AN ABSTRACT OF THE DISSERTATION OF

Alex J Brewer for the degree of Doctor of Philosophy in Toxicology presented on December 13, 2012.
Title: Addressing Wastewater Epidemiology limitations with the use of Dynamic Population Surrogates, Complementary Urinalyses and In-Situ experiments.

Abstract approved: ________________________________________

Jennifer A. Field

Wastewater epidemiology is an emerging discipline that requires collaborative research involving analytical chemists, drug epidemiologists, and wastewater engineers. Wastewater epidemiology involves the sampling and quantitative analysis of raw wastewaters from communities for illicit drugs and their metabolites. Mass loads (mass per day) and per capita (mg per day per person) are then calculated from concentrations and indicate the approximate quantity of illicit drugs used and excreted by the community. Limitations to wastewater epidemiology include that the population served by wastewater treatment plants within a day and between days is not well known. In addition, biodegradation of illicit drugs during transit in sewers may affect the concentration and mass flows that reach wastewater treatment plants. This thesis describes a series of studies conducted by an international collaboration between scientists and engineers from the United States and Switzerland to answer these two limitations. The experimental approaches for these studies used included high-frequency wastewater sampling strategies, the use of creatinine as a human urinary biomarker, as well as the use of unique locations as test sites including an open community, a prison in the state of Oregon, and a 5 km section of sewer in Zürich Switzerland.
In Chapter 2, the diurnal study on the mass flows of illicit drugs or metabolites was formed over four days in a municipality with a population of approximately 55,000 people. The diurnal trends in illicit substances vary by substance. The high (g/day) mass flows of caffeine, methamphetamine, and creatinine indicate that lower-frequency sampling (approximately one sample per h) may representatively capture the use and excretion of these substances. However, lower and episodic mass flows of cocaine and its primary human metabolite, benzoylecgonine, indicate that higher-frequency is needed to accurately assess the use of the cocaine within the municipality. Normalization of illicit substances to creatinine gave between-day trends in illicit and legal substances that differed from non-normalized trends. Resident use of cocaine and methamphetamine were indicated by normalized mass flows that increased during early morning hours while commuters are largely absent from the community.

Chapter 3 describes a series of experiments conducted at an Oregon state prison. The prison setting provided a unique opportunity to study a nearly-fixed population of individuals and their corresponding mass flows of illicit substances, the number of doses per person consumed, as well as an opportunity to quantify the level of agreement between numbers of individuals and the measured mass flows of creatinine. Methamphetamine use was more prevalent than cocaine/benzoylecgonine in the prison over the one month study in which single daily (24 h) composite samples of wastewater were collected. The hypothesis that the mass flows of methamphetamine and cocaine would be lower on days on which random urinalysis testing (RUA) is
typically conducted by the prison (Monday-Thursday) was rejected. While the mass flows (mg/d) of methamphetamine were less than those for a nearby open community, the number of estimated doses per person was higher for the prison population. A higher number of positive RUA results were obtained for methamphetamine while none were positive for cocaine, which is consistent with the data obtained from wastewater. The hourly (diurnal) trend in methamphetamine mass loads indicated continual methamphetamine use/excretion inside the prison while cocaine and benzoylcegonine were detected in five hourly composite samples. Use of methamphetamine and cocaine by inmates could not be unambiguously distinguished from that of non-inmates (employees and visitors). The observed diurnal trends in creatinine mass loads were similar to those of an open community and are indicative of the general pattern of human wakefulness/activity. Predicted creatinine mass loads based on the total prison (inmates + non-inmates) were in good agreement with the measured mass loads, which indicates the potential use of creatinine as a quantitative population indicator. Additional research on the biodegradability of creatinine is needed because the prison setting was deliberately selected to minimize the potential for creatinine biodegradation.

Chapter 4 addresses the data gap that exists on illicit drug transformation during in situ transit in sewers. The rates of in situ biodegradation have not yet been determined for conditions that are relevant to sewers, which include low to variable oxygen concentrations, the presence of a biofilm, and temperatures ≤ 20 °C. For this reason, two tracer tests were conducted in a 5 km stretch of sewer located near Zürich,
Switzerland. The stable-isotope forms (deuterated) of cocaine and benzoylecgonine were injected into flowing wastewater and three locations up to 5 km downstream were sampled over time. Breakthrough curves were constructed from measurements of cocaine-d3 and benzoylecgonine-d3 concentration with time. The area under the curve (mass) was determined by integrating concentration over time.

Benzoyllecgonine-d3 was present in the injectate that should have only contained cocaine-d3; because the benzoylecgonine-d3 formation prior to injection is not known. The injected mass of cocaine-d3 did not decline over the 5 km distance. The observed mass of cocaine-d3 at 5 km was 10% greater than at 500 m, which indicates that the transformation of cocaine was not significant over the 1.5 h experiment. At 5 km downgradient, the apparent mass of benzoylecgonine-d3 had increased by 35% over that observed at 500 m. However, the apparent increase in benzoylecgonine-d3 mass was not accompanied by a corresponding loss of cocaine-d3. While uncertainty is apparent in the increase of both cocaine-d3 and benzoylecgonine-d3, the ratio of cocaine-d3/benzoylecgonine-d3 is subject only to analytical error because any errors associated with sampling and the integration of masses cancel out. The ratio of cocaine-d3/benzoylecgonine-d3 declined from 2.98 in the injectate to 1.66 at Location 3, which indicated a greater increase in benzoylecgonine-d3 relative to cocaine over the 5 km distance. Due to the benzoylecgonine-d3 that was present in the injectate, any biodegradation of cocaine-c3 to form benzoylecgonine-d3 could not be unambiguously distinguished. During the second tracer test in which benzoyllecgonine-d3 was injected, the mass of benzoyllecgonine-d3 did not significantly decline, which suggests that the apparent loss of benzoyllecgonine-d3
during the cocaine-d3 test cannot be attributed to in-situ biodegradation. Overall, while uncertainty exists about the integrated masses for cocaine-d3 and benzylecgonine-d3, the 5 km distance was too short in order to observe a significant loss of cocaine-d3 and formation of benzylecgonine-d3. Recommendations for future research include conducting analysis on the injectate solution to ensure that only cocaine-d3 is introduced so that any formation of benzylecgonine-d3 is readily apparent and quantifiable. In addition, the tracer tests should be repeated in a longer section of sewer to increase the residence time beyond 1.5 hr and degradation products of benzylecgonine-d3 should be monitored including ecgonine and ecgonine methyl ester.
Addressing Wastewater Epidemiology limitations with the use of Dynamic Population Surrogates, Complementary Urinalyses and In-Situ experiments

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I understand that my dissertation will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my dissertation to any reader upon request.

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Alex J Brewer, Author
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CONTRIBUTION OF AUTHORS

Dr. Christoph Ort assisted with the collection of samples as well as the planning of experiments. Dr. Jean Daniel Berset and Dr. Caleb Banta-Green assisted with data interpretation presented in Chapters 2, and 3.
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Addressing Wastewater Epidemiology limitations with the use of Dynamic Population Surrogates, Complementary Urinalyses and In-Situ experiments
Chapter 1-Introduction

Illicit drug use is generally considered a problem, because of the negative impact that they have on individuals and society. The use of illicit drugs can lead to a number of wide ranging personal problems. These problems often include that include but are not limited to negative health consequences, and social and financial difficulties\(^1\) that arise from maintaining drug use habits. The consequences of these problems are not limited to the drug users themselves but they are a shared responsibility for communities. Drug use is a common problem for communities worldwide.

Estimating community drug use is needed in order to inform policy makers of drug prevention and control strategies. The methods that are typically used involve surveys,\(^2\) and workplace drug testing.\(^3\) These methods of determining community drug use contain limitations that are lagged in time from when they take place to when data is available, and are subject to self-report bias.\(^4\) In order to account for some limitations of traditionally used methods wastewater analyses have been used.

Wastewater contains not only illicit drugs but also their metabolites.\(^5\)-\(^8\) The methods that are used to collect and analyze wastewater for insights into community drug use excretion are generally called wastewater epidemiology. Community drug use via wastewater has been investigated throughout the world.\(^4\),\(^9\),\(^10\) Wastewater epidemiology interprets measured concentrations (in ng/L range) of illicit drugs and metabolites that enter a wastewater treatment plant along with volume of wastewater to calculate drug loads (mass). These drug loads are then used to determine the amount of illicit drugs used (via doses). To calculate the number of doses that are
used, pharmacokinetic data from laboratory studies are used to estimate the average mass excreted per an assumed dose a day, thus using mass to determine dose. The number of doses are then typically divided by the number of people associated with a given wastewater treatment plant to connect people contributing drug loads and doses. There are several widely used substances that are typically discussed in wastewater epidemiology research including methamphetamine, cocaine, and benzoylecgonine. The work presented in this dissertation includes these commonly used illicit substances as well as caffeine, and creatinine.

Typically wastewater epidemiology studies utilized 24 h composite wastewater samples. A 24 h composite is used to observe a time frame when the whole community is active (daytime) as well as its convenience for the back-calculation of doses from pharmacokinetic literature. A composite sample is simply a sample that is made up of sub-samples. Composite samples are often collected with various sub-sampling frequencies, which may skew loads. The skewing of loads means that there is a potential for missing a significant amount of mass, leading to either under or over-estimating the number of loads and doses. These 24 h composite samples are used along with a census population value. The number of people in a community is determined via a census or another government administered survey/register, which generally takes place every several years. Population change takes place on a much smaller scale than several years and the use of this estimate is a limitation. Population serviced by a wastewater treatment plant is not a static population. There are population influences that may include sub-populations of commuters, students, and visitors. In order to account for population there have been a number of suggestions
As of now, there have not been any chemical human urinary biomarkers (population surrogate) utilized to account for population in wastewater epidemiology studies. A chemical human urinary biomarker is a substance that every human excretes in urine.

Chapter 2 of this dissertation describes experiments that utilize high-frequency to collect 1 h composite samples, which helps inform the field of wastewater epidemiology about the daily variation in drug loads. The variation in drug loads over the day informs scientists in the wastewater epidemiology field about what sampling to representatively capture substance specific mass loads. Intuitively if the goal of a study is to observe drugs that are frequently used and excreted it may not be necessary to use a high-frequency sub-sampling regime to make a composite that accurately captures the excretion profile. It is thought that different drugs may exhibit different trends throughout the day based on use. Work described in Chapter 2 also describes the use of creatinine to normalize mass loads. This is done to take population into account and create per-capita loads. Creatinine is a compound that is non-enzymatically converted from creatine and phosphorylcreatine at an almost steady state in the body. Creatinine is excreted from the body at an almost constant rate. Work described in chapter 2 is seeking to test: (1) Diurnal trends in illicit and legal drugs may be drug specific. (2) Diurnal trends in illicit and legal drug loads are different when taking population into account by normalizing with creatinine loads.

Creatinine mass loads measured in an open system are a reflection of the variation in population. In order to minimize the amount of population movement while measuring creatinine a prison system was used. Recently wastewater exiting a
A prison system in Spain was measured to quantify mass loads. Mass loads of illicit drugs along with loads of creatinine could be measured in a prison system and compared to population. In addition, estimated loads of creatinine from the population can be compared to measured loads given that the total number of inmates and demographics are well known. Drug use by inmates is measured using random urine analyses (RUA). Wastewater epidemiology is a potential tool that can be used alongside RUAs. Chapter 3 is testing: (1) Predicted creatinine loads will correspond to measured loads. (2) Illicit drug loads are different on random urinalyses testing days vs. non-testing days.

Chemical transformation of cocaine and benzoylcegonine in-situ is also a limitation and uncertainty in current wastewater epidemiology. Given these uncertainties estimating an accurate number of doses from mass loads in wastewater remains uncertain. In-situ transformation cannot be adequately estimated without the presence of biofilm. Along with the system components (i.e. biofilm) needed to study in-situ transformation of cocaine and benzoylcgonine a chemical that is structurally similar yet identifiable from the background in wastewater is needed. Fortunately, chemicals have been used as analytical internal standards to account for matrix effects. An analytical standard typically is the same structure as the chemical of interest yet differentiable and can be used as a surrogate to study chemical transformation. When using liquid-chromatography mass spectrometry typical internal standards are deuterated which gives them a different observable mass. These analytical standards can be used to observe in-situ chemical transformation that is observable given the background in wastewater. The observation of these standards
can yield estimates of transformation, which is useful in terms of uncertainty in illicit drug loads. In total this dissertation seeks to account for the major limitations of wastewater epidemiology that include accounting for population, and in-situ chemical transformation. This work is testing if there is a difference in cocaine and benzoylcegonine in-situ transformation rates in an environmentally relevant system.
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uncertainties associated with the determination of community drug use through the

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Suspended biofilm carrier and activated sludge removal of acidic pharmaceuticals.
Normalized Diurnal and Between-Day Trends in Illicit and Legal Drug Loads that Account for Changes in Population

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Abstract

Drug concentrations in composite municipal wastewater samples and census-based estimates of population are used to derive daily loads of illicit substances that are indexed to population. However, such estimates do not provide information on the diurnal trends of substance excretion nor can they account for changes in population. To address these limitations, a series of 1 h composites created by sampling wastewater influent at 6 min intervals was collected over four consecutive days at a single wastewater treatment plant. Creatinine (a urinary indicator), caffeine, methamphetamine, benzoylecgonine (BZE), and cocaine were analyzed by liquid chromatography/tandem mass spectrometry (LC-MS/MS). Diurnal trends and between-day trends were substance specific and related to the number of estimated doses and excretory half-life. Normalization to creatinine yielded trends in substances that differed significantly from non-normalized trends by accounting for changes in population within the municipality studied. Increases in normalized substance excretion observed during early morning hours originate from individuals among the resident population of the municipality due to the absence of commuters.

Introduction

Illicit substance use in the United States is a social and economic problem. There is an ever present need for information regarding the use, distribution and presence of illicit drugs of concern at small (e.g., individual municipalities) and larger
(e.g., regional and national) geographic scales. Conventional methods for assessing drug use utilize indirect indicator data sources such as self-report surveys, drug treatment admissions, and fatal overdose data that are limited by low numbers of observations that are lagged in time and subject to known bias. Consequently, there is a need for data that directly measures drug consumption, which is obtained at smaller time intervals in order to determine temporal changes in usage patterns and impacts of interventions.

Municipal wastewater influent is used as a means for examining the loadings and source characteristics of chemicals used by humans including those contained in drugs of abuse, personal care products, household chemicals, and pharmaceuticals. Recent work indicates that illicit drug loads in wastewater influent and associated dose estimates are potentially complementary data sources to traditional indicators on substance abuse. The current method that uses wastewater influent concentrations to calculate per capita loads relies upon two measured values (concentration and wastewater flow) and one estimated value (population). Population is suggested as one of the greatest uncertainty when estimating per capita substance use from wastewater data. Annual population estimates obtained from census data are lagged in time. For example, the US census is conducted every 10 years with the last occurring on April 1st, 2010. Moreover, current census-based population estimates cannot reflect within-day or between-day changes in population. Indirect population indicators such as nitrogen, chemical oxygen demand, biological oxygen demand, phosphorus in wastewater are proposed as strategies to account for changes in population in addition to non-
traditional indicators such as prescription pharmaceuticals and electricity use within a municipality.

Creatinine is an endogenous urine indicator that has potential for use as a surrogate population indicator. Creatinine is produced at steady state in the body and is routinely used to verify urine authenticity and to account for dilution when testing human urine for illicit substances. Water soluble, urinary-derived creatinine is excreted with every void and it remains in the dissolved phase along with water-soluble substances including illicit drugs.

Average creatinine concentrations and corresponding 95% confidence intervals (95%CIs) in urine are well known for large (tens of thousands) numbers of individuals. For example, a U.S. study on 22,245 people including Non-Hispanic white, Non-Hispanic black, and Mexican Americans with individuals ranging in age from 6 to 90, gave an average creatinine concentration of 1,300 mg/L with a 30 mg/L 95% CI. A German study involving 45,000 people gave an average creatinine concentration of 1,000 mg/L with a 450 mg/L 95% CI that was not statistically different from that reported by Barr et al. Given the scale, creatinine concentrations obtained from studies on large number of individuals are viewed as likely representative of municipalities with mid to large populations. In contrast, creatinine concentrations for small numbers of individuals (8-2,000) appear more variable. Although creatinine occurs in cooked meat, the discharge/disposal of cooked meat via municipal wastewater system is considered inconsequential compared with solid waste routes of disposal. The biological variability of creatinine excretion for outlying sub-populations within a specific population is impossible to measure when aggregate
data is used. When creatinine is used as a normalization factor to account for a whole population this variation is captured as part of the total load. As a matter of fact this is true for any substance that is excreted and enters a WWTP. For these reasons, we hypothesize that creatinine can serve as a qualitative normalization factor to qualitatively account for changes in population, which addresses a current limitation in wastewater epidemiology. Normalization of illicit substances to creatinine potentially provides insight into diurnal and between day trends in loads of illicit substance.

The uncertainty about computed loads for illicit and other substances is usually not assessed, with few exceptions. Representatively capturing packets (e.g., “pulses”) of wastewater influent containing urine when creating composite samples is critical for more accurate data and in turn more accurate interpretations of temporal and spatial trends for a wide range of environmentally-relevant contaminants and illicit substances. With some exceptions, wastewater is typically collected as 24 h composites in a flow- or time-proportional manner. However, diurnal trends are masked by the current practice of collecting a single 24 h composite sample for each day. Alternatively, hourly (diurnal) trends for substances in wastewater influent can provide information on the daily patterns of substances used and consumed by humans. For example, a more detailed understanding of human consumption of illicit substance potentially aids in interpreting substance abuse within municipalities. Others have employed diurnal sampling to detect changes in concentrations of personal care products, steroids, and alkylphenols in wastewater effluent. Hourly data can then be compared to human urinary excretion of substances that are reported in the literature on the time scale of minutes to hours. Sampling to create single daily composites
typically occurs at hourly time intervals; however, there is an increasing trend toward more frequent sampling because frequencies of > 20 min are likely to miss significant pulses of urine.

The first objective of this study was to determine diurnal trends in selected substances with high sampling frequency on a 6 min interval to create sets of hourly composite samples over a period of 4 days at a single wastewater treatment plant (WWTP). Four days was selected only to demonstrate diurnal variability in the selected substances and the methodology to assess such trends. The time period used for this study was selected because a significant change in population was expected to occur due to university and public school students and families leaving for spring break. Substances selected for study include caffeine, which is a legal and widely-used substance that is considered a marker for wastewater contamination. Methamphetamine is a stimulant that is largely used as an illegal substance with only minor legitimate use in the US. Cocaine is a widely-used illicit stimulant and its major metabolite, benzoylecgonine (BZE), has been used for back-calculating numbers of cocaine doses from wastewater data. The second objective was to compare trends in selected substances with and without normalizing to creatinine in order to explore and account for changes in population within the municipality. Currently there have been no techniques to account for intra and inter day changes in substance excretion and population on the municipality scale.

**Experimental Methods**

**Location, Wastewater System, and Sampling.** The municipality selected for this study is located in the Pacific Northwest of the United States with a census-based
population of 55,000 (April 1\textsuperscript{st} 2010) and is characterized by the following age
distribution: 0-17 yrs (20%), 18-64 yrs (68%), and +65 yrs (12%).

The WWTP collects wastewater from a mainly gravity-fed sewer system (two
short sections are pressurized) consisting of 350 km of sewer piping and seven lift
stations that are located throughout the catchment. Two main lift stations pump ~25%
of the total daily wastewater volume (Figure 2.1.A). Although pump activity at lift
stations could significantly impact the temporal loads of illicit substances, discerning
the contributions of individual pumps was beyond the scope of this study. Ongoing
work is focused on understanding the potential influences of lift station pumps and the
information will be used to inform sampling practices for systems in which pumps
move a significant fraction of the total wastewater flow.

At the influent works of the WWTP, sewer flows are collected in a wet well
that is continuously pumped at variable flow rates to maintain a constant level in the
wet well. All raw wastewater samples were taken after the wet well and after the
water had passed through a series of screens. The flow provided by the WWTP was
recorded at 1 min intervals and used to compute hourly flows. Intra-hour variation in
flow within the four days was characterized by a relative standard deviation (RSD) of
less than 34% with only 15 of 96 hrs exceeding a RSD of 20% (see Table A1.1 in the
Appendix). The wastewater temperature was 14-15°C over the four sampling days
and there was no precipitation.

Sampling was carried out from March 17\textsuperscript{th} 2010 at 8 am through 8 am on
March 21\textsuperscript{st}. The sampling device (ISCO 3700 auto-sampler; Teledyne Isco Inc.,
Lincoln NE) was filled with ice to maintain a temperature of 4 °C and configured to
acquire 1 h composite samples. The ISCO sampler collected a sample every 6 min, which resulted in ten 25 mL samples to form each hourly composite. Following each day of sample collection, the 24 x 1 h composite samples were retrieved from the autosampler at 8 am and transported on ice (4 °C) to the laboratory. A 40 mL subsample of each composite was transferred to a HDPE centrifuge tube and stored at -20 °C without chemical preservation.

Chemical Analyses. Samples were analyzed by large volume injection liquid chromatography/tandem mass spectrometry as described by Chiaia et al.\textsuperscript{8} with minor modifications. Briefly, wastewater was centrifuged and the supernatant was spiked with a mixture of deuterated internal standards. Calibration curves were constructed by using five standards. For more information see the SI. Sequences contained blanks (buffer), quality control standards, and a third party, certified-reference standard (UTAK, INC Valencia, CA). Details on transitions, instrument parameters, preparation of calibration standards and curves as well as the preparation of the certified-reference standard are given in Table A1.2.

Analytical precision of the whole method, as indicated by the RSD, was determined from four replicate analyses of a single wastewater sample within each analytical sequence and typically were below < 20% (Table A1.3). Accuracy, as indicated by the % agreement with the certified third party reference standard, ranged from 68-91% (Table S4).

Uncertainty Analysis. Total uncertainties about the measured total hourly loads for each substance were calculated as the square root of the sum of the squares of the individual RSDs associated with the chemical analyses, the selected
subsampling frequency, and flow measurements made by the WWTP (Equation A1.1, Table A1.5). The uncertainties about the ratios of substances normalized to creatinine were calculated as the square root of the sum of the squares of the individual RSDs associated with the analysis and the sampling uncertainty (Equation A1.2). Sampling uncertainties were assigned considering the nature of the diurnal trend for each substance and the short 6 min sampling frequency. A sampling uncertainty of 5% was applied to caffeine and creatinine given their non-episodic (e.g., cyclic with one ‘wave’ or peak per day) diurnal trends and for the remaining substances a value of 15% was estimated. Flow measurement uncertainty was ± 5%, according to WWTP calibration protocols.

**Stability of Analytes During Storage.** The stability of analytes in wastewater samples stored for up to 24 h during collection at 4 °C was investigated by collecting single 500 mL grab samples of influent in a high density polyethylene bottle on each of three different days. The 500 mL samples were stored at 4 °C and aliquots were subsampled at 1–7, 12, and 24 h after collection and immediately frozen until analysis. For further details on the stability study see the SI.

First-order disappearance rate constants for each analyte were calculated by plotting the natural log of concentration versus time (Figures A1.1-A1.5). The mass lost during storage was computed by dividing the measured analyte concentration for each hourly composite sample by the term e^{-kt} where k is the average first-order rate constant (Table A1.6) and reaction times (t) that ranged from 24 h for the first composite sample collected to 1 h for the last hourly composite collected. Average rate constants for caffeine (Figure A1.2), methamphetamine (Figure A1.3), and
cocaine (Figure A1.5) were not significantly different from zero but variation about the measured rate constants resulted in computed losses ranging from 5-16% (Table A1.6). The estimated mass lost for each substance for each hourly composite sample was added to the measured mass to compute daily total loads for each substance. Error bars indicating the uncertainty about the estimated load of each analyte lost during storage were constructed using the upper and lower rate constants as indicated by the 95% CI about the average rate constant.

**Results and Discussion**

**Hydraulics.** The total wastewater flow decreased 17% from $3.82 \times 10^7$ L/d on Wednesday to $3.18 \times 10^7$ L/d on Saturday (Table 2.1) for the WWTP investigated. Hourly flows reflect a regular diurnal pattern with a factor of two change in flow from a minimum at night to a morning maximum (Figure 2.1A). A mean wastewater in-sewer residence time ($R_t$) of $\leq 1.5$ hrs was estimated from the location of the population’s highest density and an average wastewater velocity of 0.6 m/s in the gravity fed system. For convenience, a $R_t$ of 1 h can be used to back estimate the time of excretion within the municipality. For example, substances captured in a hourly composite collected from 8 to 9 am are attributed to excretion events within the municipality occurring from 7 to 8 am. However, no information can be inferred regarding the time of substance ingestion.

**Creatinine.** Creatinine is the only endogenous substance investigated in this study. Creatinine is excreted with every void; therefore, it does not have a half-life (the time required for the urine concentration of a substance to decrease by 50%) per se. For creatinine and all other substances investigated in this study, the estimated mass lost
during storage is added to the measured load to represent the total daily load excreted by the entire municipality.

The temporal trends exhibited by creatinine loads (concentrations multiplied by flow; Figure 2.1B) and concentrations (Figure A1.6) are similar to the diurnal pattern of wastewater flow (Figure 2.1A). The cyclic diurnal trends in creatinine are consistent with a large population excreting a correspondingly large number of creatinine-containing urine ‘pulses’.24

The diurnal trends in creatinine observed at the WWTP are characterized by peak loads from 8 am until 12-1 pm that then drop to a plateau that lasts until 12 pm-1 am (Figure 2.1B). The rapid increase in early morning creatinine is likely due to people waking up followed by their first void of the day. First voids are characterized by only 5% higher creatinine concentrations33 and cannot fully account for the ~30% larger loads of creatinine in the morning. The decline after 12 pm (midnight) is due to a large fraction of the resident population going to sleep. Peak creatinine loads shift later (i.e., 10 am) on Saturday, presumably due to later waking times (Figure 2.1B).

Commuters also potentially contribute to the greater loads of creatinine in the morning with a net weekday influx of 9,000 commuters into this municipality, which is 17% of the census-based population of 55,000.57 Commuters are likely to be of working age (e.g., 18-64 year olds), which is the age range that describes 68% of the municipality’s base population.56 The working age range of 18-64 falls within the age range of individuals included in the studies by Barr et al.33 and Arndt34 and thus have creatinine concentrations that are likely well represented by the reported average urine creatinine concentrations. Furthermore, the reported average urine creatinine
concentrations include variations with ethnicity and age. If there is any creatinine variation from foods and supplements in small groups of people they are likely to have little impact on measured creatinine loads for a whole community (i.e. large group). For this reason, the diurnal trends and changes in daily creatinine loads are attributed to changes in total population and not to shifts in age, race, sex, or diet.

The measured daily loads of creatinine (Table 2.1), which is the sum of the hourly loads (g) from 8 am to 8 am, decrease from Wednesday (36 kg) to Saturday (19 kg). The predicted maximum range in creatinine loads for this municipality were computed using both low and high average urine creatinine concentrations of 1000 mg/L and 1300 mg/L, 1.5 L of urine per person per day, and an assumed census-based population of 55,000 and a net influx of 9,000 commuters for a maximum total population of 64,000. Predicted average creatinine loads ranged from 96 to 125 kg per day using these average values, and one value of mean urine output per day (1.5 L). In an attempt to investigate the variation regarding the predicted creatinine loads, demographic data was used along with the median, 10th, and 90th percentile (± 95%CI) for age and ethnic group specific creatinine concentrations (See Appendix). When using the demographic data and median creatinine concentrations to calculate the predicted median loads they range from 116 kg to 130 with an average of 123 kg per day, which is within the 95%CI of total daily mass when the average is used (See Appendix). Urine volume and its variation, which is far less studied than creatinine concentrations may have the greatest influence on creatinine mass predicted per day. The average (n=654) urine volume per day was found to be 1.10 ± 0.05 L (95%CI). When taking volume of urine and its variation into account and using
median creatinine concentrations the range of the greatest measured creatinine load which occurred on Wednesday (Table 21) was between 36-43% of the predicted load (Table A1.7). When using average values of creatinine concentrations and 1.5 L of urine output, measured daily creatinine loads are 29-38% of the predicted load for this estimated maximum total population, which may be a result of a poor estimation of urine volume.

As indicated, this time period was deliberately selected because the population is known to decline due to the departure of students for spring break. The movements of the university’s student population, which is 40% of the municipality’s census-based population of 55,000, along with public school students and their families are likely to significantly affect measured creatinine loads. Given the lack of independent population measures and uncertainties, creatinine is used to measure apparent changes in population.

There is no independent information on the actual population served by this WWTP during the study period nor on actual changes in population associated with weekend days, (e.g., Saturday) so the accuracy of the maximum estimated population (64,000) is unknown. In the absence of data on the actual water consumption for each day of the study period, the annual estimate of the daily average drinking water consumed (1.73x10^7 L/d) was compared to the wastewater flows recorded during this dry weather study period (Table 2.1). Assuming no loss of drinking water in the distribution system and if it all entered the wastewater system, 1.45 x10^7 L/d to 2.09 x10^7 L/d (45-54%) of the total wastewater flow must originate from other sources. In fact, the municipality’s sewer system can be characterized as having a substantial
infiltration and inflow impact (personal communication WWTP operator). Detailed investigations in over 200 European sewer catchments reveal normal infiltration and inflow impacts between 35-65% of total wastewater flows during dry weather flow conditions, even in relatively well-maintained systems. For this reason, it was not possible to correlate drinking water consumption and total wastewater flow as an independent estimate of the municipality’s population during the study period.

Because ‘open’ municipalities are subject to changes in population within and between-day, more research is needed to refine our understanding of the correlation between creatinine loads and actual numbers of individuals. On-going research in this laboratory is focused on testing hypotheses for creatinine in prisons, which are closed systems with very well-defined hourly and daily populations. In addition, creatinine biodegradation during transit in sewers may decrease measured loads. Biodegradation is likely affected by factors related to the infrastructure of sewer systems including wastewater residence time, oxygen concentrations, lower temperatures (e.g., 14-15 °C), and the presence of an active biofilm. Although the biodegradation of creatinine and the other substances may be occurring during transit, the rates of biodegradation may be effectively constant for the infrastructure of a given municipality. Thus, hourly and daily trends in creatinine loads are assumed to be proportional to population. For this reason, the loads of all substances investigated in this study were ratioed (normalized) to those of creatinine in order to investigate dynamic changes in apparent per-capita excretion, which would not be possible if using static population estimates.

The observed patterns for creatinine are similar to those observed for ammonia, a more conventional urine indicator, for WWTPs and individual households.
Peak ammonia loads occurring from 6 to 10 am followed by nearly constant loads until 12 noon are governed by the circadian rhythm of urination.\textsuperscript{69,70} Therefore, the observed trends in creatinine loads reflect known patterns of human urine output. Ammonia was not measured in this study because, many other sources of ammonia exist within municipalities and because the WWTP investigated receives landfill leachate that is rich in ammonia.\textsuperscript{71}

Fecal-associated substances such as coprostanol, were suggested as potential biomarkers to estimate population size.\textsuperscript{8,16} However, in addition to the recognized challenges related to sampling for suspended and settleable solids, additional confounding factors exist when considering fecal-associated biomarkers for normalizing the mass flows of water-soluble substances. For example, sedimentation and non-continuous movement of feces cause storage of feces in sewer systems (house connections) and consequently leads to unknown lag times. In contrast to urination events that occur throughout the day, on average, there is only one fecal-related toilet flush per person per day.\textsuperscript{67,68} Therefore, most commuters may not contribute to the total feces load but proportionally to the urine load. For these reasons, fecal-associated biomarkers were not considered for this study.

**Caffeine.** Caffeine was selected to benchmark illicit substances for which consumption patterns are less well known. As expected, the wide consumption of caffeine is reflected in its higher loads (Figure 2.1C) and concentrations (Figure A1.6) when compared to those of the illegal substances. The non-normalized hourly loads (Figure 2.1C) depict cyclic diurnal trends that repeat on each of the four sampling days.
The first peak in non-normalized caffeine hourly loads observed at the WWTP occurs from 8-9 am and lasts until 12 am -1pm (Figure 2.1C). The largest loads in caffeine occur slightly later than those of creatinine and they shift to later times due to the population waking later in the morning on Saturday. The trends in loads for the two substances may also differ because caffeine ingestion typically begins after the first morning void. Furthermore, caffeine has a 6-9 h excretory half-life (the time required for the urine concentration of a substance to decrease by 50%), whereas creatinine is excreted with every void.

The daily loads of caffeine normalized to creatinine (Figure 2.1D) offer a significantly different picture of caffeine excretion than that by non-normalized loads (Figure 2.1C) with greater apparent per capita excretion of caffeine in the middle of the day rather than the morning. Non-normalized daily total loads of caffeine decrease from 1,600 g on Wednesday to 1,400 g on Saturday (Table 2.1) and indicate decreased consumption over the four day period. In contrast, apparent per-capita caffeine consumption increases from Wednesday to Saturday when loads are normalized to creatinine (Table 2.1). Such an increase requires that students and commuters leaving the community for spring break/ weekend consume less caffeine than those who remain during this time period. The example of caffeine illustrates that normalizing substances to creatinine changes the interpretation of trends in diurnal and between-day trends in loads. The amount of creatinine at any given hour is originating from the population excreting creatinine for that period of time. If you were to normalize to the entire population you would not change the observed trends, and have no idea how many people are actually in the community.
The number of caffeine doses was estimated assuming that 1%\textsuperscript{75,76} of a caffeine dose containing 150 mg\textsuperscript{73} is excreted (Equation A1.3). A large number of caffeine doses (9.6 \times 10^5 to 1.1 \times 10^6; Table 2.2) indicates a large number of caffeine-containing urine ‘pulses.’ A large number of pulses combined with a urinary half-life of 6 to 9 hrs\textsuperscript{73,74,76-78} is consistent with the observed cyclic diurnal trends in caffeine. However, the number of estimated caffeine doses (17-20 doses per person) is much larger than expected based on consumption alone, which indicates that other sources, such as the disposal of unused caffeine-containing beverages, which may contain high levels of caffeine, are entering the wastewater system. For this reason, caffeine is not an unambiguous human urine indicator.

**Methamphetamine.** Methamphetamine was quantified in each hourly composite sample over the four sampling days and is consistent with the characterization of this municipality as a high-methamphetamine use municipality as described in Chiaia et al.\textsuperscript{8} Diurnal trends in non-normalized methamphetamine hourly loads were relatively uniform (Figure 2.2A) with maximum to minimum loads exhibiting the least variability among the substances studied. In contrast, normalized-methamphetamine displays a distinct peak from 3-7 am (Figure 2.2B). Methamphetamine is a potent, relatively long-acting stimulant so activity among methamphetamine users late at night/early morning is not unexpected. Increased per-capita excretion during this time frame, when commuters are largely absent, is assumed to result from individuals within the resident population of the municipality.

The total daily methamphetamine loads, both non-normalized and normalized, increase Wednesday through Saturday (Table 2.1). However, normalized loads show
a greater increase in methamphetamine compared to non-normalized loads (Table 2.1). The apparent increase in per-capita excretion of methamphetamine on Friday and Saturday is due to the lower measured creatinine loads at the beginning of spring break and the onset of the weekend. This increase is assumed to result from the resident population of the municipality.

The total number of estimated methamphetamine doses ranged from 270 to 330 (Table 2.2) and were computed assuming a dose of 100 mg and that 40% of the dose is excreted (Equation A1.4). In the region where this municipality is located, legal use of methamphetamine as a component of prescription medications is estimated to account for only 3-8% of the observed methamphetamine loads.

Because the estimated number of methamphetamine doses is lower (270-330; Table 2.2) than that of caffeine, the number of methamphetamine-containing urine pulses is lower. While one might expect episodic (e.g., multiple peaks of shorter duration) trends in methamphetamine loads, methamphetamine has a longer half-life (9-24 h) than caffeine, which may explain why the diurnal trends in methamphetamine are cyclic and not episodic.

**Benzoylcegonine and Cocaine.** Benzoylcegonine, the major human metabolite of cocaine, was observed in each hourly composite sample (Figure 2.3A), which was not the case for the parent substance, cocaine (Figure 2.3C). The non-normalized hourly loads of BZE and cocaine are episodic with no discernible diurnal or between-day trends, which is in contrast to methamphetamine. Normalized loads of BZE and cocaine have weak but discernible peaks during the very early morning hours from 2-7
am (Figure 2.3B and 2.3D) when commuters are largely absent and, as such, are attributed to cocaine users within the resident population.

The non-normalized total daily loads of BZE and cocaine indicate no apparent trend between days (Table 2.1). In contrast, normalization to creatinine provides evidence that the per capita BZE and cocaine loads actually increase from Wednesday to Saturday (Table 2.1). Given the decrease in students and commuters as the weekend approaches, increased excretion of cocaine and BZE is interpreted as resulting from individuals among the municipality’s resident population due to the absence of the commuters during this time period. Increased cocaine usage on the weekend is consistent with other report indicating increases on weekends,\(^{22,47}\) holidays,\(^{81}\) and sporting events\(^{46}\) that rely on non-normalized cocaine/BZE data and that assume a constant population. However, such ‘weekend effects’ may actually be larger than reported if population changes are taken into account by normalizing to creatinine loads.

The estimated number of doses per day of cocaine calculated from the total daily load of BZE (Table 2.1; Equation A1.5\(^9\)) ranged from 35 to 45 (Table 2.2), which is low compared to other municipalities.\(^{15,47}\) Alternatively the number of doses if cocaine itself\(^{82}\) is used for the estimate ranged from 160-330 (Table 2.2; Equation A1.6). When considering the more accepted approach of estimating doses from BZE, the number of cocaine doses is the lowest among the substances investigated. The relatively low number of doses combined with the short excretory half-life of cocaine (1-4 hrs)\(^{41}\) is consistent with episodic cocaine loads. Even though BZE is excreted over long time periods (e.g., 3 days)\(^{41}\) that could then result in cyclic diurnal patterns,
the low number of cocaine doses may explain the observed episodic nature of BZE loads.

The ratio of BZE (Figure 2.3A) to cocaine (Figure 2.3C) ranged from 2-32 (data not shown), which is consistent with the reported ratios for 24 h wastewater composites that range from 2 up to 10. Ratios of BZE/cocaine for wastewater indicate that BZE is only ten times greater than cocaine, which is much lower than those reported in detailed datasets describing the mass balance and continuous monitoring of cocaine and its metabolites in human subjects over the time scale of minutes to hours. Others speculate that impacts of alcohol co-consumption and biodegradation in transit to WWTPs as well as deliberate release (e.g., dumping) of cocaine result in low BZE/cocaine ratios. While ‘dumping’ could result in low BZE/cocaine ratios, one would also expect such events to occur as rather large discrete peaks in the diurnal trends in cocaine loads. However, no large peaks are apparent among the hourly composites (Figure 2.3D). For this reason, ‘dumping’ does not appear to account for the low ratios in this system. Alternatively, “wash off”, may occur over the course of the day when washing cocaine-contaminated hands, surfaces, and implements involved in cocaine administration. In addition, wash off may occur after packaging or re-formulating powder cocaine to “crack”, which is often done at the local level following importation. If cocaine is washed off then 100% of the cocaine mass enters the wastewater stream instead of 1% had it been metabolized. Therefore, even small amounts of unmetabolized cocaine entering the waste stream due to wash off could significantly shift BZE/cocaine ratios to the low observed values.
Implications. The overarching goal of this study was to develop an approach that can account for qualitative changes in population when estimating illicit substance loads for municipalities. The methodology was applied to discern diurnal trends of illicit substances that, by their very nature of their use, are likely substance-, time-, and location-specific. The generalized methodology can be applied to determine the diurnal trends for these and other substances to any other series of days, seasons or locations.

Diurnal sampling offers detailed insight into substance loads within a day on the scale of a whole municipality that single 24 h composites simply cannot provide. Diurnal trends in creatinine were attributed to apparent per-capita changes in population and used to qualitatively account for within and between day changes in substance loads. Normalization to creatinine changed the interpretation of within and between-day trends. Thus, normalization to creatinine is a tool that shows potential for adjusting loads of substances obtained from single daily 24 h composites in order to account for changes in population. Normalizing to creatinine revealed the late night and early morning excretion of illicit substances interpreted as resulting from individuals among the municipality’s resident population. Interventions to address substance use can be better targeted with more precise information about the population (resident and/or non-resident) consuming substances that is gained from within-day patterns of excretion.

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Table 2.1. Total wastewater flow entering the WWTP and total loads of measured plus average estimated loss during storage for creatinine, caffeine, methamphetamine, benzoylecgonine (BZE), and cocaine, and normalized load (load substance /load creatinine) for each sampling day starting at 8 am.

<table>
<thead>
<tr>
<th>Substance</th>
<th>Wednesday</th>
<th>Thursday</th>
<th>Friday</th>
<th>Saturday</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total wastewater flow (L)</td>
<td>3.82x10^7</td>
<td>3.65x10^7</td>
<td>3.48x10^7</td>
<td>3.18x10^7</td>
</tr>
<tr>
<td>Creatinine (kg)</td>
<td>36</td>
<td>27</td>
<td>22</td>
<td>19</td>
</tr>
<tr>
<td>Caffeine (g)</td>
<td>1600</td>
<td>1600</td>
<td>1500</td>
<td>1400</td>
</tr>
<tr>
<td>normalized</td>
<td>0.05</td>
<td>0.06</td>
<td>0.07</td>
<td>0.08</td>
</tr>
<tr>
<td>Methamphetamine (g)</td>
<td>11</td>
<td>11</td>
<td>13</td>
<td>13</td>
</tr>
<tr>
<td>normalized</td>
<td>3.1 x10^{-4}</td>
<td>4.1 x10^{-4}</td>
<td>5.9 x10^{-4}</td>
<td>6.8 x10^{-4}</td>
</tr>
<tr>
<td>BZE (g)</td>
<td>2.4</td>
<td>2.2</td>
<td>1.9</td>
<td>2.1</td>
</tr>
<tr>
<td>normalized</td>
<td>6.7 x10^{-5}</td>
<td>8.1 x10^{-5}</td>
<td>8.6 x10^{-5}</td>
<td>1.1 x10^{-4}</td>
</tr>
<tr>
<td>Cocaine (g)</td>
<td>0.15</td>
<td>0.33</td>
<td>0.23</td>
<td>0.21</td>
</tr>
<tr>
<td>normalized</td>
<td>4.2 x10^{-6}</td>
<td>1.2 x10^{-5}</td>
<td>1.0 x10^{-5}</td>
<td>1.1 x10^{-5}</td>
</tr>
</tbody>
</table>
Table 2.2. Estimated number of doses of caffeine, methamphetamine, cocaine (computed from BZE), and cocaine (from cocaine). For assumptions and calculations see Equations A1.3-A1.6.

<table>
<thead>
<tr>
<th>Substance</th>
<th>Wednesday</th>
<th>Thursday</th>
<th>Friday</th>
<th>Saturday</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caffeine</td>
<td>$1.1 \times 10^6$</td>
<td>$1.0 \times 10^6$</td>
<td>$1.0 \times 10^6$</td>
<td>$0.96 \times 10^6$</td>
</tr>
<tr>
<td>Methamphetamine</td>
<td>270</td>
<td>280</td>
<td>320</td>
<td>330</td>
</tr>
<tr>
<td>Cocaine (computed from BZE)</td>
<td>60</td>
<td>50</td>
<td>40</td>
<td>50</td>
</tr>
<tr>
<td>Cocaine (computed from cocaine)</td>
<td>160</td>
<td>330</td>
<td>220</td>
<td>210</td>
</tr>
</tbody>
</table>
Figure Legends

Figure 2.1. Total hourly wastewater flows (gravity and pumped) from March 17th - March 21st (A), hourly loads (mg) of creatinine (B), caffeine (C), and caffeine/creatinine (D). Blue bars indicate sum of lift station wastewater flow. Red bars indicate measured load and the corresponding error (See Appendix for error calculations). Clear bars indicate the estimated load loss during collection and storage at 4 °C and the corresponding error calculated from the 95% CI of the rate constant measured during the stability study. Black hatched bars indicate 8 am as a time reference for each day.¹

Figure 2.2. Hourly load (mg) of methamphetamine (A) and methamphetamine/creatinine (B) for Wednesday March 17th – Saturday March 21st. Colored bars indicate measured load and the corresponding error (See Appendix for error calculations). Clear (but very small) bars indicate the estimated load loss during collection and storage at 4 °C and the corresponding error calculated from the 95% CI of the rate constant measured during the stability study. Black hatched bars indicate 8 am as a time reference for each day.¹

Figure 2.3. Hourly load (mg) of benzoylecgonine (A), benzoylecgonine/creatinine (B), cocaine (C), and cocaine/creatinine (D) for Wednesday March 17th – Saturday March 21st. Colored bars indicate measured load and the corresponding error (See Appendix for error calculations). Clear bars indicate the estimated load loss during collection and storage at 4 °C and the corresponding error calculated from the 95% CI of the rate constant measured during the stability study. Black hatched bars indicate 8 am as a time reference for each day.¹
The bottle collecting the 7-8 am sample on Saturday broke during collection.
Figure 2.2

The bottle collecting the 7-8 am sample on Saturday broke during collection.
The bottle collecting the 7-8 am sample on Saturday broke during collection.

*Samples with signal-to-noise ratios <10 determined from calibration curves are included for qualitative purpose.*
Literature Cited


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Wastewater testing as an alternative to random urinalyses for the surveillance of illicit drug use in prisons

Alex J. Brewer, Caleb Banta-Green, Christoph Ort, Jennifer Field
Chapter 3- Wastewater testing as an alternative to random urinalyses for the surveillance of illicit drug use in prisons

Abstract

Illicit drug use is known to occur among inmate populations of correctional (prison) facilities. Conventional approaches to illicit drug use surveillance in prisons include random urine analyses (RUAs) and surveys. Both approaches are prone to bias because prisoners are aware of which days of the week that the prison conducts RUAs. The hypotheses that 1) the mass loads of methamphetamine will exceed that of cocaine and 2) the loads for methamphetamine and cocaine are lower on non-RUA testing days were tested. The objectives was to collect daily (24 hr) composite samples of wastewater collected by continuous sampling for one month and to compare the frequency of illicit drug detection to the number of positive RUAs for methamphetamine and cocaine. Hourly data also were collected for a subset of days in order to determine the diurnal trends in illicit drugs and creatinine, a human urinary biomarker. Methamphetamine was observed in each daily sample of prison wastewater with no difference in mass loads or bias between RUA testing and non-testing days. Although daily and hourly mass loads for methamphetamine were lower than those for a nearly community, the per capita use (number of doses per 1,000 people) was higher inside the prison. Cocaine and its major metabolite, benzoylecgonine, were observed at levels below quantification in wastewater, which indicated lower use of cocaine compared to methamphetamine in the prison. The wastewater data were complementary to the RUA data with six RUAs positive for methamphetamine while none were positive for cocaine out of the 243 RUAs
conducted. The diurnal trend in creatinine mass loads due to human
wakefulness/activity in the prison was similar to those of an open community. Daily
measured creatinine loads were in good agreement with those estimated for inmate
and non-inmate populations, which suggests that creatinine can be used as a
quantitative population indicator.

Introduction

Correctional (prison) facilities are small communities with a well-known
population that typically discharge wastewater via prison-specific sewer lines and
many have their own wastewater treatment plants. Because a majority of the people
entering prisons are in need of substance-abuse treatment, surveys and random
urine analyses (RUAs) are used as a means of illicit drug use surveillance. Concerns for prisoner safety stem from the control of illicit drug availability/use by
prison inmate gangs. Subjects are selected for RUA either randomly or based upon
suspicion. The temporal frequency of RUAs is either random or on a schedule of
convenience (e.g., Monday-Thursday), which are well known to inmates. Therefore,
RUAs capture only a small percent of the inmate population on any given day, the
data may be biased due to prisoner knowledge, and tests are largely negative.

Wastewater epidemiology is an emerging area of research that focuses on the
estimation of drug use within a community by interpreting drug loads. Drug
loads are typically calculated by multiplying drug concentrations by wastewater flow,
thereby obtaining mass for a given sampling period. To date, only a single report
documents illicit drug use in prisons using wastewater epidemiology approaches.
While this study reported quantifiable loads of illicit drugs and estimated the number
of doses over time, no comparisons of measured loads to conventional indicators of
drug use (e.g., RUAs) were made. Because wastewater sampling can be performed
without prisoners’ knowledge, it was used to test the hypothesis that illicit drug loads
on RUA testing days are lower than on non-RUA testing days due to prisoner
knowledge and avoidance of drug use. Wastewater testing has the potential of
providing more representative information on illicit drug use within the prison system
that is complementary to RUA data.

Prison systems offer an opportunity to further test the hypothesis that
creatinine, a human urinary biomarker, can be used to normalize drug loads for
changes of population in open communities. The actual population served by the
wastewater treatment plants of open communities is one of the greatest uncertainties
when calculating per capita illicit drug use in wastewater epidemiology. Brewer et
al. reported creatinine-normalized illicit drug loads that differed from non-normalized
loads and revealed resident drug use in the absence of commuters. However,
uncertainties remain in interpreting the temporal trends in creatinine mass loads for
open communities and the ability to use creatinine to quantitatively estimate
population. For example, the actual population of open communities on any given day
is not known, in part, due to the movement of commuters, and biodegradation may
also decrease creatinine concentration during transit in sewer systems. By
comparison, the total population and the demographics of prisons are well known and
the transit time/distance between the locations of illicit drug excretion and sample
collection is short. For these reasons, prison settings provide a unique opportunity to
measure the temporal trends and mass loads of creatinine for a well-known population both in terms of numbers and demographics.

The first objective of this study was to collect single, 24 h wastewater composites by continuous sampling of a prison’s wastewater effluent each day in a single month (August 2011). The mass loads of methamphetamine, cocaine, and benzoylecgonine were then compared to the number of positive RUAs over the same one-month time period. The second objective of this study was to measure methamphetamine, cocaine, benzoylecgonine, and creatinine loads in 24 individual hourly composite samples over a single day.

**Experimental Methods**

**Prison Location.** A prison facility constructed in 1866 and located in the Pacific Northwest of the United States housing 2,083 male inmates (Figure 1A) was selected for this study. Inmates within the prison are predominately Caucasian (68%) with a normally distributed age range (See Appendix, Table A3.1). The inmates within the prison are transferred to other facilities during various times but are replaced by incoming inmates according to state prison housing needs. Thus, a constant inmate population of 2,083 is maintained. The prison system also has non-inmates comprised of employees who are present at all times of day as well as visitors who are allowed inside from 7:15 to 10:15 and again at 12:30 to 15:45 each day. Numbers of employees, separated out from visitors to the prison and by work shift, were not made available due to security reasons. From August 8–16, the average number of non-inmates (employees and visitors) per hour was 162. The overnight, when visitors are not allowed, minimum of 20 non-inmate employees was ~1% of the
prison population. The maximum number of non-inmates (employees and visitors) was 375 (18% of the prison population) and occurred during visiting hours (Figure 1A). The prison was not able to supply data on the number of visitors and employees for July 30 – Aug 7 and Aug 17 – Aug 28. The average number of non-inmates (employees plus visitors) for a day was computed by counting the total number of non-inmates once per hour and dividing by 24 h because the non-inmate data was provided by the prison as a running total. Permission for the study was obtained but the activities were deemed exempt from Institutional Review Board (IRB) oversight. The IRB-exemption is consistent with the analysis by Hall et al. who found no ethical issues related to using wastewater analysis to monitor drug use in prisons.90

The major water uses within the prison are personal inmate water use (toilet and shower), a commercial laundry operation, and food preparation. All prison wastewater exits the facility via gravity and is the only contribution to an exposed 45.72 cm parshall flume located ~45 m outside the prison wall in locked area. Wastewater residence time (minutes) in the system is considered negligible due to the small footprint of the prison (9 ha) and the proximity of the sampling point to the prison. Total flow was recorded using an ISCO 4230 (Lincoln, NE) flow meter that was set to record total hourly flows for the month studied. Uncertainty with regard to the measured flow is 0.1% and is assumed to be negligible (personal communication with manufacturer).91 Percent agreement between the flow measured during this study and records of the prison’s municipal water use (personal communication with State Department of Corrections employee)92 during the month was 99%. Wastewater flow
exhibited a regular “cyclic” pattern for the hourly sampling period (Figure 1B). There was no precipitation during the sampling period.

**Wastewater Sampling.** Representatively sampling wastewater in close proximity to a single source with relatively few people, such as a prison, requires continuous sampling or sampling at a very high frequency in order to capture all urine pulses potentially containing illicit drug residues.\(^{14,31}\) Daily composite (24 h) samples were collected starting from 23:05 July 30 to 23:05 August 26 (28 days). For the collection of daily samples, a Masterflex peristaltic pump was used (3.2 mm Norprene® tubing x 7.62 m) to deliver wastewater at a flow rate of 8 mL/min. A total volume of 12 L was collected in a closed container (HDPE) located inside a refrigerator (4ºC).

Hourly composite samples were collected daily for 7 days from August 9-16. An ISCO 2900 was set to collect hourly composites and fitted with the same type of tubing/pump as described above. The peristaltic pump was operated continuously at 8 mL/min for a total collected volume of 480 mL per hour in 500 mL HDPE bottles. Daily and hourly samples were transferred to 50 mL centrifuge tubes every night and transported at 4ºC to the laboratory (Oregon State University) and directly frozen until analyses.

**Chemical Analyses.** Samples were analyzed using large volume injection liquid chromatography/tandem mass spectrometry as described by Brewer et al., 2012.\(^7\) The lower limits of quantification (LOQ) for analytes were 40 ng/L (methamphetamine), 5 ng/L (cocaine), 40 ng/L (benzoylcegonine), and 50,000 ng/L (creatinine). The LOQ was defined as any chromatographic peak with S/N (signal to
noise) > 10 and above the lowest standard concentration. The limit of detection (LOD) was defined as any peak above a S/N >3. Accuracy, as determined from duplicate analysis of a third party reference standard, ranged from 83 to 94% (Table A3.2). Precision, determined from a wastewater sample containing all analytes that was analyzed four times, ranged from 1 to 12 % RSD (Table A3.2). Details on preparation of calibration standards, curves, and blanks as well as the preparation and use of the certified-reference standard for use in determining accuracy and precision are given in the Appendix. Wastewater concentrations were multiplied by appropriate daily or hourly flows to yield mass loads (mg or kg).

**Analyte Stability During Collection and Storage.** No loss of methamphetamine or cocaine occurred during storage for 24 h at 4 °C; however, a loss of 32 and 13% were observed for benzoylecgonine and creatinine.\(^7\) Uncertainty about the measured loads was calculated as the percent relative standard deviation (RSD) about the analytical method.

After one month of collecting wastewater samples through the 7.62 m x 3.2 mm i.d. tubing, a biofilm developed. Thus, analyte stability in the presence of a biofilm was investigated after completion of the field study. To simulate the continuous sampling scenario, a peristaltic pump was set up at the local wastewater treatment plant to continuously pump raw wastewater for 4 weeks at a flow rate of 8ml/min through an initially new piece of 7.62 m x 3.2 mm i.d. (7.5 min retention time) Norprene® tubing.

Sampling took place on days 1, 2, 3, 7 and then every 7 days thereafter. For each sampling day, a 500 ml bottle was filled with fresh municipal wastewater influent
and spiked to give quantifiable concentrations of all analytes and mixed thoroughly. This bottle was then sampled to determine the initial concentrations (Co) of all analytes. The tubing that had had wastewater continuously pumped through it was then placed into the spiked 500 ml bottle and 240 mL over 30 min (4 void volumes) were pumped from the bottle. Triplicate samples were then collected in sequence as the spiked wastewater passed through and exited the tubing.

As a control, a piece of new tubing was evaluated on each sampling day in a similar manner except that no biofilm was present. Spiked wastewater that had passed through the new tubing was collected as wastewater exited the tubing. All samples were then immediately placed on ice (4°C), transported to the lab, and frozen until analyses (-20°C). The concentrations that were initially determined in the 500 mL bottle of spiked wastewater were then compared to the concentrations that exited the tubing with and without biofilm on each sampling day.

There were no significant differences at the 95% confidence interval (CI) for methamphetamine and benzoylecgonine after four weeks of biofilm growth in the tubing that had been continuously exposed to raw wastewater. There was a significant decrease in cocaine concentrations but only after the third week. Creatinine concentrations decreased significantly (95%CI) after three days of biofilm development; thus, only the first three days of creatinine data are discussed.

**RUA Testing.** This prison routinely collects urine samples for RUA only on Mondays through Thursdays and only six urine samples per day are collected. No urine samples are typically collected on Friday-Sunday. However, for this study, urine samples were collected for RUA every day of the week (Monday- Sunday) and at a
higher frequency of collection (21 per day) from Aug 1-5 in order to sample a greater proportion of the prison population. A RUA sampling frequency of six per day was then used for August 6-26.

A total of 243 random urine samples were collected over 28 days. The total number of RUAs given during the 28 days captures 12% of the total inmate population, while the daily sampling rate of 6 and 21/day corresponds to 0.3 to 1% of the prison population. Prisoners were monitored (watched) while urine was collected. Methamphetamine in urine was measured as an indicator of methamphetamine use while benzoylecgonine (the major metabolite of cocaine) was analyzed as an indicator of cocaine use. Urine was analyzed by Pharmatech laboratories (San Diego, CA) using Siemens EMIT reagents and Olympus AU2700 analyzers. Suspected positive tests (after screening) were confirmed using gas chromatograph mass spectrometry. Analysis of urine for the presence of cocaine (determined by benzoylecgonine) had a LOD of 150 ng/ml while methamphetamine had a LOD of 500 ng/ml.

Results and Discussion

Methamphetamine. Methamphetamine was quantified in every daily composite sample and the corresponding mass loads ranged from 467 to 2,730 mg (Figure 3.2A). In contrast, methamphetamine was only infrequently detected (5/42 days) in a Spanish prison. As expected, the prison’s daily mass loads were smaller than those for a nearby community with an estimated population of 55,000, which ranged from 7,150-20,900 mg \(^{53}\) and 11,000–31,000 mg. \(^{7}\) A range of 12-68 doses of methamphetamine per day was calculated for the prison’s 2,083 inmates (inhabitants). \(^{7}\) The corresponding range of 6-32 doses per 1,000 inhabitants (inmates)
was higher than that for the nearby community of 55,000 residents with 5-6 methamphetamine doses/1000 residents,\textsuperscript{7,53} which indicates a higher per capita use of methamphetamine within the prison than in the nearby community.

The methamphetamine loads for the prison’s routine RUA testing days (Monday-Thursday) was not statistical difference (p<0.05) from that for non-testing days (Friday-Sunday). For this reason, the hypothesis that prisoner knowledge of testing days would result in lower methamphetamine loads on RUA testing days (Monday-Thursday) was rejected. However, contributions to daily measured loads by non-inmates (employees and/or visitors) cannot be excluded and could potentially obscure any patterns in inmate methamphetamine excretion/use by day of the week.

Of the 243 RUAs conducted during the sampling period, only six (2.5%) were positive for methamphetamine (Figure 3.2B), which is in contrast to the methamphetamine loads measured for each day (Figure 3.2A). The lack of agreement between the measured loads and the number of positive RUAs indicate that the RUA program underestimates actual methamphetamine use within this facility.

Additional information on methamphetamine use within the facility was obtained from the diurnal profile compiled from the hourly composite samples collected on a single day (August 13; Figure 3.2C). Methamphetamine was present in every hourly sample with mass loads ranging from 12 to 150 mg and an average of 54 mg (Figure 3.2C). The hourly average in methamphetamine load (50 mg) is approximately ten times less than those for the nearby open community (500-1,200 mg).\textsuperscript{7} Although more episodic, the general trend in hourly mass loads is similar to those observed on individual days for an open community.\textsuperscript{7} The episodic nature of the
prison’s hourly mass loads is likely due to less dilution and mixing, as proximity of
the sampling location to the small population.\textsuperscript{14}

Methamphetamine use is evident during visiting (7:15-10:15 and 12:30-15:45) and
non-visiting hours, which is when only inmates and employees are present.
Because employee numbers and the timing of shift changes were not provided by
prison, it is not possible to differentiate between inmate and employees contributions
to the observed methamphetamine loads during non-visiting hours. Use of
methamphetamine by employees is shown to lessen the fatigue associated with work
activities\textsuperscript{93}, night shift work\textsuperscript{94} and increase alertness.\textsuperscript{95} Alternative sampling locations
inside the prison and more detailed records on the number and work shifts of
employees would be needed to differentiate inmate from employee use and excretion
of methamphetamine.

**Cocaine and Benzoylecgonine.** All cocaine and benzoylecgonine
concentrations were below the LOQ of 10 and 40 ng/L, respectively, for each daily
composite sample collected over the month. All cocaine and benzoylecgonine
concentrations in the daily composite samples were either below the LOD or between
the LOD and LOQ (LOD>\(X<\)LOQ). Cocaine and benzoylecgonine were detected for
\(~50\%\) of the days during the month although concentrations were below the LOQ
(Table 1). Cocaine levels in wastewater from the nearby community also were at or
near the LOQ\textsuperscript{7} while cocaine and benzoylecgonine gave 100\% frequency of detection
(42/42 days) above the LOQ at a Spanish prison.\textsuperscript{18}

A Fisher's Exact Test was used to test if there was any difference (two-sided,
\(\alpha<0.05\)) between samples that were above detection (LOD>\(X<\)LOQ) and the
number of samples <LOD for days on which RUA typically are collected (Monday-Thursday) and non-testing days (Friday-Sunday). A 2x2 contingency table (Table 3.1) was constructed but here was no statistical difference between the routine RUA testing days (Monday-Thursday) and the non-testing days (Friday-Sunday) for both cocaine and benzylecgonine. The hypothesis that prisoner’s knowledge would result in lower loads of cocaine/ benzylecgonine on routine RUA testing days was rejected.

No positive RUAs for cocaine and benzylecgonine were reported during the month. The lack of positive RUAs for cocaine is consistent with the low concentrations of cocaine and benzylecgonine detected in wastewater. Wastewater measurements support the RUA test results with both sets of measures indicating that methamphetamine use is more prevalent inside the prison than is cocaine use.

**Creatinine.** The prison offered an opportunity to study the daily and hourly mass loads of creatinine for a fixed inmate population and a small percent (~10%) variation in total population due to movement of employees and visitors. Greater variation in the hourly (diurnal) creatinine mass loads was observed for the prison than for the open community, which is likely to the smaller number of total people in the prison, less total dilution of the wastewater, and a sampling location closer to the small population sampled.

The diurnal trend in creatinine mass load was similar to those of an open community except the increase in creatinine mass loads of the prison begin early at 5:00, which in contrast to 8:00 for the open community. The sharp increase in creatinine loads from 5:00-6:00 is consistent with the prescribed prisoner waking time of 5:00 followed by the inmate population’s first urine void of the day (Figure
The creatinine mass load increase from 6:00-7:00 is likely due to the arrival of employees, which is represented by the increase in population before visitation hours begin at 7:15 (Figure 3.1A). Independent validation of employee work shift times was not possible due to reasons related to prison security.

Creatinine loads decline over the day and reach a minimum (Figure 3.1C) that is attributed to inmates (2,083) and employees (31-50) working the night shift from midnight to 5:00 (Figure 3.1A). An overnight minimum also was observed for the open community and was attributed to the resident population when commuters were largely absent. A general pattern of human wakefulness/activity and creatinine is indicated by the similar diurnal trends in creatinine mass loads for the nearly-closed (e.g., prison) and an open nearby community. Therefore, creatinine loads in any given community may reflect the general patterns of human wakefulness/activity.

The total daily mass loads for creatinine were determined for six individual days during this study. Data for creatinine in wastewater was obtained for the first three days on which single daily composite samples were collected (July 30-August 1; data not shown) and for the hourly composites collected on August 9-11 (Figure 3.1C). Creatinine daily mass loads for July 30-August 1 ranged from 4.17 to 4.56 kg (data not shown) while the daily creatinine loads that were taken as the sum from the hourly loads for Aug 9-11 were similar and ranged from 4.41 to 2.99 kg (Table 3.2). The average creatinine mass load of 4.05 kg for these six days gave a small relative standard error of 4.8%, which is low and consistent with the nearly-closed nature of the prison for which ~90% of the total population (e.g., the inmates) does not leave.
In order to make a prediction of creatinine mass excreted by the prison population, the Department of Corrections supplied the total number of individual inmates by racial/ethnic category including Non-Hispanic White, Non-Hispanic Black, Mexican American, and other (Table A3.1). The age distribution also was supplied but only for the total population. Therefore, the age distribution was assumed to apply equally to each racial/ethnic group and used to calculate the number of inmates within each age/race/ethnicity subgroup (Table A3.1). Creatinine concentrations (mg/L) as reported in Barr et al.\textsuperscript{33} were then applied to each age group. When the age range supplied by the Department of Corrections spanned more than one age group as reported in Barr et al.,\textsuperscript{33} the average creatinine concentrations of the two age groups reported by Barr et al. was used. To estimate the inmate’s weighted creatinine urine concentration of 1,565 mg/L, the creatinine concentration for each age/racial/ethnic group was multiplied by the fraction of each age/racial/ethnic group of the total inmate population (Table A3.1).

The weighted mean creatinine concentration for the inmate + non-inmate population (Equation A3.1) was then computed and used to estimate the total volume of urine discharged by the prison’s total population for the individual days of August 9-11. It was assumed that the inmates and non-inmates (employees and visitors) are the only source of urine in the prison’s wastewater and that only dilution affects the concentration of creatinine in the prison’s wastewater. To estimate the total urine volume discharged by the prison per day (L), the measured wastewater creatinine concentration (mg/L) was multiplied by the total volume of wastewater (L) and divided by the weighted creatinine concentration for the inmate + non-inmate
population for each day (Table A3.3; Equation A3.2). Total estimated urine volumes for the three days ranged from 2,057–2,718 L (Table A3.3). To estimate the volume of urine excreted per person per day, which ranged from 0.91 – 1.21 L/person*day, the total volume of urine for each day was then divided by the total prison population. The volume of urine excreted per person was in good agreement with the range of 1.1 ± 0.5 L/day reported in Murakami et al.\textsuperscript{61}

The next step was to estimate the total mass of creatinine excreted by the inmate and non-inmate populations. The weighted creatinine concentration for each age/racial/ethnic subgroup (mg/L) of inmates (Table A3.1) was multiplied by the estimated urine volume (L/person*day) for each day and then summed to get the predicted total mass (kg) of creatinine excreted per day (2.96 – 3.93 kg) for the inmate population. To compute the total mass of creatinine potentially originating from non-inmates, the average number of non-inmates per day was multiplied by the estimated total urine volume and the mean creatinine concentration from Barr et al.\textsuperscript{33} (1,304 mg/L). No age or racial data was available on the non-inmates; therefore, the mean creatinine concentration from Barr et al obtained for 22,245 subjects of various ages and ethnicities was used (1,304 mg/L). The final predicted total mass of creatinine (kg) for the prison was taken as the sum of the predicted inmate and non-inmate creatinine masses. The predicted total creatinine mass for August 9-11 ranged from 4.2 to 3.18 kg (Table 3.2) and is in good agreement (94 – 106%) with measured total creatinine mass loads in wastewater (Table 2).

The prison system was nearly ideal for measuring the mass loads of creatinine because of its characteristic fixed population with only ~10% variation due to the
movement of employees and visitors. The sampling location that was within 45 m of the prison minimized wastewater residence times (minutes) so that the potential for creatinine biodegradation was also minimized. These constrained conditions permitted the estimate the volume of urine excreted per person, which is a term that is needed to compute the number of individuals utilizing a wastewater treatment system from measured daily mass loads of creatinine for communities (Equation A3.3). The estimated urine output per person obtained from the prison was in good agreement with literature values, which indicates urine volumes of 0.91 – 1.21 L/person*day may be representative for use in the field of wastewater epidemiology for estimating population from creatinine mass loads. Given this output of urine per person and the estimated creatinine concentration for total prison population gave good agreement (94-106%) with measured creatinine masses. Such agreement indicates the potential for creatinine to be used as a quantitative indicator of population against which illicit drug loads can be normalized.

The sensitivity of the predicted creatinine mass to assumptions regarding the population’s demographics and estimated volume of urine excreted per person was tested. First, it was assumed that no demographic data were available for the prison population was applied to the total prison population. The resulting predicted mass load of creatinine was 80-90% of the measured creatinine mass (data not shown). Furthermore, by inserting the literature value of 1.1 L urine/person*day, agreement between the measured and predicted creatinine mass loads was obtained. The use of mean creatinine concentrations for 22,245 subjects and the per person output of urine from the literature provided reasonable agreement with measured
creatinine mass loads. This finding suggests that back calculating to population using values from the literature, in the absence of more location-specific data, and measured creatinine loads in wastewater can provide a quantitative estimate of the actual population served by a given wastewater treatment plant.

**Conclusions**

Illicit drug loads on RUA testing days were not statistically different to non-RUA testing days. RUAs given to inmates were only positive for the presence of methamphetamine six times during the month. Wastewater revealed methamphetamine excretion every day, which was not reflected in RUA tests. Therefore, wastewater analyses yield a more complete view of illicit drug use/excretion inside a prison that is complementary to RUA testing. Creatinine loads were well predicted for the total prison population using estimates of the per person volume of urine excreted and creatinine concentrations in urine. Predicted creatinine loads were in good agreement with measured creatinine loads, indicating the potential usefulness of creatinine as a quantitative population indicator.

**Acknowledgments**

We thank Paul Bellaty, Jeff Duncan, Doug Young, and Margaret Braun of the Oregon State Department of Corrections and Brent Allred and Phil Janney assistance with this research.
**Table 3.1.** Frequency of cocaine and benzoylcegonine detections that were below the limit of detection (< LOD) or above the LOD but below the limit of quantification (LOD>X<LOQ) for wastewater samples collected Monday-Thursday and Friday-Sunday.

<table>
<thead>
<tr>
<th></th>
<th>Monday-Thursday</th>
<th>Friday-Sunday</th>
</tr>
</thead>
<tbody>
<tr>
<td>COC &lt;LOD</td>
<td>8</td>
<td>7</td>
</tr>
<tr>
<td>COC LOQ&gt;X&lt;LOD</td>
<td>8</td>
<td>5</td>
</tr>
<tr>
<td>BZE &lt;LOD</td>
<td>9</td>
<td>5</td>
</tr>
<tr>
<td>BZE LOQ&gt;X&lt;LOD</td>
<td>9</td>
<td>5</td>
</tr>
</tbody>
</table>

**Table 3.2.** Total prison population (2,028 inmates + non-inmates), predicted total creatinine mass, measured total creatinine mass, and the percent agreement between predicted and measured creatinine masses.

<table>
<thead>
<tr>
<th>Date</th>
<th>Total Population (inmates + non-inmates)</th>
<th>Measured total Creatinine Mass (kg)</th>
<th>Predicted Total Creatinine Mass (kg)</th>
<th>Percent Agreement (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aug 9</td>
<td>2,083 + 173</td>
<td>4.41</td>
<td>4.20</td>
<td>105</td>
</tr>
<tr>
<td>Aug 10</td>
<td>2,083 + 180</td>
<td>3.96</td>
<td>3.72</td>
<td>106</td>
</tr>
<tr>
<td>Aug 11</td>
<td>2,083 + 183</td>
<td>2.99</td>
<td>3.18</td>
<td>94</td>
</tr>
</tbody>
</table>

NA = No non-inmate data available.
**Figure Legends**

**Figure 3.1.** The total number of inmates (shown as dashed line) and inmates (A) and total volume (L) of wastewater exiting the prison for each hour (B) from 23:00 August 8 – 23:00 August 14. Hourly loads of creatinine (mg) from 23:00 August 8– 23:00 August 15 (C). Error bars indicate 95%CI of analytical precision. Blue bars indicate visiting hours.

**Figure 3.2.** Daily loads (mg) of methamphetamine (A) and RUA data (B) for July 30 to August 26. In panel A, the * indicates the single day (August 13) for which hourly loads were determined (see panel C). Single day of hourly loads of methamphetamine (C) from 23:00 August 12 ending on 23:00 August 13. Error bars indicate 95%CI of analytical precision. Blue colors indicate visiting hours.
Figure 3.1.
Figure 3.2.
Literature Cited


(91) McManus, S., Environmental Product Support Teledyne Isco personal communication October 5, 2012.
(92) Young, D., Orgon Department of Corrections personal communication March 16, 2012.
Investigation of Cocaine and Benzoylecgonine transformation in transit through the use of a deuterated tracer test

Alex J. Brewer, Christoph Ort, Jean-Daniel Berset, Jennifer Field
Chapter 4- Investigation of Cocaine and Benzoylecgonine transformation in transit through the use of a deuterated tracer test

Abstract

Illicit cocaine use is has been typically quantified using either cocaine (COC), or benzoylecgonine (BZE) concentrations in wastewater. BZE has been used as an indicator for cocaine use in wastewater based on the assumption that COC is unsuitable because it is transformed to BZE. The in situ transformation of COC in wastewater has not been determined in an environmentally relevant system with developed biofilm and real-world wastewater conditions (temperature, dissolved oxygen, pH and conductivity). In order to determine the transformation of COC and BZE deuterated \((d^3)\) COC and BZE were injected into wastewater and sampled at three different distances \((500, 2500, \text{ and } 5000 \text{ m})\) over the time span of \(~1.5 \text{ h}\). COC-\(d^3\) was transformed to BZE-\(d^3\) in solutions that were prepared pre experiment, and it is unclear what caused the transformation of COC-\(d^3\) in the injectate solution. The results from quality control samples indicate that COC is unstable in common solvents \((\text{H}_2\text{O})\) used in a controlled setting (i.e. laboratory), although the mechanisms behind this remain unseen and are likely not a function of in situ transit. There was no significant transformation of COC-\(d^3\), and no significant transformation of BZE-\(d^3\) during the COC-\(d^3\) in situ experiment. In addition during the BZE-\(d^3\) injection there was no significant transformation of BZE-\(d^3\). Although there was transformation prior to the injection of COC-\(d^3\) the in-situ transformation did not occur after the substance was poured into the sewer pipe. No further observed transformation of
COC-\textit{d3} would suggest that it might reach static flux, which may have occurred before the experiment started. The results of this experiment and wastewater epidemiology studies that have noted higher than expected COC concentrations would suggest that there may be another cause other than in-situ transformation such as “wash-off” or cocaine entering a wastewater un-metabolized.

\textbf{Introduction}

Illicit drug use and abuse is a costly burden on society,\textsuperscript{1} one of the most widely use illicit substances is cocaine (COC). COC and its metabolites have been quantified in wastewater influent for the purpose of estimating the number of doses used on the community scale.\textsuperscript{5,31,55,83,97,98} Benzoylcgonine (BZE) is a major human metabolite of COC.\textsuperscript{99,100} The calculated numbers of doses are helpful due to the possible variability in consumption by a single person depending on level of addiction. The data that is used to calculate the amount of COC and BZE excreted, (typically over a day) comes from controlled laboratory studies in which a relatively small amount of people are given a single dose of COC. If there are different in situ transformation rates in wastewater of COC and BZE then comparing dose estimates from what was initially excreted in urine becomes inaccurate. There are questions of whether to use COC or BZE to determine the number of doses originating from mass (concentration X volume) found in municipal wastewater given the possible transformation of COC to BZE.\textsuperscript{9,26} COC may yield the better estimate of COC use when considering possible transformation because there could only be an over-
estimation of COC doses. Although, BZE may be the better choice because it can only be formed from COC that has undergone human metabolism.\textsuperscript{26}

The ratio of BZE to COC found in wastewater influent may give a clue about in situ transformation. There is a discrepancy between the ratio of COC to BZE in wastewater,\textsuperscript{40,55} and what is observed in laboratory studies that have analyzed urine for the presence of COC and BZE. In these studies the COC to BZE ratio that is expected from urine analyses is small (average of 291 times more BZE\textsuperscript{101}) due to the amount of COC that is metabolized quickly to form BZE.\textsuperscript{41,99-102} Therefore, from the data produced by these urine studies, the ratios of COC and BZE in wastewater should be dominated by BZE.\textsuperscript{41,99,100} Currently there has been no studies that have quantitatively attempted to explain this discrepancy, which may be caused by in situ transformation.

Methods have been proposed to reduce the error involved in calculating the number of doses from cocaine and metabolites, by using chemical reactions to convert COC and metabolites to a single analyte.\textsuperscript{39} Other non-laboratory methods have been attempted to achieve mass balance of COC and metabolites\textsuperscript{12}, or to predict concentrations found in wastewater of COC and metabolites with the use of mathematical models.\textsuperscript{103} In addition, attempts have been made to estimate the amount of transformation of COC in wastewater, and these “stability” studies, which are more focused on storage, within a laboratory and do not take biofilms and environmentally relevant conditions into account.\textsuperscript{7,11,48,104} In order to quantify the amount of transformation of COC, a wastewater system that contains active biofilm should be used, along with a substance that is differentiable from background wastewater COC and BZE. It is thought that COC is more susceptible to transformation that BZE in
wastewater, which has been attributed to hydrolysis of COC.\textsuperscript{104,105} Thus, it is hypothesized that there is a difference between COC and BZE in situ transformation rates in an environmentally relevant system.

In situ tracer experiments are typically done in order to characterize the movement of water.\textsuperscript{106-108} In order to accomplish this the tracer should be stable and identifiably different from anything that may be in the background.\textsuperscript{109} Chemicals are often deuterated for use in analytical chemistry to account for matrix effects during analyses. Deuterated substances are ideal to observe any in situ transformation rates because they experience the same conditions (i.e. matrix effects) that a non-deuterated substance would. Grab samples do not need to be weighted when flow is fixed, thus it would be ideal to conduct in situ experiments in a relevant system where flow can be controlled.\textsuperscript{13} The objective of this study is to calculate the in situ transformation rates of COC and BZE in an active sewer system using deuterated (d3) COC and BZE during two different days.

**Experimental Methods**

**Test solutions.** COC-d3 and BZE-d3 solutions (4 mg/ml each) were purchased (LIPOMED, Switzerland) for use as injectate. COC-d3 injectate solution (4 mg/L) was prepared using one Liter of ELGA® (Elga) water. A BZE-d3 injectate solution (4 mg/L) was also prepared separately to have the same concentration using the same water source (Elga). Each solution was made the morning of each experiment respectively.

**Quality control.** Quality control (QC) samples consisted of solutions (COC-d3 and BZE-d3) that were diluted in either MeOH, Elga (H\textsubscript{2}O) or wastewater (Table 4.1), to
concentrations of 1000 ng/L. Blank wastewater was also collected (n=3). The MeOH QC samples were collected to test the purity of the solution that was purchased (Lipomed, Switzerland, both >99.9% pure) without the influence of H₂O. The Elga QC samples were collected in order to observe any effect that Elga matrix would have on the concentrations and any transformation in solutions. Blank wastewater was collected in order to determine if there was any background COC-d₃ and BZE-d₃ in the wastewater stream during the experiment. Wastewater was collected on the days of injection (prior) and spiked to concentrations of 1000 ng/L for a matrix matched QC sample. Aliquots of the un-diluted injectate solutions (4E+06 ng/L) were also collected for QC purposes. All samples were then immediately frozen either by dry ice or in a freezer (-20°C).

**Location and sewer system.** The “Glattstollen” is a concrete tunnel with two concrete embedded single source sewer lines transporting wastewater from two suburbs northeast of Zurich to Werdhölzli. Werdhölzli is the largest wastewater treatment plant (WWTP) in the City of Zurich. The Glattstollen tunnel is one of the few real full-scale sewer segments worldwide, which had the proper access, safety, as well as being a sealed single source stretch of sewer pipe. The sewer section was underground and only accessible to personnel through a controlled fence with permission. One of the sewer lines in the Glattstollen is used as a controllable reserve sewer line during intermittent periods of high flow. Wastewater entered the sewer segment via a waterfall and was then pumped into the reserve stretch of pipe. Thus, one of the sewer lines in the Glattstollen (the reserve) was used to conduct the experiment. Power was
The pH, conductivity, dissolved oxygen and wastewater temperature within the sewer on October 13, 2011 at 10:44 when COC-$d_3$ was added into the wastewater stream were measured during the experiment over a duration of ~130 min across three locations. The pH of the wastewater ranged from approximately 8.17 to 7.34, conductivity was 0.74 to 0.76 $\mu$S/cm, dissolved oxygen ranged from 3.60 mg/L at the start of the experiment to 0.39 mg/L at the end of the experiment, and water temperature was 18.5$^\circ$C throughout. When BZE-$d_3$ was added the same parameters were measured on October 14, 2011 at 9:10 over the same length of time. The pH of the wastewater ranged from approximately 8 to 8.33, conductivity was 0.78 to 0.79 $\mu$S/cm, dissolved oxygen ranged from 4 mg/L at the start of the experiment to 0.19 mg/L at the end of the experiment, and water temperature was 19.2$^\circ$C throughout. The change in dissolved oxygen concentrations are attributed to the design of the Glattstollen, which contains a waterfall by design. The decrease in dissolved oxygen was observed during preliminary experiments. The waterfall likely increased dissolved oxygen and as the experiment was taking place dissolved oxygen decreased downstream as a function of the oxygen demand of municipal wastewater.

**Sampling.** NaCl was used to determined wastewater flow, and velocity (See Appendix). Wastewater flow was determined via NaCl standard addition with the average (n=4 peaks) flow rate being 27.95 L/sec. (See Appendix). Confetti was used as a visual tracer of water arrival time, and compared to arrival of NaCl pulse in order to determine velocity of peak. The velocity of the wastewater at sampling location 1
was 39.6 m/min and 37.2 m/min at sampling location 2 (Table A4.1). The grab
sampling interval was based on the duration of the peaks observed during the NaCl
addition, for sampling point one a 10 s sampling interval would equate to ~28 samples,
sampling points 2, and 3 had 25 and 40 s intervals respectively.

Injectate solutions were added on separate days into the sewer pipe by pouring
the 1 L of injectate directly into the pipe across the width of the stream. Grab
Sampling for the deuterated substances took place with 6 more samples before and
after the duration of the pulse respectively (a total of 12 more) to insure collection of
peaks, for a total of 40 samples at each location. Each sample was collected via a
ladle, and transferred to a 1 L HDPE bottle, aliquots were then taken and placed in 30
mL LDPE bottles and frozen immediately with dry ice. Samples were then stored (~
20°C) in Dübendorf Switzerland, until next-day air transport to Oregon State
University (Corvallis, Oregon) on dry ice, and stored (~20°C) until analysis.

Chemical Analysis. Analyses were preformed according to Brewer et al., 20127 with
minor modifications that involve moving COC-\textit{d}3 and BZE-\textit{d}3 from internal
standards to analytes with two transitions and using COC-\textit{d}5 (Alsachim, France 98%
pure), and BZE-\textit{d}8 (Cerilliant, USA, 99.8% pure) as internal standards (Table A4.2).
COC-\textit{d}3 standard calibration concentrations ranged from 5 to 3000 ng/L, BZE-\textit{d}3
concentrations ranged from 10 to 4000 ng/L with calculated limit of detection (LOD
defined as S/N >3:1) of 2 and 4 ng/L respectively. For details on transitions (Table
S2), and recovery in wastewater (Table A4.3) See Appendix. Analytical precision
(Table A4.4) was determined with replicates (n=4) of samples analyzed during each
analytical sequence. In order to determine accuracy, spiked (COC-\textit{d}3, and BZE-\textit{d}3)
and un-spiked third party reference standards were analyzed (See, Appendix). In addition COC-d3 and BZE-d3 spiked wastewater samples were also analyzed (n=4) to determine recovery in wastewater collected from the sample site. Ecgonine (EC), and ecgonine methyl ester (EME) were identified as potential transformation products but were not investigated due to a lack of mass spectrometer sensitivity.

**Data analysis.** Mass of COC-d3 and BZE-d3 in peaks were calculated by using the sum of each concentration multiplied by flow and time interval for each sampling location.

**Results and Discussion.**

**Quality control.** QC samples (including injectate) that were prepared in H2O prior to the test underwent varying degrees of transformation (hydrolysis) from COC-d3 to BZE-d3 (Table, 4.1). These included; Elga water QC, aliquot of the injectate, as well as the wastewater QC (Table 4.1, Figure A4.1). The Elga QC that was prepared 10 days prior to the COC-d3 injection and immediately frozen until analysis contained seven percent BZE-d3 (of total [COC-d3] + [BZE-d3]). The MeOH QC sample that was made at the same time as the Elga QC was blank for BZE-d3 (Table 4.1). The injectate contained 13% BZE-d3 (of total [COC-d3] + [BZE-d3])(Table 4.1). Spiked wastewater QC samples that were made the day of the injection contained 25% BZE-d3 (of total [COC-d3] + [BZE-d3]) as BZE-d3. The solution that was used to make up the injectate was 100% MeOH and was stored at -20ºC before the injectate (Elga solution) was made. All QC samples that were prepared before the BZE-d3 injection only contained BZE-d3. The analytical precision was measured using replicates of either the injectate for the Elga, MeOH, and injectate samples as 2.4% for COC-d3.
and 3.2% for BZE-d3. Elga, MeOH, and injectate were all expected to have [COC-d3] of 1000 ng/L or 4 mg/L (injectate), and contained 98%, 169%, and 110% ([COC-d3]+[BZE-d3]) respectively (Table 4.1). The precision of the spiked wastewater samples was measured using replicates (n=4) of the spiked wastewater and was 2.9% for COC-d3 and 22% for BZE-d3. Wastewater was spiked to a [COC-d3] of 1000 ng/L and contained 115% of [COC-d3]+ [BZE-d3].

**Cocaine-d3 tracer test.** COC-d3 was added into the Glattstollen first during the study. After the injectate was added (poured), it took ~12 min for the COC-d3 peak to reach sampling location 1, ~45 min for the peak to reach point 2, and ~127 min to reach sampling point 3 (Figure 4.1A). There were roughly 12 samples that made up the COC-d3 peaks at each location. In addition each peak lasted ~50 s, 8 min, and 12 min at each sampling location respectively, and maintained a Gaussian shape (Figure 4.1A). The maximum concentrations in each peak were 2,655, 1,025, and 638 ng/L respectively (Figure 4.1A). Mass of COC-d3 at the first sampling location was 4.9 mg (Table 4.2), which was 22% higher than the 3.83 mg injected (Table 4.1). At location 2 there was a 1% decrease in mass of COC-d3, and finally there was an 11% increase in mass from location 2 at location 3. For the total time after injection until the left location 3 there was a total increase in mass of 10%. The amount of COC transformation has been observed to be -7.7% +/- 1 (+/- SD) for unfiltered wastewater stored inside of a laboratory at 19ºC for 12 h (pH=7.4)\textsuperscript{19,104}. The linear regression model of the natural log the highest concentration point in each peak as a function of time did not show a statistically significant (from zero) decrease over time (Figure 4.2).
BZE-d3 was co-injected on the first day of the study due to transformation prior to the addition of COC-d3 solution (Figure 4.1A). It took the same amount of time for each of the BZE-d3 peaks to reach the sampling locations (i.e. same peaks contained both substances). BZE-d3 was contained in the same ~12 sample at each location, and accordingly lasted the same amount of time. The maximum concentrations in each peak were 1,624, 719, and 640 ng/L respectively. Mass of BZE-d3 at the first sampling location was 2.4 mg (Table 4.2), which is 49% of the mass of COC-d3 that was injected and more than 4 times higher than the 0.55 mg in the injectate (Table 4.1). The %RSD for BZE-d3 for the wastewater QC sample the total error in calculated is 5%, with all deviation in QC samples being negligible. Elga water QC samples contained 7 and 14 % of the COC-d3 mass expected (Table 4.1). The main difference between these two difference QC samples was the time between when they were made prior to the start of the tracer test (10 vs 1 day). During that time the COC-d3 solution (MeOH) was being refrigerated (4°C). At location 2 there was a 1% decrease in mass, and finally there was a 35% increase in mass from location 2 at location 3. For the total time after injection until the left location 3 there was a total increase in mass of 34%. The increase in mass is ~3 times the increase in COC-d3 mass, it is unclear why both COC-d3 and BZE-d3 are increasing during the experiment. In accordance with COC-d3 the linear regression model for BZE-d3 did not show a statistically significant (from zero) increase over time (Figure 4.2B). The percent of change in wastewater stored at 19°C for 12 h at a pH of 7.4 has been measured at 5.5% +/- 3%\textsuperscript{19,104}. Ratios of BZE-d3/COC-d3 area during the first COC-d3 injection were used to confirm that
there was no significant transformation during the experiment. The change in integrated peak ratios from location 1 to location 3 (2:1 to 1.7:1) is a change of 18% over 1.9 h (Table 4.2). 18% is not a large amount of considering that analytical error is typically ~20% for BZE-d3 and ~5% for COC-d3 (See SI). Interestingly the ratios did match the ratios of ~2/1 of the background BZE/COC present at the sampling locations. If a true linear trend were observed then BZE-d3 transformation would happen on the time scale of minutes after which a much slower reaction would be taking place.

**Benzoylcegonine-d3 tracer test.** BZE-d3 was also independently injected on the second day by itself (Figure 4.1B). It took ~12 min for the peak to reach sampling location 1, which is identical to the time during the injection a day earlier, 54 min for the peak to reach sampling point 2, and 126 min for the peak to reach sampling point 3. Transit time in the sewer pipe is an important variable for in situ transformation, and residence time of peaks as indicated by arrival, were similar. The maximum concentrations in each peak were 2,651, 971, and 785 ng/L respectively, in addition each peak lasted ~2 min, 5 min and 10 minutes respectively. The total time each peak was observed was also comparatively similar indicating that conditions for diffusion were the same. Mass of BZE-d3 at the first sampling location was 4.3 mg, which was close to expected. This result was close to the QC samples that were made in Elga water prior to the experiment in terms of percent difference (7.5% vs 3.75% of expected, Table 4.1). At location 2 there was a 10% decrease in mass, and finally there was a 15% increase in mass from location 2 at location 3. For the total time after injection until the left location 3 there was a total increase in mass of 5%. Given that
there was no COC-\textit{d3} that was co-injected with BZE-\textit{d3} it is unclear how there could be an increase in BZE-\textit{d3} mass other than analytical uncertainty. This change is within the range of typical error in analyses (~20\%). The linear regression model of the natural log the highest concentration point in each peak as a function of time did not show a statistically significant (from zero) increase over time (Figure 3).

\textbf{Implications} There was no statistically significant in situ transformation of COC-\textit{d3} and BZE-\textit{d3}. However there was transformation of COC-\textit{d3} before the in situ experiment was preformed. Hydrolysis that occurred before the injection was caused by an unknown factor within the water used. Regardless of COC-\textit{d3} being transformed to BZE-\textit{d3} prior to injection the COC-\textit{d3} that was injected was not significantly transformed in situ. Given the residence time of ~2 h, which is relevant given typical transit times of wastewater from households/businesses to WWTPs the lack of transformation would signify that what is collected at WWTP is representative of what is leaving households/businesses.

\textbf{Conclusion/Limitations.} This transformation/hydrolysis reaction that occurred before the in situ experiment did not allow for an un-ambiguous view of COC-\textit{d3} in situ transformation. The conditions of the sewer system during the experiment were representative of typical system conditions and transit times. The influence of lift stations on residence time, which are common, was not investigated and may yield transformation that was not observed in this study. If in situ transformation experiments were attempted in the future perhaps sampling locations that equate to longer residence times would be beneficial.

\textbf{Acknowledgements}
We thank Aurea Chiaia, Alexander Heisele, Philipp Beutler, David Machac, Ralf Kagi, David Durrenmatt, Joao Mimoso, and Philipp Staufer for their help during sample collection. This publication was made possible, in part by, a grant from the National Science Foundation (OSIE-1132954).
**Table 4.1.** Name of quality control samples during both cocaine and benzoylecgonine injections, solvent type, time made before injection, concentrations of both cocaine-d3 (COC-d3), and benzoylecgonine-d3 (BZE-d3), the sum of the concentrations, expected concentration as injected substance, and % of expected of QC samples.

<table>
<thead>
<tr>
<th>Name indicating injection</th>
<th>Solvent</th>
<th>Time Before Injection</th>
<th>COC-d3</th>
<th>BZE-d3</th>
<th>Sum on Conc.</th>
<th>Expected (as Injected substance)</th>
<th>% of expected</th>
</tr>
</thead>
<tbody>
<tr>
<td>COC-d3 elga</td>
<td>H2O</td>
<td>10 d</td>
<td>910</td>
<td>70</td>
<td>980</td>
<td>1000</td>
<td>98</td>
</tr>
<tr>
<td>COC-d3 MeOH</td>
<td>MeOH</td>
<td>10 d</td>
<td>1690</td>
<td>nq</td>
<td>1690</td>
<td>1000</td>
<td>169</td>
</tr>
<tr>
<td>COC-d3 injectate</td>
<td>H2O</td>
<td>~2 h</td>
<td>3.83</td>
<td>0.55</td>
<td>4.38</td>
<td>4</td>
<td>109.5</td>
</tr>
<tr>
<td>COC-d3 WW</td>
<td>Wastewater</td>
<td>~15 min</td>
<td>863</td>
<td>289</td>
<td>1152</td>
<td>1000</td>
<td>115.19</td>
</tr>
<tr>
<td>BZE-d3 elga</td>
<td>H2O</td>
<td>10 d</td>
<td>nq</td>
<td>754</td>
<td>754</td>
<td>1000</td>
<td>75.4</td>
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<tr>
<td>BZE-d3 MeOH</td>
<td>MeOH</td>
<td>10 d</td>
<td>nq</td>
<td>765</td>
<td>765</td>
<td>1000</td>
<td>76.5</td>
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<tr>
<td>BZE-d3 injectate</td>
<td>H2O</td>
<td>~2 h</td>
<td>0.01</td>
<td>3.85</td>
<td>3.86</td>
<td>4</td>
<td>96.5</td>
</tr>
<tr>
<td>BZE-d3 WW</td>
<td>Wastewater</td>
<td>~15 min</td>
<td>2.45</td>
<td>1075.85</td>
<td>1078.30</td>
<td>1000</td>
<td>107.83</td>
</tr>
</tbody>
</table>

nq = not quantifiable
### Table 4.2. Mass (ng) and % change from previous location of COC-d3, and BZE-d3 peaks at each sampling location observed during the COC-d3 injection

<table>
<thead>
<tr>
<th>Distance from Injection (m)</th>
<th>Mass COC-d3 (mg)</th>
<th>COC-d3 (C/Co)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Mass BZE-d3 (mg)</th>
<th>Ratio COC-d3/BZE-d3</th>
</tr>
</thead>
<tbody>
<tr>
<td>500m</td>
<td>4.95</td>
<td>1.43</td>
<td>2.42</td>
<td>2.05</td>
</tr>
<tr>
<td>2500m</td>
<td>5.02</td>
<td>1.46</td>
<td>2.53</td>
<td>1.98</td>
</tr>
<tr>
<td>5000m</td>
<td>5.47</td>
<td>1.58</td>
<td>3.29</td>
<td>1.66</td>
</tr>
</tbody>
</table>

<sup>a</sup>C/Co = ratioed integrated mass to injected COC-d3 mass (3.45 mg)

### Table 4.3. Mass (ng) and % change from previous location of BZE-d3 collected during the BZE-d3 injection

<table>
<thead>
<tr>
<th>Distance from Injection (m)</th>
<th>Mass of BZE-d3 (mg)</th>
<th>BZE-d3 (C/Co)&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>500m</td>
<td>4.41</td>
<td>1.02</td>
</tr>
<tr>
<td>2500m</td>
<td>4.11</td>
<td>0.96</td>
</tr>
<tr>
<td>5000m</td>
<td>4.67</td>
<td>1.08</td>
</tr>
</tbody>
</table>

<sup>a</sup>C/Co = ratioed integrated mass to injected BZE-d3 mass (4.30 mg)
Figure legends

Figure 4.1. (A) Deuterated cocaine and benzoylecgonine concentrations sampled at three locations during the deuterated cocaine injection. (B) Deuterated benzoylecgonine sampled at three locations during the deuterated benzoylecgonine injection. Error bars indicate 95% CI of analytical accuracy.

Figure 4.2. Linear regression of natural log of peak cocaine-d3 (A) and benzoylecgonine-d3 (B) concentrations collected during cocaine-d3 injection as a function of time. Dashed lines indicate 95% CI of slope.

Figure 4.3. Linear regression of natural log of benzoylecgonine-d3 concentrations collected during benzoylecgonine-d3 injection as a function of time. Dashed lines indicate 95% CI of slope.
Figure 4.1.
Figure 4.2.
Figure 4.3
Literature Cited


Conclusion

Wastewater epidemiology is a discipline that's proving useful for the determination of community scale illicit drug use. It is also not a fully developed discipline. There are many limitations and questions that still need to be answered. The work presented in this dissertation is focused on answering the questions that remain.

Chapter 2 focuses on the major limitation of accounting for population movements and interpretation of drug loads given these movements. The interpretation of wastewater epidemiology data that is typically collected was further investigated with the use of hourly high-frequency samples. These samples demonstrated the drug specific diurnal trends of loads in municipal wastewater. Along with the diurnal trends of illicit drugs the diurnal trends of the human urinary biomarker creatinine was used to account for population. When constructing ratios (normalizing) of illicit drug divided by creatinine diurnal trends differed from non-normalized loads. This method of taking population into account demonstrated different interpretation of loads when taking population into account. The uses of creatinine as a population indicator lead to observations and conclusions what would have not been possible without using an endogenous human urinary biomarker.

Chapter 3 focused on the use and further refine the method of using creatinine as a population indicator. The use of a correctional facility (a closed system) with a well-known population helped confirm and strengthen the use of creatinine to account for population. Novel techniques were developed to sample the single wastewater source. A continuous sampling regime was used to collect a fraction of all wastewater
that was exiting the prison system. The population that was inside of the prison was compared to the estimated population of the prison using a technique that was developed using literature based creatinine urine concentrations, the prison demographics and an estimated volume of urine. Along with comparing the estimated population to the actual population the use of wastewater epidemiology was demonstrated as a tool to compare UAs given to inmates to wastewater drug loads. This comparison was done in an attempt to compare and contrast to methods of gaining drug use information. Wastewater demonstrated that there were illicit drugs being excreted inside of the prison system that were not observed with UAs alone. Wastewater epidemiology is potentially a means for collecting drug use information for corrections officials.

There are some well-known uncertainties in wastewater epidemiology. One of these unknowns is regarding the estimation of cocaine use. There is a disconnect between cocaine and cocaine metabolite data collected from urine and what is observed in wastewater. Given this uncertainty and investigation into the in-situ transformation was conducted. In-situ transformation is often referenced as a reason that this disconnect between literature values and wastewater concentrations exists. Chapter 4 is a novel experiment that attempts to observe in-situ transformation of cocaine and it major metabolite benzoylecgonine with the use of deuterated cocaine and benzoylecgonine. The novel use of a deuterated substance as a tracer in a working sewer system was done in order to observe transformation without background interference. A working sewer system was used to take into account biofilm and conditions that illicit drugs experience in-situ. This experiment is the first attempt to
account for biofilm along with actual real-world sewer conditions on illicit substance concentrations. This experiment is a great attempt to account for variables in a novel and a relative cost effective manner. All of the work that is presented in this dissertation was done with international collaboration to answer important questions that are timely and relevant to the science of wastewater epidemiology.

This dissertation was focused on testing: (1) Diurnal trends in illicit and legal drugs may be drug specific. (2) Diurnal trends in illicit and legal drug loads are different when taking population into account by normalizing with creatinine loads. (3) Predicted creatinine loads will correspond to measured loads. (4) Illicit drug loads are different on random urinalyses testing days vs. non-testing days. And (5) That there is a difference in cocaine and benzoylecgonine in-situ transformation rates in an environmentally relevant system. This dissertation demonstrates that there are drug specific diurnal trends and that there is a different trend when taking creatinine into account. Creatinine loads measured in a closed system closely matched (within 10%) predicted creatinine loads. It was observed through the use of wastewater epidemiology that there was no difference in illicit drug loads in prison wastewater on RUA testing days vs. non-testing days. Deuterated cocaine and benzoylecgonine were not observed to be significantly degraded in a sewer system for ~1.5 h. There are some important questions and limitations that have yet to be answered or addressed and this dissertation should be used as a resource to accomplish future work to address these questions and limitations.
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Appendices

Appendix 1- Normalized Diurnal and Between-Day Trends in Illicit and Legal Drug Loads that Account for Changes in Population

Experimental methods

Chemical Analyses. All wastewater samples were analyzed for all substances (except creatinine) within one of two analytical sequences with sequence one containing days 1 and 3 while sequence two was comprised of samples from days 3 and 4. Samples analyzed for creatinine were run in a single run. The concentration range for standards used to construct the calibration curve for this study ranged 50,000-10,000,000 ng/L (creatinine); 2,000-200,000 ng/L (caffeine); 40-4,000 ng/L (methamphetamine); 5-500 ng/L (cocaine); and 40-4,000 ng/L (benzoylecggonine) in 5 mM ammonium acetate buffer. The lower limits of quantification for analytes were 50,000 ng/L (creatinine), 2,000 ng/L (caffeine), 40 ng/L (methamphetamine), 5 ng/L (cocaine), and 40 ng/L (BZE). In the case of creatinine and caffeine, the LOQs represent the lowest calibration standard needed to quantify the high concentrations of creatinine and caffeine and are well above instrumental detection limits for these analytes. All calibration curves had $R^2 > 0.98$ with 1/X weighting. Analytes were assigned a concentration if the signal-to-noise (S/N) met the criteria of S/N ≥ 10 and was at or above the lowest calibration curve standard. For a given peak, if S/N was > 10 but below the lowest calibration standard, no concentration was assigned. The lower limit of quantification was determined as the lowest calibration standard. The
ratio of quantifier to qualifier ion ratios were plotted against each other with $R^2$ values of $>0.89$ for all analytes. For all analytical sequences, two quality control samples (standards) were analyzed after every eight samples and were considered acceptable with a percent agreement $\geq 70\%$.

**Sampling Stability.** Single 500 mL grab samples of raw influent were collected on three days and immediately transported to the laboratory. Prior to analysis, cocaine and benzoylecgonine were spiked to a concentration of $\sim 100$ng/L, each sample was shaken before removing three 15 mL aliquots for illicit drugs and caffeine and an additional three 1.5 mL aliquots for creatinine analysis and placed in a -20 $^\circ$C freezer until analysis (these aliquots are time = 0). The remaining sample volume was then placed in a 4 $^\circ$C refrigerator and then subsampled according to the procedure describe above at hours 1, 2, 3, 4, 5, 6, 7, 12, and 24 for a total of ten samples per day. All samples were then analyzed as described above.

**Accuracy and Precision.** A single wastewater sample was picked at random within each analytical sequence and run four multiple times for precision, as indicated by % RSD (Table A1.2). For determining accuracy, a custom made, third party reference standard (human urine) was prepared by UTAK Laboratories Inc. (Valencia, CA). The reference urine was diluted by a factor of 1000 with 5 mM ammonium acetate buffer. The dilute solution was then spiked with internal standards for all substances and analyzed twice, once in the beginning of a sequence and again at the end (Table S4).

**Predicted Creatinine Loads.** Demographic data for the municipality studied was used along with measured urine creatinine concentrations in order to determine
expected loads. Demographic data was provided for age and ethnicity groups for the whole municipality (PRC, 1010). It was assumed that every ethnic group contained the same age distribution as the whole municipality, and thus total number of people in each ethnicities age group was calculated. The total mass expected from each ethnicities age group was then calculated by multiplying creatinine concentration values in Barr 2005 (mean, median, 10\textsuperscript{th} and 90\textsuperscript{th} percentile and 95\%CI) by 1.5 L of urine excreted per day, and the total number of people in each group. The total predicted creatinine loads are the sum of the creatinine mass for all ethnic age groups (total number of people in the municipality).
Table A1.1. Intra-hour variation in wastewater flow as indicated by the percent relative standard deviation for the four sampling days.

<table>
<thead>
<tr>
<th>Hour</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>8 am-9 am</td>
<td>11%</td>
<td>6%</td>
<td>28%</td>
<td>10%</td>
</tr>
<tr>
<td>9am-10 am</td>
<td>11%</td>
<td>9%</td>
<td>13%</td>
<td>24%</td>
</tr>
<tr>
<td>10 am-11am</td>
<td>11%</td>
<td>7%</td>
<td>21%</td>
<td>7%</td>
</tr>
<tr>
<td>11 am-12 pm</td>
<td>14%</td>
<td>20%</td>
<td>9%</td>
<td>10%</td>
</tr>
<tr>
<td>12 pm-1 pm</td>
<td>13%</td>
<td>18%</td>
<td>9%</td>
<td>11%</td>
</tr>
<tr>
<td>1 pm-2 pm</td>
<td>9%</td>
<td>10%</td>
<td>13%</td>
<td>11%</td>
</tr>
<tr>
<td>2 pm-3 pm</td>
<td>11%</td>
<td>10%</td>
<td>12%</td>
<td>12%</td>
</tr>
<tr>
<td>3pm-4pm</td>
<td>20%</td>
<td>14%</td>
<td>11%</td>
<td>11%</td>
</tr>
<tr>
<td>4 pm-5pm</td>
<td>8%</td>
<td>23%</td>
<td>12%</td>
<td>13%</td>
</tr>
<tr>
<td>5pm-6pm</td>
<td>14%</td>
<td>10%</td>
<td>8%</td>
<td>8%</td>
</tr>
<tr>
<td>6pm-7pm</td>
<td>12%</td>
<td>11%</td>
<td>10%</td>
<td>10%</td>
</tr>
<tr>
<td>7pm-8pm</td>
<td>10%</td>
<td>10%</td>
<td>12%</td>
<td>14%</td>
</tr>
<tr>
<td>8pm-9pm</td>
<td>12%</td>
<td>14%</td>
<td>10%</td>
<td>9%</td>
</tr>
<tr>
<td>9pm-10pm</td>
<td>14%</td>
<td>12%</td>
<td>12%</td>
<td>12%</td>
</tr>
<tr>
<td>10 pm-11 pm</td>
<td>15%</td>
<td>11%</td>
<td>12%</td>
<td>14%</td>
</tr>
<tr>
<td>11pm-12pm</td>
<td>12%</td>
<td>11%</td>
<td>23%</td>
<td>9%</td>
</tr>
<tr>
<td>12pm-1am</td>
<td>17%</td>
<td>16%</td>
<td>17%</td>
<td>17%</td>
</tr>
<tr>
<td>1am-2am</td>
<td>18%</td>
<td>21%</td>
<td>16%</td>
<td>19%</td>
</tr>
<tr>
<td>2am-3am</td>
<td>7%</td>
<td>9%</td>
<td>27%</td>
<td>10%</td>
</tr>
<tr>
<td>3am-4am</td>
<td>17%</td>
<td>23%</td>
<td>16%</td>
<td>20%</td>
</tr>
<tr>
<td>4am-5am</td>
<td>31%</td>
<td>16%</td>
<td>22%</td>
<td>22%</td>
</tr>
<tr>
<td>5am-6am</td>
<td>11%</td>
<td>16%</td>
<td>26%</td>
<td>21%</td>
</tr>
<tr>
<td>6am-7am</td>
<td>34%</td>
<td>9%</td>
<td>15%</td>
<td>20%</td>
</tr>
<tr>
<td>7am-8am</td>
<td>5%</td>
<td>5%</td>
<td>10%</td>
<td>14%</td>
</tr>
</tbody>
</table>
Table A1.2. Precursor and product ion (m/z), cone (V), and collision voltages (eV).

<table>
<thead>
<tr>
<th>Substance</th>
<th>Precursor Ion (m/z)</th>
<th>Product Ion (m/z)</th>
<th>Cone (V)</th>
<th>Collision (eV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatinine</td>
<td>113.9</td>
<td>43.8</td>
<td>10</td>
<td>15</td>
</tr>
<tr>
<td>Creatinine</td>
<td>113.9</td>
<td>86.0</td>
<td>25</td>
<td>10</td>
</tr>
<tr>
<td>Creatinine D3</td>
<td>116.9</td>
<td>46.9</td>
<td>20</td>
<td>10</td>
</tr>
<tr>
<td>Creatinine D3</td>
<td>116.9</td>
<td>89.1</td>
<td>20</td>
<td>10</td>
</tr>
<tr>
<td>Caffeine</td>
<td>195.2</td>
<td>110.2</td>
<td>30</td>
<td>25</td>
</tr>
<tr>
<td>Caffeine</td>
<td>195.2</td>
<td>138.3</td>
<td>30</td>
<td>20</td>
</tr>
<tr>
<td>Caffeine D3</td>
<td>198.1</td>
<td>140.3</td>
<td>35</td>
<td>20</td>
</tr>
<tr>
<td>Methamphetamine</td>
<td>150.0</td>
<td>91.1</td>
<td>25</td>
<td>15</td>
</tr>
<tr>
<td>Methamphetamine D5</td>
<td>150.0</td>
<td>119.2</td>
<td>20</td>
<td>10</td>
</tr>
<tr>
<td>Benzoylecgonine</td>
<td>290.2</td>
<td>105.1</td>
<td>30</td>
<td>20</td>
</tr>
<tr>
<td>Benzoylecgonine D3</td>
<td>290.2</td>
<td>168.4</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Cocaine</td>
<td>304.1</td>
<td>105.1</td>
<td>35</td>
<td>25</td>
</tr>
<tr>
<td>Cocaine D3</td>
<td>307.3</td>
<td>185.5</td>
<td>30</td>
<td>20</td>
</tr>
</tbody>
</table>

Table A1.3. Precision (% RSD) of the whole method for wastewater for each sequence.

<table>
<thead>
<tr>
<th>Substance</th>
<th>Sequence 1&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Sequence 2&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatinine</td>
<td>3.2%</td>
<td></td>
</tr>
<tr>
<td>Caffeine</td>
<td>1.2 %</td>
<td>9.3 %</td>
</tr>
<tr>
<td>Methamphetamine</td>
<td>1.3 %</td>
<td>13 %</td>
</tr>
<tr>
<td>Benzoylecgonine</td>
<td>16 %</td>
<td>25 %</td>
</tr>
<tr>
<td>Cocaine</td>
<td>16 %</td>
<td>19 %</td>
</tr>
</tbody>
</table>

<sup>a</sup> The separate analysis for creatinine was conducted in a single sequence that included all the samples from the four sampling days.

<sup>b</sup> Sequence 1 contained samples from days 1 and 3 (March 17<sup>th</sup> and 19<sup>th</sup>).

<sup>c</sup> Sequence 2 contained samples from days 2 and 4 (March 18<sup>th</sup> and 20<sup>th</sup>).
Table A1.4. Percent agreement between the average measured (n=2) and certified UTAK values.

<table>
<thead>
<tr>
<th>Substance</th>
<th>Certified UTAK concentration (ng/L)</th>
<th>% Agreement</th>
<th>Sequence 1\textsuperscript{b}</th>
<th>Sequence 2\textsuperscript{c}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caffeine</td>
<td>60,000</td>
<td>77%</td>
<td>91%</td>
<td></td>
</tr>
<tr>
<td>Methamphetamine</td>
<td>750</td>
<td>84%</td>
<td>87%</td>
<td></td>
</tr>
<tr>
<td>Benzoylecgonine</td>
<td>350</td>
<td>68%</td>
<td>76%</td>
<td></td>
</tr>
<tr>
<td>Cocaine</td>
<td>100</td>
<td>70%</td>
<td>81%</td>
<td></td>
</tr>
</tbody>
</table>

NA = not available
\textsuperscript{a} The separate analysis for creatinine was conducted in a single sequence that included all the samples from the four sampling days.
\textsuperscript{b} Sequence 1 contained samples from days 1 and 3 (March 17\textsuperscript{th} and 19\textsuperscript{th}).
\textsuperscript{c} Sequence 2 contained samples from days 2 and 4 (March 18\textsuperscript{th} and 20\textsuperscript{th}).

Table A1.5. Estimated total uncertainty expressed as % RSD for loads and analyte to creatinine ratios (See Equations A1.1 and A1.2).

<table>
<thead>
<tr>
<th></th>
<th>Load (days 1 and 3)</th>
<th>Ratio (days 1 and 3)</th>
<th>Load (days 2 and 4)</th>
<th>Ratio (days 2 and 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatinine</td>
<td></td>
<td>7.8\textsuperscript{a}</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caffeine</td>
<td>16</td>
<td>8</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Methamphetamine</td>
<td>16</td>
<td>17</td>
<td>21</td>
<td>21</td>
</tr>
<tr>
<td>Benzoylecgonine</td>
<td>22</td>
<td>23</td>
<td>24</td>
<td>24</td>
</tr>
<tr>
<td>Cocaine</td>
<td>22</td>
<td>22</td>
<td>17</td>
<td>17</td>
</tr>
</tbody>
</table>

\textsuperscript{a} The separate analysis for creatinine was conducted in a single sequence that included all the samples from the four sampling days.
Table A1.6. Average rate constants ± 95% confidence interval and the maximum estimated percent loss for analytes in samples stored at 4°C in the autosampler for 24 h.

<table>
<thead>
<tr>
<th>Substance</th>
<th>Average first-order rate constant ± 95% CI (h⁻¹)</th>
<th>Estimated maximum loss at 24h (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatinine</td>
<td>-6.08E-03 ± -2.06E-3</td>
<td>13</td>
</tr>
<tr>
<td>Caffeine*</td>
<td>-1.18E-03 ± -1.95E-3</td>
<td>5</td>
</tr>
<tr>
<td>Methamphetamine*</td>
<td>-2.89E-04 ± -5.42E-3</td>
<td>8</td>
</tr>
<tr>
<td>Benzoylecgonine</td>
<td>-1.57E-02 ± -4.90E-3</td>
<td>32</td>
</tr>
<tr>
<td>Cocaine*</td>
<td>-2.03E-03 ± -1.13E-2</td>
<td>16</td>
</tr>
</tbody>
</table>

*Rate constant not statistically different from zero at the 95% CI.

Table A1.7. Calculated mass of creatinine (kg) using demographic data*, mean, median, 10th and 90th percentile (95%CI) of creatinine concentrations* and Liters (L) of urine excreted per day†‡.*

<table>
<thead>
<tr>
<th>Daily urine volume (L)</th>
<th>1.07 L*</th>
<th>1.10 L*</th>
<th>1.15 L†</th>
<th>1.50 L*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean*</td>
<td>78 (±3)</td>
<td>80 (±3)</td>
<td>84 (±3)</td>
<td>109 (±3)</td>
</tr>
<tr>
<td>Median*</td>
<td>87 (±4)</td>
<td>90 (±4)</td>
<td>94 (±4)</td>
<td>122 (±4)</td>
</tr>
<tr>
<td>10th percentile*</td>
<td>28 (±3)</td>
<td>29 (±3)</td>
<td>30 (±3)</td>
<td>39 (±3)</td>
</tr>
<tr>
<td>90th percentile*</td>
<td>164 (±7)</td>
<td>169 (±7)</td>
<td>176 (±7)</td>
<td>230 (±7)</td>
</tr>
</tbody>
</table>

* Concentration ranges from Barr et al., 2005
* Average Daily urine volume from Rauch et al., 2003 (n=47 95%CI NA)
† Average (95%CI) Daily urine volume from Murakami et al., 2008 (n=654)
* Using demographics from PRC., 2010
Equation A1.1. Calculation of total uncertainty from analysis, sampling, and flow.
Total uncertainty = \( \sqrt{(\text{RSD analytical})^2 + (\text{RSD sampling})^2 + (\text{RSD flow})^2} \)

Equation A1.2. Calculation of total uncertainty for analytes normalized to creatinine from analyses of analytes (A) and creatinine (Creat) and the sampling error for each analyte and creatinine.
Total uncertainty = \( \sqrt{(\text{RSD analytical}_A)^2 + (\text{RSD sampling}_A)^2 + (\text{RSD analytical}_\text{Creat})^2 + (\text{RSD sampling}_\text{Creat})^2} \)

Equation A1.3. Calculation of caffeine doses using 100 mg dose
\[
\text{Caffeine excreted (mg)} \times \frac{100 \text{ mg ingested}}{\text{day}} \times \frac{1 \text{ dose}}{1 \text{ mg excreted}} \times \frac{150 \text{ mg}}{\text{day}} = \text{dose}
\]

Equation A1.4. Calculation of methamphetamine doses using 100 mg dose
\[
\text{Methamphetamine excreted (mg)} \times \frac{100 \text{ mg ingested}}{\text{day}} \times \frac{1 \text{ dose}}{40 \text{ mg excreted}} \times \frac{100 \text{ mg}}{\text{day}} = \text{dose}
\]

Equation A1.5. Calculation of cocaine doses from mass of benzoylecgonine (BZE) using 100 mg
\[
\frac{\text{BZE excreted (mg)}}{\text{day}} \times \frac{1 \text{ mM cocaine}}{0.45 \text{ mM BZE excreted}} \times \frac{303E-4 \text{ mg cocaine}}{1 \text{ mM cocaine}} \times \frac{1 \text{ mM BZE}}{289E-4 \text{ mg BZE}} \times \frac{1 \text{ dose}}{100 \text{ mg}} = \text{dose}
\]

Equation A1.6. Calculation of cocaine doses using 100 mg dose
\[
\frac{\text{Cocaine excreted (mg)}}{\text{day}} \times \frac{100 \text{ mg ingested}}{\text{day}} \times \frac{1 \text{ dose}}{1 \text{ mg excreted}} \times \frac{1 \text{ dose}}{100 \text{ mg}} = \text{dose}
\]
Figure A1.1. Creatinine concentrations over 24 h at 4°C for three different days (A, B, and C). Dashed lines represent the 95% CI about the slope.\(^1\)

\(^1\text{n}=3\) at each time point except time = 0 where \(n=4\)
Figure A1.2. Caffeine concentrations over 24 h at 4°C for three different days (A, B, and C). Dashed lines represent the 95%CI about the slope.$^1$

$n=3$ at each time point except time = 0 where $n=4$
Figure A1.3. Methamphetamine concentrations over 24 h at 4°C for three different days (A, B, and C). Dashed lines represent the 95% CI about the slope.¹

¹n = 3 at each time point except time = 0 where n = 4
Figure A1.4. Benzoyllecgonine concentrations over 24 h at 4°C for three different days (A, B, and C). Dashed lines represent the 95%CI about the slope. 

\[ n = 3 \text{ at each time point except time } = 0 \text{ where } n = 4 \]
Figure A1.5. Cocaine concentrations over 24 h at 4°C for three different days (A, B, and C). Dashed lines represent the 95% CI about the slope.¹

¹n = 3 at each time point except time = 0 where n = 4
RESULTS

Figure A1.6. Hourly concentrations of creatinine, caffeine, benzoylecgonine, cocaine, and methamphetamine.
Figure A1.7. Ratios (mg/mg) of analytes to creatinine after applying degradation rate constant from stability study.
Appendix 2. Wastewater testing as an alternative to random urinalyses for the surveillance of illicit drug use in prisons

EXPERIMENTAL METHODS

Chemical Analyses. All wastewater samples were analyzed for all substances (except creatinine) within one of two analytical sequences with sequence one containing monthly wastewater samples. Samples analyzed for creatinine were analyzed in a single run. The concentration range for standards used to construct the calibration curve for this study ranged from 40-4,000 ng/L (methamphetamine); 5-500 ng/L (cocaine); 40-4,000 ng/L (benzoylecgonine); and 50,000-10,000,000 ng/L (creatinine) in 5 mM ammonium acetate buffer. The lower limits of quantification for analytes were; 40 ng/L (methamphetamine), 5 ng/L (cocaine), 40 ng/L (benzoylecgonine), and 50,000 ng/L (creatinine). All calibration curves had $R^2 > 0.90$ with 1/X weighting. Analytes were assigned a concentration if the signal-to-noise (S/N) met the criteria of $S/N \geq 10$ and was at or above the lowest calibration curve standard. For a given peak, if S/N was > 10 but below the lowest calibration standard, no concentration was assigned. The lower limit of quantification was determined as the lowest calibration standard.

Accuracy and Precision. Accuracy was determined by analyzing, a custom made, third party reference standard (human urine) prepared by UTAK Laboratories Inc. (Valencia, CA) and comparing nominal to measured concentrations (Table A2.5). The reference urine was diluted by a factor of 1000 with 5 mM ammonium acetate buffer. The dilute solution was then spiked with internal standards for all substances and analyzed twice, once in the beginning of a sequence and again at the end (Table
A single wastewater sample was picked at random within each analytical sequence and run four multiple times for precision, as indicated by % RSD (Table A2.5). Also, as a check on the sampling methodology 24 h composite loads were compared to the sum of the hourly loads for one day (Table A2.6).

**Methadone, Hydrocodone, and Oxycodone.** The most commonly abused drugs are opioids. Methadone is a common\textsuperscript{110} prescription drug that has been used to treat opioid abuse as well as chronic pain particularly for surgery and cancer\textsuperscript{111}. Hydrocodone and oxycodone are widely prescribed drug for the treatment of pain and is sold under the common brand names of Lortab®, Vicodin®, Hycodan® (hydrocodone containing) Oxyfast®, Percocet®, Percodan®, and OxyContin® (oxycodone containing).

Monthly methadone loads displayed a consistent trend throughout the month, which is something expected in a prescription drug (Figure A2.9). There were two days in the month (8/1 and 8/20) when there was no methadone excretion in the system. This pattern is possibly consistent with an employee that did not happen to work on these two days. It is unlikely that the absence of loads for these two days are a result of an inmate. Methadone was not prescribed to inmates during the study period this is a key factor in should appear early, and it not a common drug of abuse thus it is mainly attributed to non-inmates. It is worth noting that methadone was not present on two different days during the month. Again, employee records were not available for this time period. Hourly methadone loads were observed during August 13\textsuperscript{th} (Figure A2.10). In contrast to the daily methadone loads there was variation observed during the day. There were two hours in which no mass was observed.
followed by an increase in mass from 06:00 to 15:00. Furthermore the majority of mass is observed during visiting hours although there were observations made during non-visiting hours for which only employees and inmates could solely contribute to. There was no statistical difference between \( p<0.05 \) loads on different days of the week and urine analyses that were conducted for the month did not include any tests that were positive for opiate like substances. The presence of methadone was not tested in the inmate population during the UAs. Thus there is not a possibility of ruling out inmates contributing to the overall methadone loads if there is a contraband methadone use inside the prison.

In comparison to oxycodone, hydrocodone is a more common substance in terms of excretion from the prison. Both hydrocodone and oxycodone are not prescribed to inmates and thus all loads are attributed to, employees, visitors or illicit inmate use. Hydrocodone displayed a daily pattern within the month that displayed little variation (Figure A2.9). As with methadone there was more variation observed with the hourly loads of hydrocodone (Figure A2.11). In contrast oxycodone did was not quantified in any of the daily samples, although it was observed in the hourly samples (Figure A2.12), with all but one observation taking place during visiting hours. There were no statistical differences \( p<0.05 \) for loads between different days of the week. These drugs are also prone to tampering which can change known patterns of excretion of prescription drugs.

**Nicotine and Cotinine.** Nicotine is a contraband substance for inmates. Nicotine and cotinine diurnal trends have also never been observed in wastewater, and may be of interest to researchers interested in smoking behavior. Nicotine (Figure
A2.13) and cotinine (Figure A2.13) trends in daily loads for the month were different from one another. Nicotine loads increased starting on August 9th, and peaked on August 13th followed by a decrease until August 15th. Thus, there is slightly more variation in nicotine loads than cotinine. Cotinine loads displayed little variation during the month. Daily nicotine and cotinine (Figures A2.14, A2.15) display similar diurnal trends. There was roughly 10 times as much nicotine mass observed than cotinine. In both substances there is an increase of ~3 times during the morning hours from 5:00 to 6:00, which is possibly attributed to either a work shift change. As with the other substances under investigation in this study there is no statistical difference (p<0.05) in loads between different days of the week. The percent difference between the daily load and the total hourly loads was 35%. Although we cannot separate inmates from non-inmates within the system there is ~20 x more inmates than non-inmates at any given moment. Nicotine is a contraband substance for inmates, but not tested for in UAs. Thus, most of the nicotine and cotinine are attributed to employees and visitors.

**Caffeine.** Caffeine was investigated as an alternative biomarker and widely used substance that is found in wastewater. Caffeine is also a widely used substance inside the prison, and can potentially be used as a verification of assumptions made from diurnal trends in an open system. Daily 24 h composite loads of caffeine were compared to the sum of the hourly composite samples in order to check variance in sampling methodologies. The percent difference between the daily load and the total hourly loads were 12% (Table A2.5). Daily loads for the month exhibited a trend of having two peaks with maxima that were roughly 30 percent
higher than minimum loads (Figure A2.16). The overall trends in daily caffeine loads are in agreement with caffeine’s wide use as a stimulant by inmates and non-inmates. The hourly loads of caffeine (Figure A2.17) clearly display the effect of inmates waking and excreting caffeine. The mass loads increase from roughly nothing to \(~0.03\) kg at 5:00 in the morning. The trend in caffeine loads also clearly shows the effect of sleep on the loads of caffeine. There were no statistical differences between days of the week for the month of sampling (p<0.05).

Acesulfame. Acesulfame was investigated due to its wide use as an artificial sugar as well as wide occurrence in wastewater. Acesulfame is an un-metabolized substance thus excreted unchanged. Acesulfame was detectable in every daily sample (Figure A2.18). The average load for the month was 10,000 mg with a 33% coefficient of variation. Acesulfame loads are an order of magnitude less than caffeine. Given that caffeine is extensively metabolized (3% dose of caffeine excreted) the difference in loads highlights caffeine use inside of the prison.
Equations.

Equation S1

\[
[\text{Creat}]_{\text{total pop}} (\text{mg/L}) = f_{\text{inmate}} \frac{1,565 \text{ mg Creat}}{L_{\text{urine}}} + f_{\text{noninmate}} \frac{1,304 \text{ mg Creat}}{L_{\text{urine}}}
\]

The weighted creatinine urine concentration for the inmate + non-inmate population \([\text{Creat}]_{\text{total pop}} (\text{mg/L})\) was determined as the sum of the fraction of inmates of the total population times the weighted mean creatinine urine concentration for the inmates (1,565 mg/L; Table S1) and the fraction of non-inmates of the total population times the mean creatinine urine concentration from Barr\(^1\) because no demographic data on non-inmates was provided.

Equation S2

Total Urine Volume \(
\left( \frac{L_{\text{urine}}}{\text{day}} \right) = \frac{mg \text{ Creat}}{L_{\text{ww}} \text{ day}} \left( \frac{L_{\text{urine}}}{mg \text{ Creat}} \right)
\)

Total urine volume (L) = total urine volume excreted by the total prison population \(mg \text{ Creat/L}_{\text{ww}}\) = measured creatinine concentration in wastewater (mg/L) \(L_{\text{ww}}/\text{day} = \) measured total volume of wastewater per day (L) \(mg \text{ Creat/L}_{\text{ urine}} = \) estimated weighted mean creatinine concentration for the inmate + non-inmate population from Equation S1.

Equation S3

\[
\frac{mg \text{ Creat}}{\text{day}} \left( \frac{L_{\text{urine}}}{mg \text{ Creat}} \right) \left( \frac{\text{person} \cdot \text{day}}{L_{\text{urine}}} \right) = \text{number of persons}
\]
Tables.

Table A2.1. Demographics (age and race/ethnicity) of inmate population, mean (or averaged) creatinine urine concentration for each age and race/ethnic, and the weighted mean concentration (1,565 mg/L) for the inmate population of 2,083.

<table>
<thead>
<tr>
<th>Age Group</th>
<th>People</th>
<th>Creatinine Urine Concentration (mg/L)</th>
<th>Weighted Subgroup Creatinine Concentration (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>18 to 24</td>
<td>124</td>
<td>1690</td>
<td>101</td>
</tr>
<tr>
<td>25 to 30</td>
<td>271</td>
<td>1820</td>
<td>237</td>
</tr>
<tr>
<td>31 to 45</td>
<td>569</td>
<td>1495</td>
<td>408</td>
</tr>
<tr>
<td>46 to 60</td>
<td>344</td>
<td>1370</td>
<td>226</td>
</tr>
<tr>
<td>61 and over</td>
<td>103</td>
<td>1205</td>
<td>59.6</td>
</tr>
<tr>
<td>18 to 24</td>
<td>21</td>
<td>2095</td>
<td>21.4</td>
</tr>
<tr>
<td>25 to 30</td>
<td>46</td>
<td>2150</td>
<td>48.0</td>
</tr>
<tr>
<td>31 to 45</td>
<td>98</td>
<td>1915</td>
<td>89.7</td>
</tr>
<tr>
<td>46 to 60</td>
<td>59</td>
<td>1800</td>
<td>51.0</td>
</tr>
<tr>
<td>61 and over</td>
<td>18</td>
<td>1475</td>
<td>12.5</td>
</tr>
<tr>
<td>18 to 24</td>
<td>29</td>
<td>1610</td>
<td>22.6</td>
</tr>
<tr>
<td>25 to 30</td>
<td>64</td>
<td>1700</td>
<td>52.2</td>
</tr>
<tr>
<td>31 to 45</td>
<td>134</td>
<td>1620</td>
<td>104</td>
</tr>
<tr>
<td>46 to 60</td>
<td>81</td>
<td>1445</td>
<td>56.3</td>
</tr>
<tr>
<td>61 and over</td>
<td>24</td>
<td>1205</td>
<td>14.1</td>
</tr>
<tr>
<td>18 to 24</td>
<td>9</td>
<td>1620</td>
<td>6.6</td>
</tr>
<tr>
<td>25 to 30</td>
<td>19</td>
<td>1499</td>
<td>13.4</td>
</tr>
<tr>
<td>31 to 45</td>
<td>39</td>
<td>1315</td>
<td>24.7</td>
</tr>
<tr>
<td>46 to 60</td>
<td>24</td>
<td>1165</td>
<td>13.2</td>
</tr>
<tr>
<td>Age Group</td>
<td>Total Weighted Mean Urine Creatinine Concentration (mg/L)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-----------</td>
<td>-------------------------------------------------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>61 and over</td>
<td>1,565</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table A2.2. Accuracy and precision as indicated by agreement between nominal and measured concentrations of average (n=2) third party reference standard (UTAK) as well as one wastewater analyzed as an indication of precision (n=4).

<table>
<thead>
<tr>
<th>Analyte (Target ng/L)</th>
<th>Accuracy (% Agreement, n=2)</th>
<th>Precision (% RSD, n=4 replicate wastewater analyses)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methamphetamine (750)</td>
<td>94</td>
<td>6.2</td>
</tr>
<tr>
<td>Cocaine (100)</td>
<td>83</td>
<td>7.3</td>
</tr>
<tr>
<td>Benzoylcegonine (350)</td>
<td>89</td>
<td>12</td>
</tr>
<tr>
<td>Creatinine (2000)</td>
<td>90</td>
<td>1.3</td>
</tr>
</tbody>
</table>

Table A2.2. Input of wastewater flow, measured creatinine concentration in wastewater (mg/L), and estimated creatinine concentration for the total prison (inmate+non-inmate) population (mg/L) used to calculate the daily total volume (L) of urine for the prison. The estimated creatinine urine concentration was computed using Equation S1.

<table>
<thead>
<tr>
<th>Date</th>
<th>Measured Wastewater flow (L)</th>
<th>Measured Creatinine in Wastewater (mg/L)</th>
<th>Estimated Urine Creatinine (mg/L)</th>
<th>Total Urine volume (L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>9-Aug</td>
<td>1,966,661</td>
<td>2.14</td>
<td>1,545</td>
<td>2,718</td>
</tr>
<tr>
<td>10-Aug</td>
<td>1,925,226</td>
<td>1.93</td>
<td>1,544</td>
<td>2,410</td>
</tr>
<tr>
<td>11-Aug</td>
<td>1,946,143</td>
<td>1.63</td>
<td>1,544</td>
<td>2,057</td>
</tr>
</tbody>
</table>
Figures.

Figure A2.3. Methamphetamine concentrations in wastewater (ng/L) after being exposed to either control or experimental tubing divided by concentration of initial solution over four weeks. Error bars represent 95% CI.
Figure A2.4. Cocaine and benzoylecgonine concentrations in wastewater (ng/L) after being exposed to either control or experimental tubing divided by concentration of initial solution over four weeks. Error bars represent 95% CI.
**Figure A2.5.** Creatinine concentrations in wastewater (ng/L) after being exposed to either control or experimental tubing divided by concentration of initial solution over 7 days. Error bars represent 95% CI.
Figure A2.6. Concentrations of sample bottle, control and experimental tubing used to determine the effect of biofilm on methadone, hydrocodone, and oxycodone concentrations in wastewater over four weeks.
Figure A2.7. Concentrations of sample bottle, control and experimental tubing used to determine the effect of biofilm on caffeine, nicotine, and cotinine concentrations in wastewater over four weeks.
Figure A2.8. Hourly loads (mg) of creatinine and population (inmate and non-inmate) during August 8th through Aug 16th.

Figure A2.9. Loads (mg) of methadone and hydrocodone in prison wastewater determined from July 30th to August 26th 2011. Error bars represent (%RSD) of analytical precision.
Figure A2.10. Hourly loads (mg) of methadone during August 13th 2011

Figure A2.11. Hourly loads (mg) of hydrocodone during August 13th 2011
**Figure A2.12.** Hourly loads (mg) of hydrocodone during August 13\textsuperscript{th} 2011

**Figure A2.13.** Loads (mg) of nicotine and cotinine in prison wastewater determined from July 30\textsuperscript{th} to August 26\textsuperscript{th} 2011. Error bars represent (%RSD) of analytical precision.
Figure A2.14. Hourly loads (mg) of nicotine during August 13th 2011

Figure A2.15. Hourly loads (mg) of cotinine during August 13th 2011
**Figure A2.16.** Loads (mg) of caffeine in prison wastewater determined from July 30\textsuperscript{th} to August 26\textsuperscript{th} 2011. Error bars represent (%RSD) of analytical precision.

**Figure A2.17.** Hourly loads (mg) of caffeine during August 13\textsuperscript{th} 2011
Figure A2.18. Loads (mg) of Acesulfame in prison wastewater determined from July 30\textsuperscript{th} to August 26\textsuperscript{th} 2011. Error bars represent (%RSD) of analytical precision.
Literature Cited


Appendix 3. Investigation of Cocaine and Benzoylecgonine transformation in transit through the use of a deuterated tracer test

**Sampling.** NaCl was used as a tracer to determine wastewater flow, velocity as well as a check of sampling methodology on a separate day before the addition of the deuterated substances. Wastewater flow was determined via standard addition. NaCl solution was prepared by adding 8 Kg of NaCl solution to 52 Liters of water (tap). The concentration of the NaCl solution was 0.15 Kg/L (156 g/L). 5 Liters of wastewater was collected to preform standard addition. Briefly a conductivity meter was used to measure 42mL of NaCl solution that was pipetted to 5 Liters of wastewater (1.68 mS). A final value of 3.62 mS was reached in order to determine average g/l/mS. The changes in conductivity values were recorded with the addition of a known mass in order to obtain the average value with the units of \( \frac{g}{l*mS} \). The average conductivity from the standard addition was set equal to the known mass (g) of each injection along with the average conductivity of the observed peak (mS) to solve for the liters of each pulse (Liters = grams injected/(Avg.mS*g/l/mS)). The liters of each pulse were then divided by the number of seconds that each peak was present to determine flow rate (L/sec).

**Chemical analysis.**

Ecgonine methyl ester (EME) and Ecgonine (EC) are transformation products of COC and BZE respectively\(^39\). Analyses utilizing different liquid chromatographic injection volumes of 900 uL and 1800 uL were used to determine instrument parameters for EC-d3 and EME-d3. EC-d3 and EME-d3 were identified to be transformation products using the University of Minnesota biodegradation database.\(^114\) Briefly,
Phenyl analytical and guard columns were used due to poor retention with C18 columns. A solvent-based calibration curve was used in order to determine linearity, as well as LOQ with a 900 uL injection volume (in solvent based calibration curve). Typical area counts for EC and EME-d3 were <500 (Table A3.5). 1800uL injections were preformed in a wastewater matrix in order to determine calibration in matrix as well as LOQ within the matrix. Calibration down to 40ng/L did not work in the matrix (no peak for EME-D3-2, and low area counts for EC-D3-2 (Table A3.6)). Peak shape for both experiments were symmetrical when observed and S/N was >10. Area counts for EME-D3-2 highest standard (4000ng/L) was ~11,000. Standard #4 (2000ng/L) ~5300 area counts. Making theoretical area counts ~110 for a 40ng/L. Area counts for EC-D3-2 highest solvent based standard (4000ng/L) were ~14,000. Standard #4 (2000ng/L) produced ~8200 area counts. Making theoretical area counts ~140 for a 40ng/L standard in wastewater. Area counts for cocaine and benzoylcegonine (lowest standards) were ~10X EC, and EME-d3 in wastewater. (Figure A3.2) For EME in wastewater the 2\textsuperscript{nd} transition was visible at 2000ng/L, for EC in wastewater the 2\textsuperscript{nd} transition was visible at the same concentration (Tables A3.5, A3.6). Note for one transition only two non-zero points are used in calibration.

In conclusion EC-d3 and EME-d3 were not investigated for the following reasons

1. Unable to calibrate in wastewater down to 40ng/L (no second transition)
2. Unable to account for matrix effect with Internal standard (no peaks for low end)
3. Unable to observe either analyte in “scout” samples
4. Lack of sensitivity/matrix effects in Zurich wastewater
### Table A3.1. Wastewater speed as determined from time and location of peaks at each sampling location.

<table>
<thead>
<tr>
<th></th>
<th>min</th>
<th>m</th>
<th>m/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>COC-d3</td>
<td>12.0</td>
<td>500.0</td>
<td>41.7</td>
</tr>
<tr>
<td></td>
<td>45.0</td>
<td>2500.0</td>
<td>55.6</td>
</tr>
<tr>
<td></td>
<td>127.0</td>
<td>5000.0</td>
<td>39.4</td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td></td>
<td>45.5</td>
</tr>
<tr>
<td>BZE-d3</td>
<td>11.6</td>
<td>500.0</td>
<td>43.1</td>
</tr>
<tr>
<td></td>
<td>54.6</td>
<td>2500.0</td>
<td>45.8</td>
</tr>
<tr>
<td></td>
<td>124.7</td>
<td>5000.0</td>
<td>40.1</td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td></td>
<td>43.0</td>
</tr>
</tbody>
</table>

### Table A3.2. Precursor and product ion (m/z), cone (V), and collision voltages (eV) all operated in ESI+ mode.

<table>
<thead>
<tr>
<th></th>
<th>Parent (m/z)</th>
<th>Product (m/z)</th>
<th>Cone (V)</th>
<th>Col. Energy (eV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BZE</td>
<td>290.2</td>
<td>105.1</td>
<td>30</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>293.2</td>
<td>153</td>
<td>30</td>
<td>25</td>
</tr>
<tr>
<td>BZE d3</td>
<td>298</td>
<td>153</td>
<td>30</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>304.1</td>
<td>182.3</td>
<td>30</td>
<td>20</td>
</tr>
<tr>
<td>COC</td>
<td>307.3</td>
<td>185.5</td>
<td>30</td>
<td>20</td>
</tr>
<tr>
<td>COC d3</td>
<td>309.2</td>
<td>84.9</td>
<td>30</td>
<td>25</td>
</tr>
<tr>
<td>COC d5</td>
<td>182</td>
<td>30</td>
<td>20</td>
<td></td>
</tr>
</tbody>
</table>
Table A3.3. Recovery of spiked cocaine-d3 and benzoylecgonine-d3 (100ng/L cocaine, and 25ng/L benzoylecgonine) in blank Zurich wastewater.

<table>
<thead>
<tr>
<th></th>
<th>Recovery in wastewater</th>
<th>%RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cocaine-d3</td>
<td>105.00</td>
<td>13.77</td>
</tr>
<tr>
<td>Benzoylecgonine-d3</td>
<td>109.00</td>
<td>13.13</td>
</tr>
</tbody>
</table>
Table A3.4. Analytical accuracy and precision of four location specific as indicated by %RSD of a wastewater sample analyzed four times, spiked UTAK % recovery (cocaine-d3, and benzoylecgonine-d3), and % deviation of expected in UTAK (cocaine and benzoylecgonine).

<table>
<thead>
<tr>
<th>Location</th>
<th>%RSD</th>
<th>Spiked UTAK Recovery</th>
<th>UTAK % deviation from expected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Location 1</td>
<td>Cocaine</td>
<td>6.21</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Cocaine-d3</td>
<td>7.98</td>
<td>109.80</td>
</tr>
<tr>
<td></td>
<td>Benzoylecgonine</td>
<td>7.02</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Benzoylecgonine-d3</td>
<td>7.28</td>
<td>106.49</td>
</tr>
<tr>
<td>Location 2</td>
<td>Cocaine</td>
<td>5.97</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Cocaine-d3</td>
<td>5.88</td>
<td>108.70</td>
</tr>
<tr>
<td></td>
<td>Benzoylecgonine</td>
<td>4.48</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Benzoylecgonine-d3</td>
<td>23.55</td>
<td>107.98</td>
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<tr>
<td>Location 3</td>
<td>Cocaine</td>
<td>2.44</td>
<td>-</td>
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<tr>
<td></td>
<td>Cocaine-d3</td>
<td>2.43</td>
<td>100.00</td>
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<tr>
<td></td>
<td>Benzoylecgonine</td>
<td>11.89</td>
<td>-</td>
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<tr>
<td></td>
<td>Benzoylecgonine-d3</td>
<td>19.98</td>
<td>95.01</td>
</tr>
<tr>
<td>Benzoylecgonine Injection</td>
<td>Cocaine</td>
<td>19.13</td>
<td>93.80</td>
</tr>
<tr>
<td></td>
<td>Cocaine-d3</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Benzoylecgonine</td>
<td>21.91</td>
<td>116.00</td>
</tr>
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<td></td>
<td>Benzoylecgonine-d3</td>
<td>15.26</td>
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</tbody>
</table>

Table A3.5. Area counts and Ion ratio of analytes determined with 900uL injection in wastewater matrix

<table>
<thead>
<tr>
<th>Area</th>
<th>Cocaine (5ng/L)</th>
<th>Benzoylecgonine (40 ng/L)</th>
<th>Ecgonine D3-2 (40 ng/L)*</th>
<th>Ecgonine Methyl Ester D3-2 (40 ng/L)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Area</td>
<td>1425</td>
<td>2470</td>
<td>316</td>
<td>506</td>
</tr>
<tr>
<td>Ion Ratio</td>
<td>~10/1</td>
<td>~5/1</td>
<td>~1/1</td>
<td></td>
</tr>
</tbody>
</table>

*-2 notes the transition
Table A3.6. Area counts and Ion ratio of observed peaks of analytes determined with 1800uL injection in wastewater matrix

<table>
<thead>
<tr>
<th></th>
<th>Cocaine (5ng/L)</th>
<th>Benzoylecgonine (40ng/L)</th>
<th>Ecgonine D3-2 (40 ng/L)</th>
<th>Ecgonine Methyl Ester D3-2 (80 ng/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Area</td>
<td>1336</td>
<td>2352</td>
<td>132</td>
<td>202</td>
</tr>
<tr>
<td>Ion Ratio</td>
<td>~10/1</td>
<td>~5/1</td>
<td>1/0 no second trans.</td>
<td>1/0 no second trans.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>~1/1 at 800ng/L</td>
<td>~2/1 at 2000ng/L</td>
</tr>
</tbody>
</table>

*-2 notes the transition
Figure A3.1. Schematic of QC samples that were constructed for each experiment

Analyzed by LC/MS/MS in Berne

Analyzed by LC/MS/MS in Corvallis

Cocaine-d3 solution (LIPOMED)

Cocaine-d3 in ELGA "Injectate"

MeOH QC
Made: 10 Days before injection
Expected: 1000 ng/L (coc-d3)
Measured: 1690 ng/L COC-d3

"Traces" BZE-d3 of (COC+BZE)

ELGA QC
Made: 10 Days before injection
Expected: 1000 ng/L (coc-d3)
Measured: 910 ng/L COC-d3
70 ng/L BZE-d3

Wastewater QC
Made: Min.
before injection
Expected: 1000 ng/L (coc-d3)
Measured: 863 ng/L COC-d3
289 ng/L BZE-d3

Injectate QC
Made: ~2 h before injection
Expected: 4 mg/L (coc-d3)
Measured: 3.83 mg/L COC-d3
0.55 mg/L BZE-d3

MeOH

10 Days Elapsed stored at -20°C

~2 h Elapsed stored at 4°C

Dilution

Spike

Spike before injection

Aliquot when injectate was made

Into Sewer
**Figure A3.2.** Comparison between wastewater calibration and solvent calibration with 1800 uL injection.
Literature Cited
