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Title: Rapid assessment of opioid exposure and treatment in cities through robotic collection and chemical analysis of wastewater

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MM is the CEO and co-founder of Biobot Analytics, Inc. NE, CD, KF are employees of Biobot Analytics, NG is President and co-founder of Biobot Analytics

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Key Words: Waste Water, opioids, overdose, robot, Map, data visualization

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Abstract:

Introduction: Accurate data regarding opioid use, overdose and treatment is important in guiding community efforts at combating the opioid epidemic. Wastewater-based epidemiology (WBE) is a potential method to quantify community-level trends of opioid exposure beyond overdose data, which is the basis of most existing response efforts. However, most WBE efforts collect parent opioid compounds (e.g. morphine) at wastewater treatment facilities, measuring opioid concentrations across large catchment zones which typically represent an entire municipality. We sought to deploy a robotic sampling device at targeted manholes within a city to semi-quantitatively detect opioid metabolites (e.g. morphine glucuronide) at a sub-city community resolution.

Methods: We deployed a robotic wastewater sampling platform at ten residential manholes in an urban municipality in North Carolina, accounting for 44.5% of the total municipal population. Sampling devices comprised a robotic sampling arm with in-situ solid phase extraction, and collected hourly samples over 24 hour periods. We used targeted mass spectrometry to detect the presence of a custom panel of opioids, naloxone and buprenorphine.

Results: Ten sampling sites were selected to be a representative survey of the entire municipality by integrating sewer network and demographic GIS data. All eleven metabolites targeted were detected during the program. The average morphine milligram equivalent (MME) across the nine illicit and prescription opioids, as excreted and detected in wastewater, was 49.1 (standard deviation of 31.9) MME/day/1000-people. Codeine was detected most frequently (detection rate of 100%) and buprenorphine was detected least frequently (12%). The presence of naloxone correlated with city data of known overdoses reversed by emergency medical services in the prehospital setting.

Conclusion: Wastewater-based epidemiology with smart sewer selection and robotic wastewater collection is feasible to detect the presence of specific opioids, naloxone, methadone and buprenorphine within a city. These results suggest that wastewater epidemiology could be used to detect patterns of opioid exposure and may ultimately provide information for opioid use disorder (OUD) treatment and harm reduction programs.

Introduction:

Exposure to opioids continues to cause significant morbidity and mortality in the United States. In 2017, over 47,000 deaths in the United States were attributed to opioid overdose, making it the deadliest year of the recent opioid epidemic [1]. Measuring the community-level success of opioid harm reduction and treatment interventions is important to understanding their public health impact. Most available public health data report changes in opioid-related overdoses, deaths, incarceration, or trends in opioid prescriptions filled [1,2]. These data are used to measure large scale changes in the opioid crisis but may miss granular community level responses to the opioid epidemic. Additionally, these public health outcome metrics are often delayed and reported on a quarterly or annual basis. Non-fatal overdoses, as estimated by emergency calls or visits to emergency departments, have recently emerged as a timelier data source but they may miss important clusters of individuals with opioid use who may experience overdose and reversal with naloxone, yet never seek medical care.

In 2018, the United States Department of Health and Human Services (HHS) described the need for higher resolution, timely opioid-related analysis and outcomes data [3]. Innovative sources of opioid metrics should ideally help communities gauge the effectiveness of harm reduction and opioid use disorder treatment programs on a frequent basis and allow communities to adjust opioid outreach efforts based on empiric data of opioid and naloxone use locally. A noninvasive real-time community-level measurement of opioid exposure may help harm reduction interventions and advocacy groups improve their agility in responding to community challenges related to the opioid epidemic.

One potential source of opioid outcome data is wastewater-based epidemiology (WBE) [4, 5]. Analysis of wastewater at treatment plants and local bodies of water has been successfully implemented to detect the presence of drugs including opioids and inform public health efforts on a large scale [6,7]. However, current sampling practices focused on treatment

plants are not sufficiently granular for stakeholders such as municipalities or hospitals, who deploy and evaluate public health intervention programs at the community-level. Furthermore, because of the long transport times to treatment plants, certain urinary metabolites (e.g. glucuronidated drug metabolites) may only be amenable to detection if sampling is conducted in city manholes located far upstream of treatment plants [8]. By measuring the presence of opioid urinary metabolites, WBE techniques can not only measure the community level burden of opioids, but also assess the human exposure to opioids [9-11].

Sampling wastewater from city manholes instead of treatment plants requires novel methods to be developed. In this manuscript, a novel platform to enable timely detection and reporting of community-level opioid exposure through WBE is described. The platform consists of computational selection of optimal sampling sites in the sewer network, robotic sampling of wastewater from city manholes, and semi-quantitative analysis of human opioid exposure. We present the first pilot program of this novel WBE platform implemented in a municipality in North Carolina in 2018. Insights from opioid focused WBE strategies combined with other public data sources like census data and Emergency Medical Services (EMS) overdose reports may help direct harm reduction interventions to the places and communities of greatest need.

Materials and methods:

WBE opioid exposure monitoring program

From June to November 2018, we implemented the WBE opioid exposure monitoring program in a suburban municipality in North Carolina as a pilot demonstration project. In 2017, the rate of fatal overdose in the municipality was 6.7 per 100,000 people, approximately one third lower than the national average. The town recently experienced a local outbreak of increasing opioid misuse resulting in a 40% increase in fatal overdoses and a 135% increase in non-fatal overdoses between 2016 and 2017. We therefore sought to deploy our WBE opioid exposure monitoring program to understand where opioids were being consumed, and the use

of naloxone in the community. This investigation was determined to be not human subjects research and therefore did not require review from an Institutional Review Board.

Wastewater samples were taken every two weeks from June to August, 2018 and monthly from September to November, 2018 (total of seven sampling weeks; Samples 1-7). In addition, the incidence and locations of fatal and non-fatal overdoses recorded from calls to the local EMS service in 2018 were obtained from the municipality and aggregated at the catchment-level to serve as a comparison for the wastewater-based data.

Selection of optimal sampling sites in the sewer network

We selected ten wastewater catchments for sampling (Sites a-j) representative of the entire municipality. These sites were selected through a two-step process: computational identification of potential sewer sampling sites (Figure 1A), and confirmation of sampling sites after physical inspection.

To identify potential sampling sites, we first obtained the town wastewater network from the municipality's public works department. This network was overlaid with land use data and census-based demographic data. The parcel-level land use data was obtained from the municipality. Demographic data was procured through SimplyAnalytics, Inc. (New York, NY), for the year 2018 at spatial resolution of block group. All geographic information systems (GIS) analyses were performed using ArcMAP v.10.6 (Esri, Redlands, CA).

We applied a routing analysis in MATLAB (v. 2019a) in order to compute maximum wastewater retention time and demographic characteristics in the catchment area associated with every segment of sewer network (e.g., the number of people that live in buildings that are located upstream of the each segment of sewer network). We identified potential sewer sampling sites (*i.e.* manholes) along the wastewater network that satisfied the following inclusion criteria: a minimum residential population of 4,000 to ensure individual anonymity; a

maximum hydraulic retention time of 4 hours to ensure molecular stability; and a minimum residential ratio of 90% to obtain data most relevant to human activity.

Once potential sampling sites were identified, we selected 10 final sampling sites among them such that the associated catchment areas together represented the average demographic composition of the municipality (Table 1). The final selection of sampling sites were also conducted in conjunction with the Mayor's office and the municipal public works department to confirm that none of the catchment areas included buildings whose discharge could significantly affect data (e.g. hospitals and factories) and that the selected manholes were accessible for sampling (e.g. not on busy roads).

Robotic sampling of wastewater

Wastewater samples were collected using battery-powered sampling devices installed inside selected sewer manholes (Figure 1B). We designed the robotic sampling device consisting of a filter, a water receptacle, and a solid-phase extraction (SPE) cartridge (Figure 1C). The sampling device was programmed to collect a composite wastewater sample, by sampling every hour over 24 hours (time-proportional sampling). At each hour, 450 mL of wastewater are collected from the sewer using a peristaltic pump. Collected wastewater is immediately processed through a Durapore 0.22µm filter to remove large particles and an Oasis HLB Solid-Phase Extraction (SPE) cartridge to concentrate small molecules. The effluent from the SPE cartridge is discarded back to the sewer [12]. In each sample, a total of 10L of wastewater are processed through the SPE cartridge over 24-hours. This device is contained within a waterproof container and installed using a suspension wire cable under a manhole cover (Figure 1B).

All devices were installed inside manholes and retrieved 24 hours later. The SPE cartridge was removed and shipped on ice for analysis upon sample retrieval. We considered a composite sample suitable for analysis if at least 20 out of 24 hourly sampling collections were

successful (*i.e.*, total volume collected was at least 9L). Out of 70 sampling events (7 time points at each of the 10 catchments), 59 (84%) passed for analysis.

Semi-quantitative wastewater analysis by HPLC-MS/MS

SPE cartridges were eluted with 100% methanol, dried down using a Vacufuge, and analyzed by High Performance Liquid Chromatography Tandem-Mass Spectrometry (HPLC-MS/MS) [13]. We used a library of human metabolites of heroin, fentanyl, codeine, morphine, hydrocodone, hydromorphone, oxycodone, oxymorphone, tramadol, methadone, buprenorphine, and naloxone, as well as substances that serve as positive controls, such as nicotine, acetaminophen, and caffeine (Table 2). Measurement of drug metabolites, as opposed to parent drugs, provides a technical advantage in estimating actual drug exposure, because the data are not confounded by the amounts of drugs dispensed in the sewer system or “flushed down the toilet” without being used.

The presence of analytes was determined semi-quantitatively by HPLC-MS/MS using an external standard curve. An HPLC-MS/MS method was developed using certified reference materials purchased from Cerilliant Corporation. An analyte was considered ‘detected’ if it had the same retention time, precursor ion and fragmentation pattern as the reference standard. The concentration of each analyte was measured using an external standard curve of standards prepared in Milli-Q water. The aim of this study is to provide a proof of concept of upstream WBE analysis. Conducting fully-quantitative analysis of human opioid exposure is outside the scope of this study.

Data analysis

Sample concentrations obtained through HPLC-MS/MS analysis (ng/mL) were converted to sewage concentration (ng/L), collective excretion rates (mg/day/1000 people), collective consumption rates (mg/day/1000 people), and total opioid exposure rate (MME/day/1000 people) (Fig. 1D, supplemental information) [9]. The collective consumption rates of most substances in

our panel can be estimated directly from a urinary metabolite that uniquely identifies the parent drug (e.g. heroin was estimated from 6-monoacetylmorphine). However, some opioids lack urinary metabolites that uniquely identify them. Consumption of morphine, hydromorphone, and oxycodone were estimated after correcting for other sources of their metabolic products (Table 2, supplemental information). The dosage rate (doses/day/1000-people) of naloxone was estimated by dividing the naloxone collective consumption rate (mg/day/1000-people) by an average naloxone dose (4 mg).

The data was further analyzed for correlations and visualized in maps. Spearman correlation analysis was conducted in Python 3 using the “spearmanr” function from scipy.stats. The presence of opioids, buprenorphine, and naloxone was visualized by overlaying the total opioid exposure rate and the detection rate of the compounds in each catchment onto the map of the municipality.

Results:

Sampling sites

The 10 catchment areas selected for sampling encompassed 44.5% of the population of the entire municipality, and the demographic characteristics of the catchments were comparable to those of the municipality (Table 1). For example, the population-weighted average of the median age among the 10 selected catchments was 38.8 years, and that of the municipality was 38.7 years (the population-weighted variance was 7.5 years). The sampling catchments were also spatially distributed across the municipality (Figure 2).

Exposure to illicit and prescription opioids

The total opioid exposure rate (MME/day/1000 people) for illicit and prescription opioids was calculated for each sample. The mean and standard deviation of the total opioid exposure across samples was 49.1 and 31.9 MME/day/1000-people, respectively. We compared the rate

of total opioid exposure for each catchment (mean MME across all sampling time points, June – November 2018) with the reported overdose rate in 2018 (Figure 2A and 2B), and no correlation was obtained (Spearman's correlation coefficient $\rho = -0.042$, p -value = 0.91).

All of the opioid metabolites targeted (Table 2) were detected during the program, but with different detection rates in each site (Figure 3, Supplemental Table 1). Codeine (average detection rate of 100%), tramadol (96%), hydrocodone (80%), oxycodone (68%) were detected frequently; fentanyl (41%), heroin (39%), morphine (35%), oxymorphone (18%), and hydromorphone (15%) were detected less frequently. All the positive-control molecules (nicotine, acetaminophen, and caffeine), which are expected to be ubiquitous in sewer samples, were present in all samples.

Treatment and overdose reversal drugs

Exposures to methadone, buprenorphine, and naloxone was also detected. The detection rate for methadone, buprenorphine, and naloxone was 64%, 12%, and 55%, respectively (Figure 3, Supplemental Table 1). The detection rate of buprenorphine and naloxone was mapped (Figure 2C and D, respectively) and compared with the reported overdose rate in 2018 (Figure 2A). While our sample size was small and baseline incidence of opioid overdose was low in this sample, we found correlations between the catchment-level opioid overdose rates and the detection rates of treatment and overdose reversal drugs. Buprenorphine exposure was significantly correlated with opioid overdose rate, naloxone exposure was weakly correlated, and methadone exposure did not correlate with opioid overdose rate (buprenorphine: $\rho = 0.79$, p -value = 0.028; naloxone: $\rho = 0.58$, p -value = 0.080; methadone: $\rho = 0.41$, p -value = 0.23).

In 2018, the rate of reported overdose in the sampled communities was 0.40/year/1000-people. If we assume that one dose of naloxone is used at every reported overdose event, the

corresponding naloxone dosage rate in the sampled communities would be 0.40/year/1000-people. In contrast, using the standard dose of naloxone (4 mg/dose) and our wastewater data, the average collective dosage rate of naloxone was calculated to be 0.027 doses/day/1000-people or 10 doses/year/1000-people from our samples.

Discussion:

This investigation demonstrates the feasibility of robotic collection of wastewater from city manholes to measure exposure to opioids in communities anonymously through upstream wastewater analysis. We collected wastewater samples from municipal sewer lines using an in-house robotic instrument that automates *in situ* wastewater filtration and solid phase extraction. This technical innovation permits the capture of opioid exposure data with high spatial resolution and accuracy due to smaller molecule degradation as compared to analyzing wastewater samples at wastewater treatment plants [14-16]. Indeed, we detected glucuronidated metabolites of opioids indicating that we can directly measure human opioid exposure. This technique also allowed us to detect exposure to naloxone, methadone and buprenorphine in specific areas of the city, providing insight into treatment and overdose reversals. Additionally, selection of optimal maintenance hole sites using our algorithm enhances our ability to generate data representative of all demographic groups in a city [17].

This WBE strategy is a practical system to implement within a municipality and is scalable to other municipalities. After obtaining approval from the Mayor's office and the department of public works, deploying upstream WBE requires GIS-based wastewater network analysis and minimally invasive installation of the sampling device, in addition to publicly available demographic data. We were able to train public works department employees to install our robotic collection devices, which required minimal maintenance during the study period. Our sampling method was also feasible; sampling success rate during the investigation was 84%.

Working closely with officials within the municipality also reduced the potential for data misuse and allowed us to ensure that citizens were informed about the project and could have a voice in its eventual applications. We also ensured that catchment sizes were at least several thousand people to guarantee personal anonymity. In future broad deployments of WBE, it will be critical to continue partnering with city officials and to engage privacy advocacy groups and legislators to create best practices that align with the interest of citizens.

This investigation faced several technical and analytical limitations. First, wastewater sampling with our device failed to produce a 100% success rate. The major mode of sampling failure in this program was clogging of the inlet pickup strainer. Pickup strainers less prone to clogging have been developed since this program which we anticipate will alleviate this issue. Second, uncertainties that derive from variability of wastewater data need to be addressed. Our 24-hour composite samples indicated significant variability of opioid concentration in sewage. This variability is attributable to both short-term (~a few minutes) [18,19] and daily variation of opioid concentration in wastewater [9, 20]. To reduce uncertainty, near-continuous sampling over seven consecutive days is suggested [19]. Our robotic sampling device was designed to be able to operate at a user-defined sampling mode (e.g., sampling frequency, duration, and volume), and it can be easily adapted to take more representative data in the future.

Finally, in order to obtain fully-quantitative data, future work remains to correct for the fidelity of extraction from the SPE, the effects of the wastewater matrix on mass spectrometry measurements, and the stability of molecules in sewage. In this program, the extraction efficiency of SPE was assumed to be 100%, but the actual extraction efficiency is known to be molecular dependent and can be lower than 50% [21]. For more accurate data analysis, the actual SPE extraction efficiency should be obtained for specific matrix and analysis methods and used to correct the data. In our study, we also ignored the effects of the wastewater matrix on the mass spectrometer instrument, but future programs can correct for these effects by using internal calibration curves or matrix-matched standard curves. Furthermore, we assumed the

molecular stability in sewage to be 100%. This assumption is reasonable given the short maximum retention time (4 hours) that we designed, but some opioid metabolites may still be broken down in sewage, raising the potential to underestimate consumption or even to report false negatives [20,22].

Despite these limitations, there are several major implications from this investigation. First, opioids and treatments metabolized by humans can be mapped into specific communities and visualized using GIS techniques to generate maps of community-level opioid exposure. As our opioid panel broadens, patterns of use for specific opioids and treatment drugs can be combined with existing overdose and other opioid-related data to: detect increasing opioid use portending a local outbreak of increased opioid use disorder; help policymakers and city planners allocate resources such as EMS, needle exchange programs or even safe injection sites to specific areas of high opioid use; detect communities in which outreach, naloxone distribution, and increased surveillance may be needed; and allow for mobile harm reduction teams to relocate in anticipation of rising opioid use in different communities. This data may also serve as a noninvasive early warning sentinel, detecting increasing concentrations of opioids in low consumption counties. In contrast to current data sources, our WBE platform can generate reports four weeks after data is collected. This is already a major improvement in terms of timeliness, and iterative improvements and automation in sample collection and processing will further shorten the time to reporting. These real-time insights can help cities anticipate novel opioids and position resources in advance of clusters of overdoses.

Second, the detection of naloxone glucuronide in sampling catchment areas imply local concentrations of opioid overdose reversals, which may not be reported through EMS calls. The average dosage rate of naloxone inferred from our wastewater data (10 doses/year/1000-people) was 25 times greater than the doses inferred from reported overdoses (0.40/year/1000-people), suggesting that WBE monitoring may provide a more comprehensive view of community overdose reversals. However, naloxone is found in both Narcan (overdose reversal

drug) as well as Suboxone (buprenorphine/naloxone treatment). Future studies should address how to correct for the use of Suboxone, so wastewater naloxone data can be fully leveraged to estimate the number of overdoses and evaluate public health programming. The initiation of a naloxone distribution program in an area of high opioid overdose could be monitored by measuring the change in naloxone metabolites found in specific catchment areas, demonstrating that naloxone was distributed by the program and used in the same area to reverse overdoses. Similarly, concentrations of buprenorphine and methadone metabolites suggest local communities where MAT may have penetrated and could be used to measure uptake of buprenorphine as MAT during rollout of treatment programs.

Overall, we have demonstrated the feasibility of deploying a robotic wastewater sampling device to collect upstream wastewater among communities thereby providing high resolution visualization of opioid use across a city. We were able to measure several important opioid metabolites and detect the presence of naloxone glucuronide, a potential indicator of the incidence of opioid overdose reversal. We were able to develop simple guidelines to commence WBE monitoring programs, including the assessment of sampling locations, deployment of a novel robotic device, and rapid assessment of opioid exposure within a targeted municipality which can be extrapolated to other vulnerable communities. In collaboration with local communities and stakeholders, these analyses can be expanded upon and used to detect patterns of opioid exposure and overdose and may ultimately provide information for opioid use disorder treatment and harm reduction programs.

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FIGURE LEGENDS:

Figure 1. Schematics of upstream wastewater-based epidemiology platform

(A) Sampling sites and associated catchments. As compared to sampling at a wastewater treatment plant (WWTP), sampling at upstream sites with smaller catchments (Site A, B) can achieve smaller hydraulic retention time and higher spatial resolution. (B) Installation of sampling device in manhole. Our sampling device is installed in a manhole using a suspension cable. (C) Robotic wastewater sampling and in-situ solid phase extraction. The sampling device consists of two peristaltic pumps, a filter, a water receptacle, and a SPE cartridge. (D) Data analytics and main parameters used. See Methods for details.

Figure 2. Reported opioid overdoses (A) compared to wastewater measured opioid exposure (B), naloxone (C), and buprenorphine use (D) in selected 10 sampling sites (a-j).

Overdose rate per site was calculated based on the fatal and non-fatal overdose incidents recorded from calls to the local EMS services and the residential population in each catchment for 2018. Average opioid exposure rate (MME/day/1000-people) was obtained by averaging the total opioid exposure rate, except for methadone and buprenorphine (see Methods), over successful sampling points for each site. Detection rates for buprenorphine and naloxone were obtained by calculating the detection frequency of those drugs inferred from their respective metabolites (see Table 2 and Methods). Detection rate per site was calculated based on the total successful samples in each site (59 samples: Site **a** (n=7), Site **b** (n=7), Site **c** (n=4), Site **d** (n=6), Site **e** (n=7), Site **f** (n=6), Site **g** (n=5), Site **h** (n=7), Site **i** (n=4), Site **j** (n=6)).

The maps were reproduced to maintain the spatial relationship between the sampling sites, but do not represent their actual geospatial locations or the actual city-wide boundary.

Figure 3. Detection rate of opioids, treatment and overdose reversal drugs per site.

Exposure to parent drugs (y-axis) was inferred from detection of their respective metabolites (see Table 2 and Methods). Detection rate per site was calculated based on the total successful samples in each site (59 samples: Site **a** (n=7), Site **b** (n=7), Site **c** (n=4), Site **d** (n=6), Site **e** (n=7), Site **f** (n=6), Site **g** (n=5), Site **h** (n=7), Site **i** (n=4), Site **j** (n=6)).

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Tables and Figures:

Table 1. Average demographic characteristics of the municipality and the ten residential catchments sampled in this study.

Standard deviation (std) for municipality was calculated based on block-level data.

* Population-weighted average.

	Municipality	Residential catchments in this study
Population	162,200	72,300 (44.5%)
Area	59.4 sq. mile	25.6 sq. mile (43.0%)
Median age*	38.7 (std=7.5)	38.8 (std=4.5)
Median household income*	\$129,200 (std=46,700)	\$122,400 (std=48,600)
White*	71.6% (std=13.9%)	71.2% (std=7.8%)
Black*	8.8% (std=6.8%)	9.4% (std=4.0%)
Asian*	13.4% (std=10.4%)	12.5% (std=7.8%)
Overdose rate in 2018 (year/1000 people)	0.388	0.402

Table 2. Analytes used in the HPLC MS/MS method to estimate consumption of the listed substances.

All analytes in the method are urinary metabolites that indicate consumption and excretion.

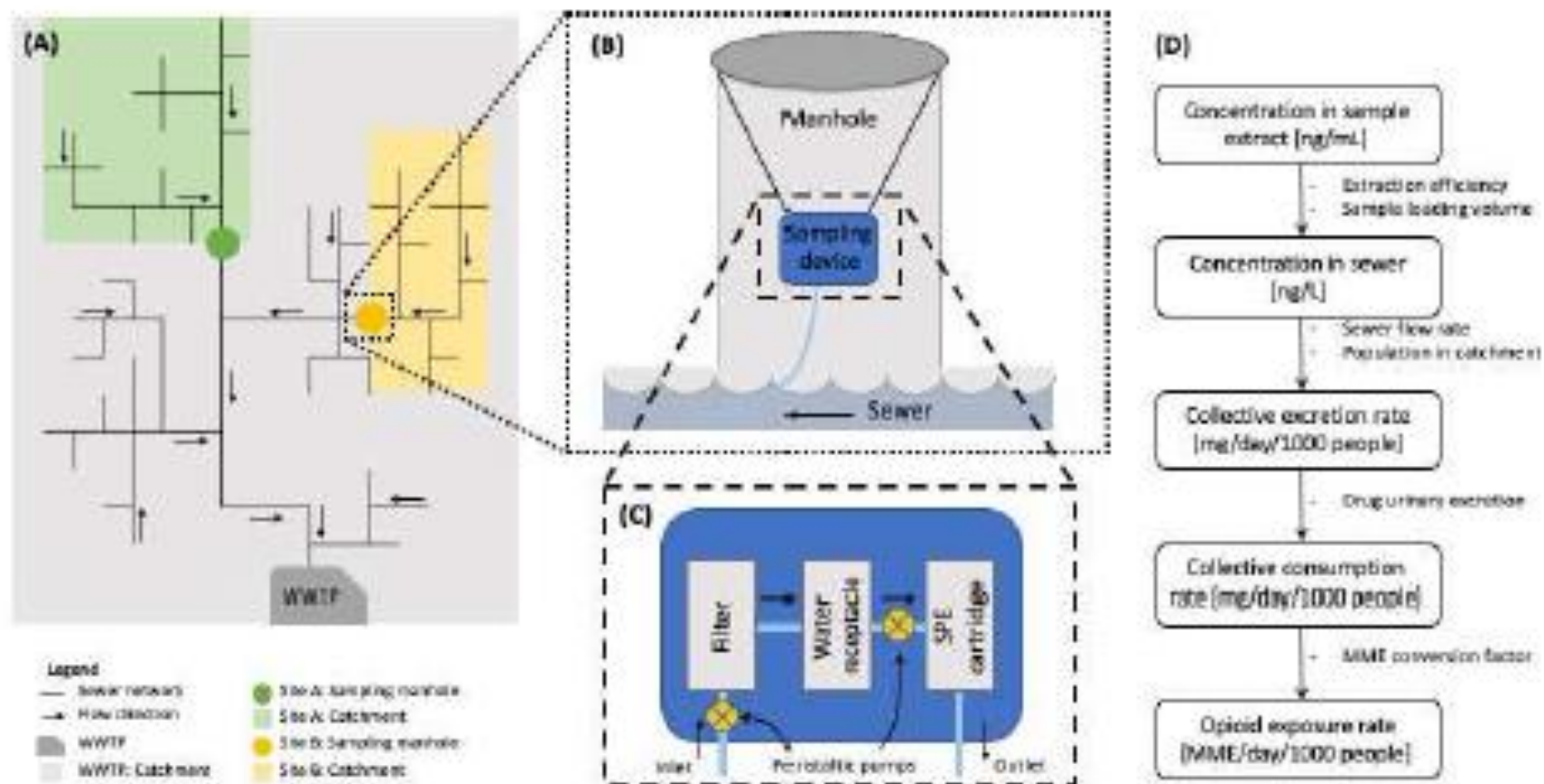
* Analytes marked with * are excreted after consuming the respective parent drug, but were not used to calculate consumption of that parent drug. These analytes were used in the consumption calculation for morphine, hydromorphone, and oxycodone (see supplemental information).

** MME conversion factors for methadone and buprenorphine are available, but they were not counted towards total opioid exposure rate in this study.

Substance	Type	Analyte metabolite in HPLC MS/MS method	MME conversion factor
Heroin	Illicit	6-monoacetylmorphine Morphine-3-glucuronide*	3
Fentanyl	Illicit/Prescription	Norfentanyl	100
Codeine	Prescription	Codeine-6 β -D-glucuronide Morphine-3-glucuronide*	0.15
Morphine	Prescription	Morphine-3-glucuronide	1
Hydrocodone	Prescription	Norhydrocodone Hydromorphone-glucuronide*	1
Hydromorphone	Prescription	Hydromorphone-glucuronide	4
Oxycodone	Prescription	Noroxycodone Oxycodone-glucuronide*	1.5

Oxymorphone	Prescription	Oxymorphone-glucuronide	3
Tramadol	Prescription	O-desmethyl-cis-tramadol	0.1
Methadone	Treatment	2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP)	4**
Buprenorphine	Treatment	Norbuprenorphine-glucuronide	75**
Naloxone	Overdose reversal	Naloxone-3-glucuronide	-
Nicotine	Positive control	Trans-3-hydroxycotinine	-
Acetaminophen	Positive control	Acetaminophen	-
Caffeine	Positive control	Caffeine	-

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