Environmental Science & Technology
Article
Cite This: Environ. Sci. Technol. 2018, 52, 1561−1570
pubs.acs.org/est

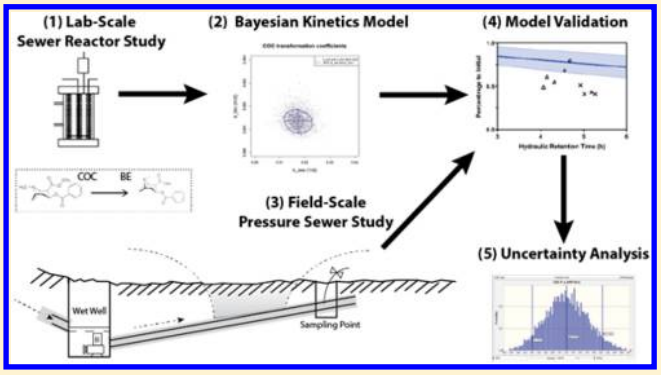
Stability of Illicit Drugs as Biomarkers in Sewers: From Lab to Reality

Jiaying Li,[†] Jianfa Gao,[‡] Phong K. Thai,[§] Xiaoyan Sun,[†,∥] Jochen F. Mueller,[‡] Zhiguo Yuan,[†] and Guangming Jiang*[,†]

[†]Advanced Water Management Centre, The University of Queensland, St. Lucia, Queensland 4072, Australia
[‡]Queensland Alliance for Environmental Health Sciences, The University of Queensland, Brisbane, Queensland 4108, Australia
[§]International Laboratory for Air Quality and Health, Queensland University of Technology, Brisbane, Queensland 4001, Australia

Supporting Information

ABSTRACT: Systematic sampling and analysis of wastewater samples are increasingly adopted for estimating drug consumption in communities. An understanding of the in-sewer transportation and transformation of illicit drug biomarkers is critical for reducing the uncertainty of this evidence-based estimation method. In this study, biomarkers stability was investigated in lab-scale sewer reactors with typical sewer conditions. Kinetic models using the Bayesian statistics method were developed to simulate biomarkers transformation in reactors. Furthermore, a field-scale study was conducted in a real pressure sewer pipe with the systematical spiking and sampling of biomarkers and flow tracers. In-sewer degradation was observed for some spiked biomarkers over typical hydraulic retention time (i.e., a few hours). Results indicated that sewer biofilms prominently influenced biomarker stability with the retention time in wastewater. The fits between the measured and the simulated biomarkers transformation demonstrated that the lab-based model could be extended to estimate the changes of biomarkers in real sewers. Results also suggested that the variabilities of biotransformation and analytical accuracy are the two major contributors to the overall estimation uncertainty. Built upon many previous lab-scale studies, this study is one critical step forward in realizing wastewater-based epidemiology by extending biomarker stability investigations from laboratory reactors to real sewers.



INTRODUCTION

Wastewater-based epidemiology (WBE) has been widely studied for its application to assess drug consumption and community health in the past decade.[1] Through analyzing wastewater samples for target drug residues (namely biomarkers), WBE estimates per capita drug consumption by integrating biomarkers concentration with the information on wastewater flow, catchment inhabitation size, and drug excretion factors. Recognized as a reliable tool for drug monitoring, WBE has been under improvement for more accurate applications. Concentration changes of biomarkers due to transformation in wastewater, including sorption, abiotic, and biological degradation/formation, could lead to over- or underestimate of drug consumption in a catchment.[2,3]

In-sewer stability of biomarkers has been evaluated by several lab-scale studies considering the effects of sewer types (pressure vs gravity sewer), biomass (suspended sludge vs biofilm), and conditions (aerobic vs anaerobic).[4−9] These laboratory studies suggested that degradation of some biomarkers could be considerably amplified by sewer biofilms. Sewer networks act as an active bioreactor with microbial, chemical, and physical processes.[10] Heterotrophic microorganisms, e.g. sulfate reducing bacteria, have the ability to transform wastewater components including biomarkers.[4,5,8−10] It is also reported that methanogenic archaea has the potential for cometabolic enzymatic transformation of organic micropollutants.[11] Besides, previous studies revealed that microorganisms in sewer biofilms had significantly higher contribution to in-sewer processes compared to the suspended microorganisms in wastewater.[5,12,13]

Biotransformation affects the stability of biomarkers to different levels based on the hydraulic retention time (HRT) of wastewater. In a sewer system, HRT of wastewater is usually dependent on that in pressure sewer compared to gravity sewer by reason for the operational design.[10] The HRT in pressure sewer, which could be as long as a few hours, usually shows diurnal patterns due to the wastewater generation with the daily routine of the subpopulation.[10,14] This HRT dynamics is critical for in-sewer processes including biological activities[10,12,15−18] and biomarkers transformation.[19] Besides, at the upstream of a catchment, pressure sewer usually consists of sewer pipes with different sizes, leading to different area-to-volume (A/V) ratios which also could impact biomarkers transformation.

Received: October 3, 2017
Revised: December 23, 2017
Accepted: December 29, 2017
Published: December 29, 2017

DOI: 10.1021/acs.est.7b05109
Environ. Sci. Technol. 2018, 52, 1561−1570

To date, the investigation of biomarkers stability in real sewers is scarce, as most previous research employed lab-scale reactors without[20−22] or with biofilms.[4,5,7,9] The findings from these lab-scale studies need to be validated against data from real sewers. One field-scale study was conducted in a pressure sewer pipe to monitor biotransformation of native pharmaceuticals over 21 h using daily composite samples.[23] Another study employed a controllable sewer pipe with constant flow rate and HRT (2 h) to monitor native biomarkers transformation through 24-h composite samples.[24] However, research has not moved further to investigate biomarker stability in a pressure sewer with dynamic hydraulics over representative HRT, which is critical for WBE application in a real catchment.

This study aims to investigate the stability of selected illicit drug biomarkers in a real pressure sewer with typical dynamics of wastewater hydraulics and compositions. This field-scale study employed the systematic spiking and sampling of biomarkers and water tracers to understand the in-pipe sewer flow pattern. Moreover, the stability data obtained from lab-scale reactors were used to determine the transformation kinetic models using the Bayesian statistics method, which was subsequently extended to the conditions in real sewers. The validation of modeling results against field data provided critical insights about the applicability of lab-scale findings to WBE in real sewers.

MATERIALS AND METHODS

Biomarkers and Chemicals. Based on the WBE applications reported in the literature, 11 biomarkers of parent illicit drugs and their metabolites were selected for investigation. These included cocaine (COC), benzoylecgonine (BE), methamphetamine (METH), amphetamine (AMP), 3,4-Methylenedioxymethamphetamine (MDMA), 6-acetylmorphine (6-AM), morphine (MOR), ketamine (KET), methadone (MTD), 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrolidine (EDDP), and codeine (CODE) (see Table S1 for more details). Mixture solutions of parent compounds and metabolites were separately prepared in Milli-Q water (S1.1).

Rhodamine and acesulfame were applied as flow tracers in the field study. Rhodamine was used as 1) a visual tracer to indicate the in-pipe transportation of the spiked wastewater slugs, 2) a stable marker to indicate wastewater mixing conditions, such as the in-pipe dispersion, and 3) the potential inflow and in/exfiltration. Acesulfame was employed as another flow tracer considering its high stability in wastewater.[8,25] The simultaneous spiking of acesulfame with rhodamine was employed for the purpose of cross-check and validation in this field study.

Batch Tests Using Lab-Scale Sewer Reactors. The stability of illicit drug biomarkers was investigated in lab-scale sewer reactors, which mimic the typical sewer condition with mature biofilms (see further description of sewer reactors in S3.1). The capability of sewer reactors to represent the real sewer environment has been demonstrated in previous studies.[17,26,27] Triplicate batch tests were conducted in one pressure sewer reactor (PR) with anaerobic biofilms on reactor wall and carriers (A/V ratio 72.5 m^{-1}) and in one control reactor (CR) without attached biofilm or carrier. Biological activity of PR in terms of sulfide generation and methanogenesis had reached pseudo-steady-state before the batch tests. Selected biomarkers were spiked into the real domestic sewer as the feeding to reactors. The spiked sewer retained 12 h in reactors, and a magnetic stirrer provided continuous mixing (250 rpm) inside the reactor. Biomarker samples were taken from both PR and CR at 0, 0.25, 0.5, 1, 2, 3, 6, 9, and 12 h for chemical analysis. Sulfide and methane concentrations in PR were also monitored during the batch tests, which respectively provided sulfide and methane production rates to indicate the biofilm activities.

The Pressure Sewer Used in the Field Study. The field study was conducted in a pressure sewer pipe named UC9 in Southeast Queensland, Australia (Figure 1). The study was

Figure 1. UC9 pressure sewer with the plug-flow hydraulics in the sewer pipe.

carried out in July when the average wastewater temperature was around 23 °C. Previous monitoring showed the active sulfide and methane generations in the UC9 sewer.[12,17] UC9 has an internal pipe diameter of 0.15 m (corresponding to the A/V ratio of 26.7 m^{-1}) and receives an average dry weather flow of 126 m^3 d^{-1}. Previous examination of a removable section of this sewer network showed no deposition of sediment.[17] A pumping event is started and stopped when the wastewater level in the pump station wet well reaches around 19.5% and 8.5% of the wet well capacity, respectively. Each pumping event lasts approximately 2 min and delivers a wastewater slug of approximate 1.8 m^3 into the pipe. HRT of a wastewater slug is defined as the time for the slug "travel" from the beginning of the pipe to the sampling point location (828 m downstream) close to the end of the pipe. HRT of each wastewater slug is calculated using Matlab R2016a based on the pump operational data recorded by the online supervisory control and data acquisition (SCADA) system.

Field Study. The field study started with the spiking events in the pump station wet well. The spiked biomarkers were separated into two groups, namely the metabolite group (Test 1 on day 1) and the parent group (Test 2 on day 2). The spiked biomarkers in the metabolite group included BE, AMP, and 6-AM, and the spiked biomarkers in parent group included COC, METH, MDMA, MTD, and KET. Besides, MOR, CODE, and

DOI: 10.1021/acs.est.7b05109
Environ. Sci. Technol. 2018, 52, 1561−1570

EDDP were not spiked, while the native compounds were investigated. For each group test, the mixture solution of biomarkers, rhodamine, and acesulfame was spiked into the wet well immediately after one pumping event, which was subsequently mixed and diluted by the continuous inflow into wet well above the pumping-start water level. In order to minimize the interference between the spiked wastewater slugs, the mixture solution was spiked once every two pumping events, and each biomarker group was spiked four times over eight pumping cycles in each experimental day (spiking protocol in Table S9).

During the field study, wastewater was collected as grab samples at both the wet well and the sampling point (828 m downstream) right before and after every pumping event. Samples were also collected from the wet well before the first spiking on each day to determine the background biomarker residues. Samples were prepared on site for the measurement of biomarkers, sulfur species, and dissolved methane (details in S1.2 and S1.4). All samples were stored in an ice cooler on site and immediately transferred to a lab fridge after a campaign. Biomarker samples were frozen in a −20 °C freezer for further pretreatment. Sulfur and dissolved methane samples were analyzed within 24 h after preparation. Samples of other wastewater parameters (i.e., volatile fatty acids (VFA), ammonia, total and volatile suspended solids (TSS and VSS), total and soluble chemical oxygen demand (TCOD and SCOD)) were prepared within 24 h for further measurement (details in S1.4).

Online Monitoring and Chemical Analysis. A S::CAN UV−vis spectro::lyser (Messtechnik GmbH, Austria) coupled with a pH probe was installed at the sampling point, providing *in situ* monitoring of sulfide and pH of wastewater, as described previously.[28] Rhodamine concentration in wastewater samples was measured by a rhodamine monitoring system, which comprised a portable Cyclops-7 Submersible Rhodamine Sensor coupled with a Cyclops Explorer. The temperature of wastewater samples was measured on site using a portable meter with a temperature probe (TPS Aqua-pH pH/Temp meter).

The analytical methods for biomarkers, acesulfame, and other parameters are specified in S1.2 and S1.4. The uncertainty of chemical analysis ($U_{analysis}$) was calculated according to a previous method integrating the relative standard error of recoveries, triplicate analysis for each sample, intraday instrumental precision, and other uncertainty factors (details in S1.3).[23]

Bayesian-Based Transformation Kinetic Models. Assuming the in-sewer transformation of illicit drug biomarkers was mainly due to the abiotic processes in the bulk wastewater and the biotransformation by sewer biofilm,[4,5,7,9,22,24,29,30] respectively, first-order (eq 1) and zero-order (eq 2) kinetics were employed to evaluate the biomarker transformation

$$C = C_0 \cdot e^{-(k_{ww}+k_{bio})\cdot t} = C_0 \cdot e^{-(k_{ww}+k'_{bio}\cdot\frac{A}{V})\cdot t} \quad (1)$$

$$C = -(k_{ww,0} + k_{bio})\cdot t + C_0 = -\left(k_{ww,0} + k'_{bio,0}\cdot\frac{A}{V}\right)\cdot t + C_0 \quad (2)$$

where C is biomarker concentration (ppb), C_0 is the initial concentration at time 0, t is time after spiking (h), k_{ww} (h^{-1}) represents abiotic transformation of biomarker in the bulk wastewater such as hydrolysis and sorption to suspended solids, k_{bio} (h^{-1}) and k'_{bio} (m h^{-1}) represent the biotransformation by sewer biofilms (the anaerobic biofilm in this study) with and without the normalization with respect to the A/V (i.e., biofilm area to wastewater volume) ratio (m^{-1}), and $k_{ww,0}$ (ppb h^{-1}), $k_{bio,0}$ (ppb h^{-1}), and $k'_{bio,0}$ (ppb m h^{-1}) are the transformation coefficients for the zero-order kinetics.

Drug transformation coefficients (mean with 95% credible intervals) were determined using Bayesian statistics, or known as the Markov Chain Monte Carlo (MCMC) method, with the propagation of the associated uncertainties. This study applied R[31] to execute the Bayesian method in OpenBUGS[32] and to generate the statistics and graphs based on the simulation results. A Bayesian model was developed on OpenBUGS with an execution of 10,000 iterations to simulate the posterior distributions of modeling parameters (model structure in S2.1). An error term with variance *tau* was applied to include all potential uncertainties. In order to select the proper prior information for the modeling transformation coefficients, several commonly used priors were examined, including normal, uniform, gamma, and flat distributions (the hyperparameters in Table S5). Uniform prior distribution was assigned to C_0 as suggested by similar studies.[4,24,33]

Data used for model simulation were obtained from both the lab-scale sewer reactor batch tests and the data reported in the study of Thai et al.[5] The parameter of k_{ww} ($k_{ww,0}$) was estimated based on the experimental data of CR (without biofilm). The parameter of k'_{bio} ($k'_{bio,0}$) was estimated based on the determined posterior information on k_{ww}, the A/V ratio, and the experimental data of PR (with biofilm). Posterior distributions of the modeling parameters were used to calculate a specific Deviation Information Criterion (DIC) value and to generate the figures of density distributions and a joint highest-posterior-density (HPD) region for each investigated biomarker. The HPD region presented the mean value and 95% confidence bounds for the pairwise transformation coefficients (e.g., k_{ww} and k'_{bio}). Besides, the determined transformation coefficients were further used to calculate the half-life ($t_{1/2}$, h) of biomarkers in CR and PR.

Furthermore, for the biomarkers which presented limited degradation in sewer reactors, linear regression was applied to assess the deviation from zero. A pretty small R^2 reported by linear regression would suggest a horizontal line as the best fit. The stability of biomarkers was thus verified by the deviation of concentration changes from zero over the investigated time frame.

Assessment of in-Sewer Stability of Biomarkers. The in-sewer stability of biomarkers was assessed by determining the change of biomarker concentration with HRT. This change was calculated as the ratio in percentage (P) of the sampling concentration at time t (C_t) against the spiking concentration at time 0 (C_0) (eq 3).

$$P = \frac{C_t}{C_0} \times 100\% \quad (3)$$

According to the modeling results of k_{ww} and k'_{bio} ($k_{ww,0}$ and $k'_{bio,0}$) as well as the A/V ratio of sewer reactor, the simulated transformation regions (mean with 95% confidence bounds) for CR (P_{WW}) and PR (P_{PR}) were generated for each investigated biomarker. The fits between the measured transformations in reactors and the simulated P_{WW} and P_{PR} were examined using the R^2 value on GraphPad Prism 7.

For the field study in the UC9 sewer, in-sewer stability was assessed by comparing the biomarker concentrations at the

DOI: 10.1021/acs.est.7b05109
Environ. Sci. Technol. 2018, 52, 1561−1570

downstream sampling point to the spiking concentration in the same wastewater slug at upstream, assuming an ideal plug flow regime in the pipe. To account for the potential in-pipe dilution/dispersion, the change of biomarker in real sewer (P_{SEWER}) was normalized by the corresponding rhodamine concentration in the same wastewater slug (eq 4) as proposed previously.[34] Furthermore, for each spiked biomarker in the field study, a simulated transformation region P_{SIM} (mean with 95% confidence bounds) was generated according to the estimated k_{ww} and k'_{bio} ($k_{ww,0}$ and $k'_{bio,0}$) and the A/V ratio of the UC9 sewer pipe. The measured P_{SEWER} was then compared to the simulated P_{SIM} to assess the applicability of the lab-scale biomarker stability to the real sewer

$$P_{SEWER} = \frac{C_{i,j}^{down}/C_{rho,j}^{down}}{C_{i,j}^{up}/C_{rho,j}^{up}} \times 100\% \quad (4)$$

where $C_{i,j}^{up}$ and $C_{i,j}^{down}$ represent the upstream and downstream concentrations of biomarker i in wastewater from the j-th spiking event, while $C_{rho,j}^{up}$ and $C_{rho,j}^{down}$ represent the upstream and downstream concentrations of rhodamine in wastewater from the same j-th spiking event.

Uncertainty and Sensitivity Analyses. For the parameters determined by the Bayesian-based kinetic models, uncertainty and sensitivity analyses were conducted on Oracle Crystal Ball, aiming to evaluate the correlation and contribution of the variability of each parameter to the overall uncertainty of the simulated biomarker transformation (details in S2.3). The assumptions of transformation coefficients (e.g., k_{ww}, k'_{bio}) and compound concentration (C_0) were defined according to the modeling posterior distribution results and the specific $U_{analysis}$ of each biomarker, respectively. Based on these input definitions, the forecasting cells of biomarker transformation in different experimental scales (i.e., in lab-scale control and pressure sewer reactors and in the UC9 sewer) were simulated with HRT of 1, 6, and 12 h. Simulation for each forecasting scenario was run 5,000 times, which created a specific uncertainty chart with frequency distribution. According to the simulation results, the correlations between assumptions and forecasts were calculated. Furthermore, sensitivity analysis was carried out to evaluate the contribution of each assumption cell to the overall uncertainty of forecasts.

RESULTS AND DISCUSSION

Biomarker Stability in Lab-Scale Sewer Reactors. Lab-scale batch tests revealed different stability levels of illicit drug biomarkers in the wastewater with or without sewer biofilms (Table 2 and Figure S4). In the control reactor over 12 h, biomarkers BE, METH, MDMA, MOR, and CODE had <15% variations, biomarkers COC and KET showed partial transformation (<25% loss), while biomarkers 6-AM, MTD, and EDDP exhibited relatively higher degradation (>40% loss). These variations in the bulk wastewater were likely due to the abiotic processes (e.g., hydrolysis and sorption) depending on their specific physicochemical properties.

In comparison to the control reactor, higher transformation was observed for all biomarkers in the pressure sewer reactor with mature anaerobic biofilm. During the batch tests, biofilm in PR exhibited sulfide and methane generations as comparable to those previously reported (Table 1 and Figure S5). The degradation of CODE, which was relatively negligible in CR, was significantly accelerated by the sewer biofilm. Biomarkers BE, METH, MDMA, and MOR still remained stable in PR but with larger deviations compared to their higher consistency in CR. These deviations could come from the fluctuation of sewer conditions (e.g., biological activities and the resulted wastewater compositions) over the triplicate experiments in PR. All the unstable biomarkers, as observed in CR, showed more degradation in the presence of sewer biofilms, supporting the role of biofilm in biodegradation of these biomarkers. Indeed, biodegradation in PR reduced the half-life of COC, 6-AM, CODE, MTD, and EDDP to within 6 h, except for KET which remained 60% over 12 h.

Table 1. Biofilm Activities of the Lab-Scale Pressure Sewer Reactor, the UC9 Pressure Sewer, and Literature Values

	pressure sewer reactor	UC9 pressure sewer	lit. values
sulfide production rate (gS m^{-2} d^{-1})	1.60 ± 0.54	1.63 ± 0.12 (Tests 1 and 2)	0.48−2.4[10]
methane production rate (gCOD m^{-2} d^{-1})	4.09 ± 1.10	3.88 ± 0.38 (Test 1)	5.03,[16] 4.8, and 5.3[37]
		5.32 ± 0.38 (Test 2)	

Bayesian Kinetic Model Based on Lab-Scale Data. Model simulations were operated based on the data obtained from the lab-scale sewer reactors. After examining different prior probability distributions for the modeling parameters k_{ww} and k'_{bio} (also $k_{ww,0}$ and $k'_{bio,0}$), it was found that the kinetic models using normal prior distribution usually resulted in higher R^2 value (Table S5). According to this finding, the estimation results of the first-order and zero-order kinetic models with the application of normal prior distribution were reported in Table 2. Comparing these two kinds of models, it was further revealed that the P_{WW} and P_{PR} simulated by the first-order kinetic model usually had better fits to the measured transformations compared to the zero-order kinetic model in terms of higher R^2 and lower DIC values. Therefore, the first-order kinetic model with the normal prior distribution to transformation coefficients was chosen for further evaluation.

According to the HPD regions of k_{ww} and k'_{bio} estimates, they were reliable with limited variance (Figure S1). Therefore, the estimated k_{ww} and k'_{bio} were used to generate the simulated biomarkers transformation regions with the integration of the A/V ratio of sewer reactor (Figure S4). When comparing the findings of this study to the literature, it was found that the transformation coefficients of some biomarkers (e.g., COC, BE, METH, MDMA, 6-AM, and KET) determined by the sewer reactors were commensurable and comparable to other recent studies which investigated biomarkers stability in the sewer conditions with biofilms.[4,24] In general, results of this study implied that the lab-scale sewer reactors were useful in determining the kinetic parameters which are difficult to measure in real sewers.

For the relatively stable biomarkers (i.e., BE, METH, MDMA, and MOR in both reactors and CODE in CR), the estimated values of k_{ww} (mean ≤0.005) and k'_{bio} (mean ≤0.0001) are very low (10-fold to 100-fold lower) in comparison to those for the less stable biomarkers (i.e., COC, 6-AM, KET, MTD, and EDDP in both reactors and CODE in PR). The evaluation by linear regression also confirmed that the transformation of these stable biomarkers had insignificant deviation from zero over 12 h HRT (Table 2). The R^2 values of these stable biomarkers were estimated to be negative, indicating that a horizontal line might be more appropriate to describe their measured stability in wastewater.

DOI: 10.1021/acs.est.7b05109
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Table 2. Simulation Results of Drug Transformation Kinetic Models Using Bayesian Statistics[a,b,c,d,e,f]

		zero-order kinetic model		first-order kinetic model			zero-order kinetic model		first-order kinetic model
COC	DIC	−91.35	DIC	**-97.59**	6-AM	DIC	−49.31	DIC	**-67.54**
	$k_{ww,0}$	0.0150 (0.0108, 0.0195)	k_{ww}	0.0176 (0.0123, 0.0231)		$k_{ww,0}$	0.0278 (0.0216, 0.0340)	k_{ww}	0.0367 (0.0274, 0.0466)
	$k'_{bio,0}$	0.0006 (0.0005, 0.0008)	k'_{bio}	0.0014 (0.0009, 0.0019)		$k'_{bio,0}$	0.0008 (0.0005, 0.0010)	k'_{bio}	0.0034 (0.0022, 0.0050)
	CR	R^2 = 0.59 $t_{1/2}$ = 31.64 (24.87,42.50)	CR	**R^2 = 0.61** $t_{1/2}$ = 39.38 (30.01,56.58)		CR	R^2 = 0.64 $t_{1/2}$ = 16.80 (14.06, 21.28)	CR	**R^2 = 0.68** $t_{1/2}$ = 18.90 (14.87,25.29)
	PR	R^2 = 0.65 $t_{1/2}$ = 7.76 (6.29, 10.39)	PR	**R^2 = 0.78** $t_{1/2}$ = 5.85 (4.26, 8.54)		PR	R^2 = 0.68 $t_{1/2}$ = 5.62 (4.57, 7.49)	PR	**R^2 = 0.89** $t_{1/2}$ = 2.43 (1.68, 3.72)
	UC9	$t_{1/2}$ = 15.69 (12.24, 21.48)	UC9	$t_{1/2}$ = 12.67 (9.31, 18.44)		UC9	$t_{1/2}$ = 10.39 (8.30, 13.79)	UC9	$t_{1/2}$ = 5.42 (3.83, 8.08)
BE	DIC	−87.12	DIC	−87.23	MOR	DIC	−48.06	DIC	−48.08
	$k_{ww,0}$	0.0035 (−0.0027, 0.0098)	k_{ww}	0.0029 (−0.0034, 0.0097)		$k_{ww,0}$	0.0047 (−0.0060, 0.0162)	k_{ww}	0.0054 (−0.0085, 0.0177)
	$k'_{bio,0}$	0.0000 (−0.0002, 0.0001)	k'_{bio}	0.0000 (−0.0001, 0.0001)		$k'_{bio,0}$	0.0000 (−0.0002, 0.0002)	k'_{bio}	0.0000 (−0.0002, 0.0003)
	CR	***Insignificant Deviation from Zero (IDZ)***	CR	R^2 < 0 $t_{1/2}$ = 235.36 (71.20, \)		CR	***IDZ***	CR	R^2 < 0 $t_{1/2}$ = 129.56 (39.09, \)
	PR	***IDZ***	PR	R^2 < 0 $t_{1/2}$ = 239.16 (37.43, \)		PR	***IDZ***	PR	R^2 < 0 $t_{1/2}$ = 136.73 (19.15, \)
	UC9	$t_{1/2}$ = 171.87 (39.84, \)	UC9	$t_{1/2}$ = 236.75 (53.46, \)		UC9	$t_{1/2}$ = 111.32 (22.99, \)	UC9	$t_{1/2}$ = 132.11 (28.27, \)
METH	DIC	−83.71	DIC	−83.73	CODE	DIC	−11.21	DIC	**-46.99**
	$k_{ww,0}$	0.0014 (−0.0047, 0.0076)	k_{ww}	0.0012 (−0.0048, 0.0076)		$k_{ww,0}$	−0.0003 (−0.0122, 0.0119)	k_{ww}	0.0003 (−0.0119, 0.0133)
	$k'_{bio,0}$	0.0001 (−0.0001, 0.0003)	k'_{bio}	0.0001 (−0.0001, 0.0003)		$k'_{bio,0}$	0.0013 (0.0009, 0.0016)	k'_{bio}	0.0099 (0.0068, 0.0140)
	CR	***IDZ***	CR	R^2 < 0 $t_{1/2}$ = 579.55 (90.73, \)		CR	***IDZ***	CR	R^2 < 0 $t_{1/2}$ = 2178.34 (52.23, \)
	PR	***IDZ***	PR	R^2 = 0.42 $t_{1/2}$ = 80.78 (24.54, \)		PR	R^2 = 0.49 $t_{1/2}$ = 4.82 (3.61, 7.77)	PR	**R^2 = 0.86** $t_{1/2}$ = 0.97 (0.67, 1.44)
	UC9	***IDZ***	UC9	$t_{1/2}$ = 177.23 (45.56, \)		UC9	$t_{1/2}$ = 14.65 (8.97, 38.24)	UC9	$t_{1/2}$ = 2.63 (1.79, 4.08)
MDMA	DIC	−54.74	DIC	−54.87	MTD	DIC	−37.38	DIC	**-53.05**
	$k_{ww,0}$	−0.0027 (−0.0068, 0.0016)	k_{ww}	−0.0029 (−0.0066, 0.0013)		$k_{ww,0}$	0.0523 (0.0444, 0.0604)	k_{ww}	0.0888 (0.0722, 0.1080)
	$k'_{bio,0}$	0.0000 (−0.0001, 0.0001)	k'_{bio}	0.0000 (−0.0001, 0.0002)		$k'_{bio,0}$	0.0003 (0.0001, 0.0005)	k'_{bio}	0.0018 (0.0007, 0.0036)
	CR	***IDZ***	CR	R^2 < 0 $t_{1/2}$ = \ (549.24, \)		CR	R^2 = 0.79 $t_{1/2}$ = 9.04 (8.14, 10.36)	CR	**R^2 = 0.90** $t_{1/2}$ = 7.80 (6.42, 9.60)
	PR	***IDZ***	PR	R^2 < 0 $t_{1/2}$ = \ (54.61, \)		PR	R^2 = 0.43 $t_{1/2}$ = 6.43 (5.00, 9.50)	PR	**R^2 = 0.84** $t_{1/2}$ = 3.13 (1.86, 5.69)
	UC9	$t_{1/2}$ = \ (92.80, \)	UC9	$t_{1/2}$ = \ (126.83, \)		UC9	$t_{1/2}$ = 8.35 (6.73, 10.90)	UC9	$t_{1/2}$ = 5.04 (3.38, 7.67)
KET	DIC	−99.59	DIC	**-102.50**	EDDP	DIC	32.61	DIC	**-31.03**
	$k_{ww,0}$	0.0194 (0.0128, 0.0260)	k_{ww}	0.0213 (0.0139, 0.0286)		$k_{ww,0}$	0.0944 (0.0596, 0.1342)	k_{ww}	0.3767 (0.1538, 0.7752)
	$k'_{bio,0}$	0.0002 (0.0001, 0.0004)	k'_{bio}	0.0004 (0.0002, 0.0007)		$k'_{bio,0}$	0.0000 (−0.0007, 0.0072)	k'_{bio}	0.0455 (0.0260, 0.0753)
	CR	R^2 = 0.59 $t_{1/2}$ = 26.47 (20.42, 38.59)	CR	**R^2 = 0.59** $t_{1/2}$ = 32.48 (24.25, 49.97)		CR	R^2 = 0.77 $t_{1/2}$ = 4.96 (4.02, 7.21)	CR	**R^2 = 0.86** $t_{1/2}$ = 1.84 (0.89,4.51)
	PR	R^2 = 0.67 $t_{1/2}$ = 13.37 (9.12, 27.66)	PR	**R^2 = 0.74** $t_{1/2}$ = 13.58 (9.05, 26.74)		PR	R^2 < 0 $t_{1/2}$ = 4.66 (0.75, 47.87)	PR	**R^2 = 0.88** $t_{1/2}$ = 0.19 (0.11, 0.34)
	UC9	$t_{1/2}$ = 19.40 (13.41, 34.98)	UC9	$t_{1/2}$ = 21.48 (15.00, 37.87)		UC9	$t_{1/2}$ = 5.26 (1.53, 12.20)	UC9	$t_{1/2}$ = 0.44 (0.25, 0.82)

[a]The modeling results of k_{ww} (h^{-1}), k'_{bio} (m h^{-1}), $k_{ww,0}$ (ppb h^{-1}), and $k'_{bio,0}$ (ppb m h^{-1}) are provided with the mean value with 95% credible intervals. [b]The R^2 value is determined by fitting the measured changes of the biomarker in CR/PR with the accordingly simulated transformation. [c]Half-life ($t_{1/2}$, h) of the biomarker in CR, PR, and UC9 is calculated based on k_{ww} ($k_{ww,0}$), k'_{bio} ($k'_{bio,0}$), and the corresponding A/V ratios. Half-life is not calculated for the biomarker with negative transformation (i.e., formation). [d]The reported *insignificant deviation from zero* (*IDZ*) by linear regression indicates the limited measured change of the biomarker in wastewater. [e]Kinetic models with lower DIC and higher R^2 are bolded except where *IDZ* is reported. [f]Model simulation is based on the lab-scale stability experiments in the control (CR) and pressure (PR) sewer reactors.

Generally, results suggested that these relatively stable biomarkers had insignificant transformation in the lab-scale sewer reactors.

The uncertainty and sensitivity analyses evaluated the correlations and contributions of the variabilities of k_{ww}, k'_{bio}, and C_0 to the overall uncertainty of the simulated biomarker transformation in sewer reactors. For an HRT of 6 h in CR, the variability of C_0 due to $U_{analysis}$ contributed more than 80% of the uncertainty to most biomarkers except for MTD and EDDP. In PR over 6 h, the variability associated with $U_{analysis}$ still had the dominated contribution for the relatively stable biomarkers, while the variability of k'_{bio} became the highest contributor for those less stable biomarkers. It should be noted that the proportions of uncertainty contributors to the overall estimation uncertainty change with HRT (Table S7). Generally speaking, analyte concentration plays an important role in determining biomarker stability for shorter HRT, while biotransformation k'_{bio} dominates for the scenario with longer HRT.

Sewage Flow and Biological Activities in the UC9 Sewer. The UC9 sewer had typical flow dynamics and intraday HRT variations (Figure 2). The interval of each pumping cycle

DOI: 10.1021/acs.est.7b05109
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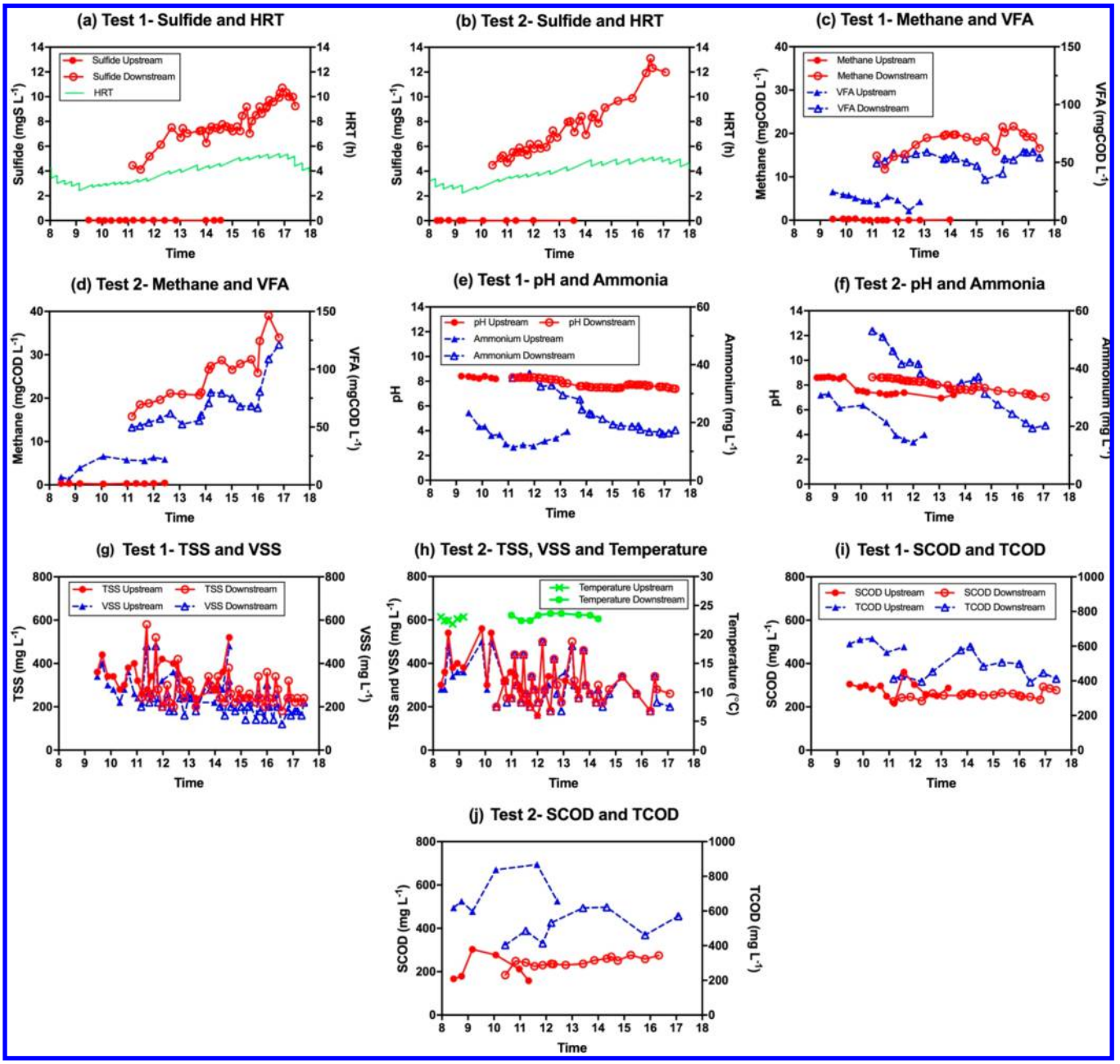

Figure 2. Profiles of HRT, sulfide, methane, VFA, ammonia, pH, TSS and VSS, temperature, and SCOD and TCOD in the UC9 pressure sewer from the upstream pump station to the 828 m downstream sampling point over time during the field study (Tests 1 and 2).

(namely the pump-off period) varied in the range of 15 to 37 min during the whole study. For the wastewater slugs containing the spiked biomarkers, HRTs ranged from 3.6 to 5.4 h, which was the typical diurnal HRTs for sewer system.[15,19,24,35] Flow tracer rhodamine and acesulfame reflected the plug-flow nature of the UC9 sewer pipe, which were in good agreement with the HRT profile (Figure 3). In addition, both rhodamine (88 ± 13%) and acesulfame (96 ± 12%) showed good recoveries from the wet well to the sampling point (Figure S6), suggesting that neither infiltration/exfiltration nor sampling error had the major contribution to uncertainty in this study.

Biological activities were measured through monitoring changes in wastewater compositions, including sulfate, methane, VFAs, pH, ammonia, TSS and VSS, temperature, and TCOD and SCOD (Figure 2). Sulfide and methane were produced in the anaerobic condition of the UC9 pressure sewer over HRTs (Figure S7). Sulfide production rate was similar in Test 1 and Test 2 as 1.67 ± 0.21 gS m^{-2} d^{-1}. The methane production rate was measured as 3.88 ± 0.38 gCOD m^{-2} d^{-1} in Test 1, while it was elevated to 5.32 ± 0.38 gCOD m^{-2} d^{-1} in Test 2 likely due to the higher VFAs concentration as the preferable substrates for methanogens. Wastewater pH slightly declined with time as a response to fermentation.[36] TSS (305 ± 89 mg L^{-1}) and VSS (270 ± 90 mg L^{-1}) presented variations in the range of 160−580 mg L^{-1} over the experimental period. Wastewater temperature kept stable at 22.9 ± 0.5 °C in the sewer pipe between the wet well and the sampling point. TCOD was consumed over HRT, while SCOD had similar levels from upstream (261 ± 53 mg L^{-1}) to downstream (250 ± 20 mg L^{-1}), the consumption of which was actually compensated by the hydrolyzable substrates and fermentation

DOI: 10.1021/acs.est.7b05109
Environ. Sci. Technol. 2018, 52, 1561−1570

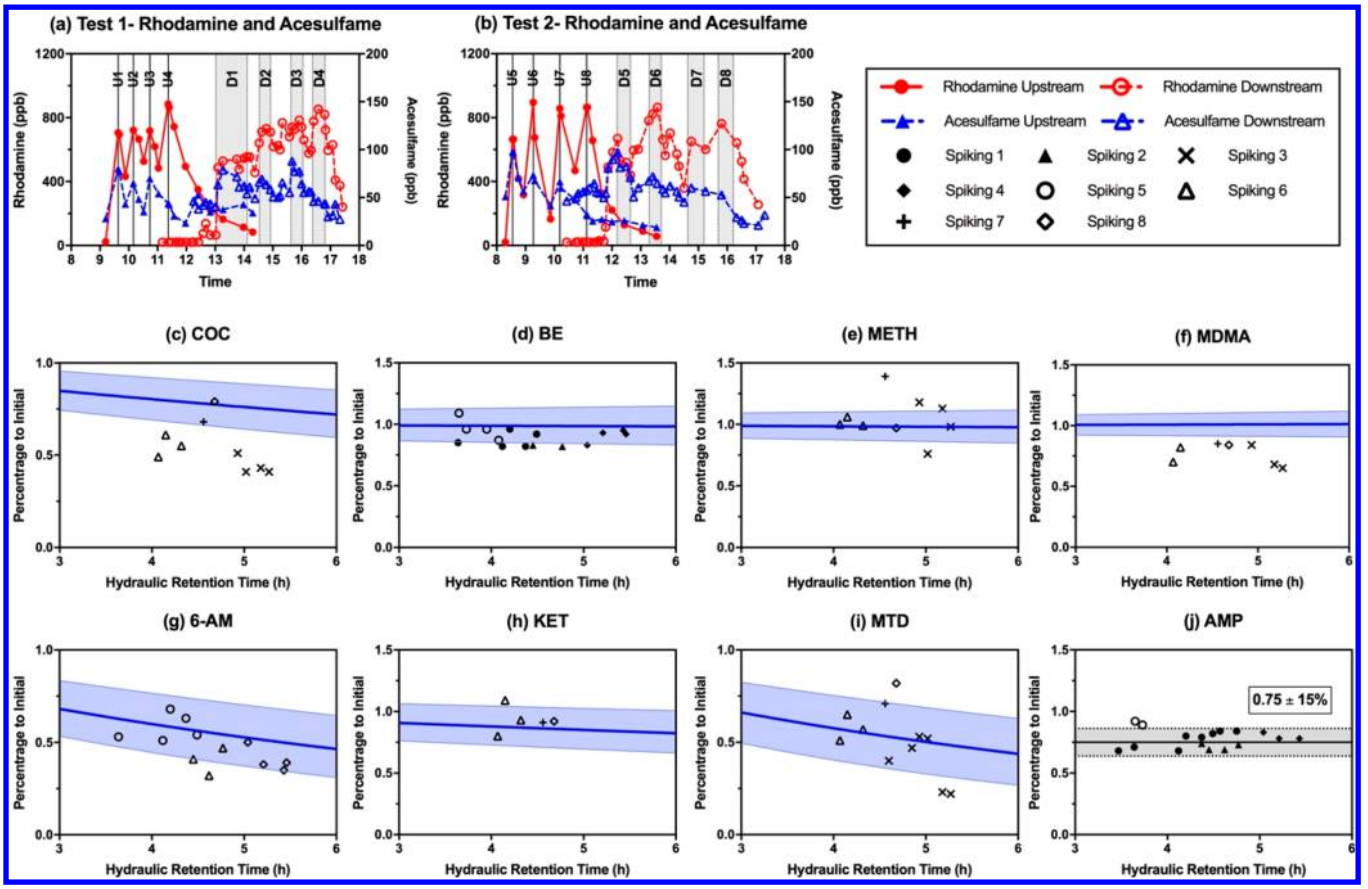

Figure 3. Transportation of flow tracers in the UC9 sewer over time from the upstream pump station to the downstream 828 m sampling point and transformation of the spiked illicit drug biomarkers over HRTs during the field study. (a, b) The upstream spiking concentrations (U1-4 in Test 1 and U5-8 in Test 2) and the downstream sampling concentrations (D1-4 in Test 1 and D5-8 in Test 2) of rhodamine and acesulfame. The black vertical lines at upstream indicate the activation of the pumping events with compounds spiking. The gray areas at downstream indicate the pump-off period when certain spiked wastewater slug stayed at the sampling point. (c-j) Measured changes of the spiked biomarkers at UC9 (symbol) and the simulated transformation (blue line) with 95% confidence bounds (blue area). For AMP without the lab-based simulation, the straight line indicating 75% transformation with 15% deviations is provided.

process over time. Collectively speaking, the UC9 sewer and the lab-scale sewer reactors had similar conditions in terms of wastewater compositions (Table S8) and biological activities which were also comparable to data from the literature (Table 1).[10,16,37]

Biomarkers Stability in the UC9 Sewer. Transformation of biomarkers in the UC9 sewer over HRTs is presented in Figure 3 and Figure S8, showing different in-sewer stability levels in the real sewer. Background concentration of the spiked biomarkers was very low compared to their spiking concentration (Figure S8).

COC and BE. The spiked COC in parent group tests showed a relatively strong transformation in the UC9 sewer. A clear trend of COC degradation with HRT was observed at a level similar to previous studies, due to the combined effects of hydrolysis and biotransformation.[4,5,7] As a major metabolite of COC, the upstream concentration of BE promptly increased when COC was spiked into the wet well, and subsequently BE peaks appeared in the COC-spiked wastewater slugs at downstream. Statistical analysis revealed the insignificant difference between the BE formation rate and COC degradation rate in wastewater (P = 0.6651, Figure S9a). On the other hand, the metabolite group tests with BE being spiked instead of COC showed the relative stability of BE in the real sewer, with P_{SEWER} of BE being at 90 ± 7% over 3.6−5.4 h. Such high stability of BE was also reported by previous studies.[4,5] This field study thus confirmed that BE can serve as the target biomarker for back-estimating COC consumption when the in-sewer transformation ratio of BE/COC is considered. The transformation ratio of COC-to-BE was determined as 0.38 ± 0.08 according to their measured changes in the UC9 sewer (details in S4.2), which was similar to the typical excretion rates of BE after COC consumption (e.g., 29−45%).[38] Furthermore, the global correction factor (CF_{global}) was calculated by integrating excretion percentage and in-sewer transformation of the target biomarker, which was determined as 25.82 for COC and 3.82 for BE (calculation details in Table S10 and Figure S12). However, the CF_{global} in this study only considered human excretion and in-sewer transformation of COC and BE for back-estimation without taking the direct disposal of COC into account and could only be applied to other sewer systems with similar hydraulic and bioactivity conditions.

Amphetamine-like Stimulant: METH, AMP, and MDMA. METH presented high stability in the UC9 sewer with P_{SEWER} = 105 ± 16% within 6 h, which is comparable to its reported highly stable characteristic in wastewater.[5] Different than the reported high stability,[5] MDMA showed certain degradation with P_{SEWER} = 77 ± 8% in the real sewer. However, the P_{SEWER} of MDMA kept consistent for the experimental HRT between

DOI: 10.1021/acs.est.7b05109
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4 and 5.5 h. Thus, this diminished stability of MDMA might suffer from other defects such as analytical uncertainty. For the spiking tests with AMP as a metabolite, a medium degradation with P_{SEWER} = 78 ± 7% was measured in the UC9 sewer, which agreed well with its reported medium stability.[4]

KET. KET had relatively good stability in the UC9 sewer with P_{SEWER} = 93 ± 9% over 5 h. The transformation behavior of KET in the real sewer agreed with its reported stability in lab-scale sewer conditions.[4]

Opioids: 6-AM, MOR, CODE, MTD, and EDDP. The spiked 6-AM exhibited substantial degradation in the UC9 sewer with a half-life around 4 h, which agreed well with its reported rapid decrease in the anaerobic wastewater with biofilms.[4,5,7] As a major metabolite of 6-AM, the behavior of MOR (nonspiked) in the real sewer was also assessed. MOR formation was observed during sewer transportation, which was higher than the concomitant loss of the spiking 6-AM (Figures S9 and S10). Therefore, MOR formation could be derived from the degradation of parent compounds such as 6-AM and heroin and from the deconjugation of MOR-glucuronide by fecal bacteria in wastewater. This investigation of MOR in the real sewer suggested that, when used as the target biomarker for back-estimating heroin consumption, the manifold sources of MOR need to be considered.

MTD was considerably degraded with approximately 60% loss over 5 h in the UC9 sewer. It was reported that the instability of MTD was mainly attributed to its high sorption tendency,[4,6,7] which could be influenced by the TSS in wastewater and the in-pipe hydraulic mixing condition. In addition, the transformation by sewer biofilms also contributed to the substantial in-sewer degradation of MTD. As the human metabolite of MTD, the behavior of EDDP (nonspiked) was also investigated in the UC9 sewer, where significant formation of EDDP was observed during sewer transportation (Figure S10).

CODE (nonspiked) was found to increase during sewer transportation in UC9 (Figure S10). Since CODE can be excreted as the conjugated form into sewer system,[3,9] the elevated CODE concentrations could be due to the deconjugation process in wastewater.

Application of Laboratory Kinetic Model to the Field Data. For biomarkers investigated in the field study, their stability indicators (P_{SIM} and $t_{1/2}$) were estimated using the transformation coefficients (k_{ww} and k'_{bio}) determined in the lab-scale study, the HRT as well as the A/V ratio of the UC9 sewer (Table 2). The measured stability P_{SEWER} of most biomarkers, including BE, METH, 6-AM, KET, and MTD, fall within the 95% confidence bounds of predictions (Figure 3). These compounds cover a range of stability as measured in sewer reactors. Results thus suggest that the lab-scale stability of BE, METH, 6-AM, KET, and MTD is successfully validated and could be applied to WBE studies in real sewers.

However, relatively significant deviations between the simulated and the measured transformations in the real sewer were found for COC and MDMA (Figure 3 (c, f)). The measured P_{SEWER} of COC was higher than its P_{SIM}, indicating stronger degradation of COC in the real sewer. This was also reflected by the $t_{1/2}$ value, which was around 6 h for the UC9 sewer in contrast to the estimated value of 12.7 h (Table 2). The lab-based kinetic model also underpredicted the transformation of MDMA in the UC9 sewer. Similarly, certain degradation of MDMA in real sewers was reported in a previous study as well, where >20% MDMA loss was observed in 12 h after spiking.[4] This was explained by the different transformation potentials of divergent biofilms, especially the biofilm with prominent microbial diversities.[4] Thus, the stability discrepancy observed in this study could also be relative to the more diverse microbial communities in the UC9 sewer than in the lab-scale sewer reactors.

For COC and MDMA which could not be well predicted by the kinetic model, the uncertainty and sensitivity analyses further evaluated proportions of the variabilities derived from modeling transformation coefficients (k_{ww} and k'_{bio}) and chemical analysis (C_0) to the overall uncertainty of their estimated transformation in the UC9 sewer. Compared to the variability from modeling, the variability associated with $U_{analysis}$ had the major contribution (i.e., 65.3% for COC and 92.6% for MDMA) to their overall estimation uncertainty over 6 h HRT (Table S7). Results thus imply that the possible analytical inaccuracy could also lead to the less fit between the measured and the simulated transformations of COC and MDMA in this study.

The current WBE is based on the biomarker concentration at the inlet of WWTP, which is located at the end of a whole sewer system. This work confirmed that transformations of some illicit drug biomarkers in real sewer pipes can cause significant changes to their concentrations. More importantly, this study revealed that the degradation of some biomarkers in real sewers can be reliably estimated using the kinetics determined based on lab-scale experiments. In general, although the models for some biomarkers require improvement, this work demonstrates that modeling could be an important tool to bridge the lab-scale stability studies, which are much easier to conduct than the actual field measurements, to the real-life WBE applications.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.7b05109.

Additional information about the analytical methods of wastewater samples, modeling development and simulations, experimental setup and results of the lab-scale sewer reactor batch tests, experimental protocol, and supplementary data analyses of the field study (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*Phone: +61 7 3346 3219. Fax: +61 7 3365 4726. E-mail: g.jiang@uq.edu.au.

ORCID

Jiaying Li: 0000-0002-2132-3330
Phong K. Thai: 0000-0003-0042-3057
Guangming Jiang: 0000-0001-5399-8239

Present Address

‖Center for Microbial Ecology and Technology (CMET), Ghent University, Coupure Links 653, 9000 Gent, Belgium.

Author Contributions

J.L. and J.G. contributed equally.

Notes

The authors declare no competing financial interest.

DOI: 10.1021/acs.est.7b05109
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■ ACKNOWLEDGMENTS

This research was supported by the ARC Discovery project (DP150100645). J.L. acknowledges the scholarship support from China Scholarship Council. J.G. received an ARC scholarship and a UQI scholarship. P.K.T. is funded by the QUT VC Fellowship. G.J. is the recipient of an Australian Research Council DECRA Fellowship (DE170100694). We acknowledge the collaboration with Gold Coast Water for the field measurement campaign and the assistance of Ian Johnson and Lisa Bethke during the field study.

■ REFERENCES

(1) Daughton, C. G. Pharmaceuticals and Personal Care Products in the Environment. *Overarching Issues and Overview* **2001**, *791*, 2−38.
(2) Thai, P. K.; Lai, F. Y.; Bruno, R.; van Dyken, E.; Hall, W.; O'Brien, J.; Prichard, J.; Mueller, J. F. Refining the excretion factors of methadone and codeine for wastewater analysis - Combining data from pharmacokinetic and wastewater studies. *Environ. Int.* **2016**, *94*, 307−314.
(3) McCall, A. K.; Bade, R.; Kinyua, J.; Lai, F. Y.; Thai, P. K.; Covaci, A.; Bijlsma, L.; van Nuijs, A. L.; Ort, C. Critical review on the stability of illicit drugs in sewers and wastewater samples. *Water Res.* **2016**, *88*, 933−47.
(4) McCall, A. K.; Scheidegger, A.; Madry, M. M.; Steuer, A. E.; Weissbrodt, D. G.; Vanrolleghem, P. A.; Kraemer, T.; Morgenroth, E.; Ort, C. Influence of Different Sewer Biofilms on Transformation Rates of Drugs. *Environ. Sci. Technol.* **2016**, *50* (24), 13351−13360.
(5) Thai, P. K.; Jiang, G.; Gernjak, W.; Yuan, Z.; Lai, F. Y.; Mueller, J. F. Effects of sewer conditions on the degradation of selected illicit drug residues in wastewater. *Water Res.* **2014**, *48*, 538−47.
(6) Ramin, P.; Libonati Brock, A.; Polesel, F.; Causanilles, A.; Emke, E.; de Voogt, P.; Plosz, B. G. Transformation and sorption of illicit drug biomarkers in sewer systems: understanding the role of suspended solids in raw wastewater. *Environ. Sci. Technol.* **2016**, *50* (24), 13397−13408.
(7) Ramin, P.; Brock, A. L.; Causanilles, A.; Valverde Perez, B.; Emke, E.; de Voogt, P.; Polesel, F.; Plosz, B. G. Transformation and sorption of illicit drug biomarkers in sewer biofilms. *Environ. Sci. Technol.* **2017**, *51* (18), 10572−10584.
(8) O'Brien, J. W.; Banks, A. P. W.; Novic, A. J.; Mueller, J. F.; Jiang, G.; Ort, C.; Eaglesham, G.; Yuan, Z.; Thai, P. K. Impact of in-Sewer Degradation of Pharmaceutical and Personal Care Products (PPCPs) Population Markers on a Population Model. *Environ. Sci. Technol.* **2017**, *51* (7), 3816−3823.
(9) Gao, J.; Banks, A.; Li, J.; Jiang, G.; Lai, F. Y.; Mueller, J. F.; Thai, P. K. Evaluation of in-sewer transformation of selected illicit drugs and pharmaceutical biomarkers. *Sci. Total Environ.* **2017**, *609*, 1172−1181.
(10) Hvitved-Jacobsen, T.; Vollertsen, J.; Nielsen, A. H. *Sewer Processes: Microbial and Chemical Process Engineering of Sewer Networks*, 2nd ed.; CRC Press: Boca Raton, 2013.
(11) Gonzalez-Gil, L.; Carballa, M.; Lema, J. M. Cometabolic Enzymatic Transformation of Organic Micropollutants under Methanogenic Conditions. *Environ. Sci. Technol.* **2017**, *51* (5), 2963−2971.
(12) Mohanakrishnan, J.; Sharma, K. R.; Meyer, R. L.; Hamilton, G.; Keller, J.; Yuan, Z. Variation in Biofilm Structure and Activity Along the Length of a Rising Main Sewer. *Water Environ. Res.* **2009**, *81* (8), 800−808.
(13) Gutierrez, O.; Mohanakrishnan, J.; Sharma, K. R.; Meyer, R. L.; Keller, J.; Yuan, Z. Evaluation of oxygen injection as a means of controlling sulfide production in a sewer system. *Water Res.* **2008**, *42* (17), 4549−4561.
(14) Nielsen, P. H.; Raunkjaer, K.; Norsker, N. H.; Jensen, N. A.; Hvitved-Jacobsen, T. Transformation of Wastewater in Sewer Systems − A Review. *Water Sci. Technol.* **1992**, *25* (6), 17−31.
(15) Sharma, K. R.; Yuan, Z.; de Haas, D.; Hamilton, G.; Corrie, S.; Keller, J. Dynamics and dynamic modelling of H(2)S production in sewer systems. *Water Res.* **2008**, *42* (10), 2527−2538.
(16) Foley, J.; Yuan, Z.; Lant, P. Dissolved methane in rising main sewer systems: field measurements and simple model development for estimating greenhouse gas emissions. *Water Sci. Technol.* **2009**, *60* (11), 2963−2971.
(17) Guisasola, A.; de Haas, D.; Keller, J.; Yuan, Z. Methane formation in sewer systems. *Water Res.* **2008**, *42* (6), 1421−1430.
(18) Tanaka, N.; Hvitved-Jacobsen, T. Anaerobic transformations of wastewater organic matter and sulfide production - investigations in a pilot plant pressure sewer. *Water Sci. Technol.* **2002**, *45* (3), 71−79.
(19) Kapo, K. E.; Paschka, M.; Vamshi, R.; Sebasky, M.; McDonough, K. Estimation of U.S. sewer residence time distributions for national-scale risk assessment of down-the-drain chemicals. *Sci. Total Environ.* **2017**, *603-604*, 445−452.
(20) Devault, D. A.; Levi, Y.; Karolak, S. Applying sewage epidemiology approach to estimate illicit drug consumption in a tropical context: Bias related to sewage temperature and pH. *Sci. Total Environ.* **2017**, *584-585*, 252−258.
(21) Bisceglia, K. J.; Lippa, K. A. Stability of cocaine and its metabolites in municipal wastewater−the case for using metabolite consolidation to monitor cocaine utilization. *Environ. Sci. Pollut. Res.* **2014**, *21* (6), 4453−4460.
(22) Plosz, B. G.; Reid, M. J.; Borup, M.; Langford, K. H.; Thomas, K. V. Biotransformation kinetics and sorption of cocaine and its metabolites and the factors influencing their estimation in wastewater. *Water Res.* **2013**, *47* (7), 2129−2140.
(23) Jelic, A.; Rodriguez-Mozaz, S.; Barcelo, D.; Gutierrez, O. Impact of in-sewer transformation on 43 pharmaceuticals in a pressurized sewer under anaerobic conditions. *Water Res.* **2015**, *68*, 98−108.
(24) McCall, A. K.; Palmitessa, R.; Blumensaat, F.; Morgenroth, E.; Ort, C. Modeling in-sewer transformations at catchment scale − Implications on drug consumption estimates in wastewater-based epidemiology. *Water Res.* **2017**, *122*, 655−668.
(25) Buerge, I. J.; Buser, H. R.; Kahle, M.; Muller, M. D.; Poiger, T. Ubiquitous Occurrence of the Artificial Sweetener Acesulfame in the Aquatic Environment: An Ideal Chemical Marker of Domestic Wastewater in Groundwater. *Environ. Sci. Technol.* **2009**, *43* (12), 4381−4385.
(26) Jiang, G.; Sharma, K. R.; Guisasola, A.; Keller, J.; Yuan, Z. Sulfur transformation in rising main sewers receiving nitrate dosage. *Water Res.* **2009**, *43* (17), 4430−4440.
(27) Gutierrez, O.; Sudarjanto, G.; Ren, G.; Ganigue, R.; Jiang, G.; Yuan, Z. Assessment of pH shock as a method for controlling sulfide and methane formation in pressure main sewer systems. *Water Res.* **2014**, *48*, 569−78.
(28) Sutherland-Stacey, L.; Corrie, S.; Neethling, A.; Johnson, I.; Gutierrez, O.; Dexter, R.; Yuan, Z.; Keller, J.; Hamilton, G. Continuous measurement of dissolved sulfide in sewer systems. *Water Sci. Technol.* **2008**, *57* (3), 375−381.
(29) Aymerich, I.; Acuna, V.; Barcelo, D.; Garcia, M. J. J.; Petrovic, M.; Poch, M.; Rodriguez-Mozaz, S.; Rodriguez-Roda, I.; Sabater, S.; von Schiller, D.; Corominas, L. Attenuation of pharmaceuticals and their transformation products in a wastewater treatment plant and its receiving river ecosystem. *Water Res.* **2016**, *100*, 126−136.
(30) Plosz, B. G.; Leknes, H.; Thomas, K. V. Impacts of Competitive Inhibition, Parent Compound Formation and Partitioning Behavior on the Removal of Antibiotics in Municipal Wastewater Treatment. *Environ. Sci. Technol.* **2010**, *44* (2), 734−742.
(31) R-Development-Core-Team. R: a language and environment for statistical computing. www.r-project.org (accessed Jan 9, 2017).
(32) Spiegelhalter, D.; Thomas, A.; Best, N.; Lunn, D. *OpenBUGS user manual*, version 3.0.2; www.openbugs.net (accessed Jan 9, 2017).
(33) Jiang, G.; Yuan, Z. Inactivation kinetics of anaerobic wastewater biofilms by free nitrous acid. *Appl. Microbiol. Biotechnol.* **2014**, *98* (3), 1367−1376.
(34) Barber, L. B.; Keefe, S. H.; Brown, G. K.; Furlong, E. T.; Gray, J. L.; Kolpin, D. W.; Meyer, M. T.; Sandstrom, M. W.; Zaugg, S. D.

DOI: 10.1021/acs.est.7b05109
Environ. Sci. Technol. 2018, 52, 1561−1570

Persistence and Potential Effects of Complex Organic Contaminant Mixtures in Wastewater-Impacted Streams. *Environ. Sci. Technol.* **2013**, *47* (5), 2177−2188.
(35) Ort, C.; van Nuijs, A. L. N.; Berset, J. D.; Bijlsma, L.; Castiglioni, S.; Covaci, A.; de Voogt, P.; Emke, E.; Fatta-Kassinos, D.; Griffiths, P.; Hernandez, F.; Gonzalez-Marino, I.; Grabic, R.; Kasprzyk-Hordern, B.; Mastroianni, N.; Meierjohann, A.; Nefau, T.; Ostman, M.; Pico, Y.; Racamonde, I.; Reid, M.; Slobodnik, J.; Terzic, S.; Thomaidis, N.; Thomas, K. V. Spatial differences and temporal changes in illicit drug use in Europe quantified by wastewater analysis. *Addiction.* **2014**, *109* (8), 1338−1352.
(36) Gutierrez, O.; Park, D.; Sharma, K. R.; Yuan, Z. Effects of long-term pH elevation on the sulfate-reducing and methanogenic activities of anaerobic sewer biofilms. *Water Res.* **2009**, *43* (9), 2549−2557.
(37) Foley, J.; Yuan, Z.; Keller, J.; Senante, E.; Chandran, K.; Willis, J.; Shah, A.; van Loosdrecht, M.; van Voorthuizen, E. *N2O and CH4 emission from wastewater collection and treatment systems: technical report*; Global Water Research Coalition: London, United Kingdom, 2011.
(38) Gracia-Lor, E.; Zuccato, E.; Castiglioni, S. Refining correction factors for back-calculation of illicit drug use. *Sci. Total Environ.* **2016**, *573*, 1648−1659.

DOI: 10.1021/acs.est.7b05109
Environ. Sci. Technol. 2018, 52, 1561−1570