the sewer stability of three potential population biomarkers for application in wastewater-based epidemiology. Sci. Total Environ. 671, 248–253.
USDA. Automated reports and consolidated ordering system,https://www.deadiversion.usdoj.gov/arcos/retail_drug_summary/index.html.
Van Nuijs, A.L.N., Brien, J.W., Banks, A.P.W., Jiang, G., Cai, J., Choi, P.M., et al., 2019. Evaluating the sewer stability of three potential population biomarkers for application in wastewater-based epidemiology. Sci. Total Environ. 671, 248–253.
Zuccato, E., Chiabrando, C., Castiglioni, S., Ragnati, F., Fanelli, R., 2008. Estimating community drug abuse by wastewater analysis. Environ. Health Perspect. 116, 1027–1032.
Zuccato, E., Chiabrando, C., Castiglioni, S., Ragnati, F., Fanelli, R., 2008. Estimating community drug abuse by wastewater analysis. Environ. Health Perspect. 116, 1027–1032.