Effects of sewer conditions on the degradation of selected illicit drug residues in wastewater

Phong K. Thai[a *], Guangming Jiang[b], Wolfgang Gernjak[b], Zhiguo Yuan[b], Foon Yin Lai[a], and Jochen F. Mueller[a]

[a]*The University of Queensland, The National Research Centre for Environmental Toxicology (Entox), 39 Kessels Rd., Coopers Plains QLD 4108, Australia*

[b]*The University of Queensland, Advanced Water Management Centre, St Lucia, QLD 4072, Australia*

* Corresponding Author. Tel.: +61 (07) 3274 9060. Fax: +61 (07) 3274 9003, E-mail address: p.thai@uq.edu.au

ABSTRACT

The stability of five illicit drug markers in wastewater was tested under different sewer conditions using laboratory-scale sewer reactors. Wastewater was spiked with deuterium labelled isotopes of cocaine, benzoyl ecgonine, methamphetamine, MDMA and 6-acetyl morphine to avoid interference from the native isotopes already present in the wastewater matrix. The sewer reactors were operated at 20^{o}C and pH 7.5, and wastewater was sampled at 0, 0.25, 0.5, 1, 2, 3, 6, 9 and 12 hours to measure the transformation/degradation of these marker compounds. The results showed that while methamphetamine, MDMA and benzoyl ecgonine were stable in the sewer reactors, cocaine and 6-acetyl morphine degraded quickly. Their degradation rates are significantly higher than the values reportedly measured in wastewater alone (without biofilms). All the degradation processes followed first order kinetics. Benzoyl ecgonine and morphine were also formed from the degradation of cocaine and 6-acetyl morphine, respectively, with stable formation rates throughout the test. These findings suggest that, in sewage epidemiology, it is essential to have relevant information of the sewer system (i.e. type of sewer, hydraulic retention time) in order to accurately back-estimate the consumption of illicit drugs. More research is required to look into detailed sewer conditions (e.g. temperature, pH and ratio of biofilm area to wastewater volume among others) to identify their effects on the fate of illicit drug markers in sewer systems.

Key Words: illicit drugs, biofilms, sewage epidemiology, transformation kinetics

Nomenclature

6-AM	6-acetyl morphine
A/V ratio	ratio of area of biofilms vs volume of wastewater in the sewer or sewer reactor
COC	cocaine
BE	benzoyl ecgonine

MA	methamphetamine
MDMA	3,4-methylenedioxy-N-methylamphetamine
LCMSMS	Liquid chromatography-tandem mass spectrometry

1. INTRODUCTION

After being consumed, illicit drugs are excreted and then transported from individual toilets to the sewage treatment plants via the sewer system. A decade ago, Daughton (2001) proposed that measuring loads of illicit drug residues in wastewater can be used as a tool to back-estimate the consumption of the corresponding illicit drugs in the communities. This approach, generally named as sewage epidemiology and first applied by Zuccato et al. (2005) to estimate the consumption of cocaine in different Italian communities, has now been used worldwide to estimate the consumption of a range of illicit drugs (Banta-Green et al. 2009, Daughton 2011, Thomas et al. 2012, Irvine et al. 2011, van Nuijs et al. 2011).

The accuracy of this sewage epidemiology approach may be compromised by a range of uncertainties related to aspects such as wastewater sampling, sample storage, and analytical methods for illicit drugs (Lai et al. 2011, van Nuijs et al. 2011, Zuccato et al. 2008, Castiglioni et al. 2013). Some of these recognised issues have been addressed through technical improvement related to sampling (e.g. through the use flow proportional sampling under controlled temperature) and measurement methods of target drugs (e.g. with the use of highly sensitive methods). However, one major limitation of the sewage epidemiology approach that is yet to be fully understood and addressed concerns the transformation/degradation of illicit drug residues in the sewer system and during storage (Lai et al. 2011, van Nuijs et al. 2011, Zuccato et al. 2008, Castiglioni et al. 2013).

To address this problem, there have been studies on transformation/degradation of illicit drug residues in wastewater with an initial focus on the stability of illicit drug residues during sample storage (i.e. low temperature and long period) (Castiglioni et al. 2006, González-Mariño et al. 2010, Castiglioni et al. 2011, Gheorghe et al. 2008, Chiaia et al. 2008). Recently, some stability studies have started to evaluate the fate of illicit drug residues in wastewater

65 under ambient condition (i.e. pH 7 – 7.5, 20 ^{o}C) (van Nuijs et al. 2012, Bisceglia 2010, Chen
66 et al. 2012, Plósz et al. 2013). However, those former studies only used freshly collected
67 wastewater in glass containers as the test environment. It means that the effects of biofilms in
68 the sewer system, which contain more biologically active organism than the wastewater, to
69 the stability of the illicit drug residues have not been considered.
70 A sewer system that collects and transports wastewater from residential and commercial areas
71 typically consists of rising sewer mains and gravity sewer. Rising sewer mains normally start
72 with a pump station to lift wastewater from low to high altitude. In contrast, gravity sewer, as
73 its name indicates, use gravity to transport wastewater from high to low altitude. A sewer
74 system usually requires both types of sewers but the ratio between those two sewer types is
75 dependent on the unevenness of the land in the catchment area.
76 Rising sewer mains are generally fully filled with wastewater and anaerobic biofilms
77 dominate on the pipe walls. In comparison, gravity sewer is only partially filled with sewage,
78 which may sustain both aerobic and anaerobic biofilms/sediments (Hvitved-Jacobsen 2002).
79 Since biofilms are rich in microorganisms, which are capable of transforming/degrading
80 various chemical compounds, it is hypothesized that illicit drug residues can also be
81 transformed biologically in sewers by microbes residing in biofilms. This hypothesis leads to
82 the speculation that actual sewer conditions can have different impact on the transformation
83 of illicit drug residues than wastewater alone due to the presence of different microbial
84 populations in biofilms/sediments. Indeed, it has been revealed previously that sewer biofilms
85 makes a substantially higher contribution to sulfide production compared to suspended
86 microorganisms in wastewater (Mohanakrishnan et al. 2009, Gutierrez et al. 2008). Moreover,
87 redox condition of the sewer, i.e. aerobic or anaerobic, can also influence biological
88 transformation processes of chemicals, which can also contribute to the overall

89 transformation of illicit drug residues. It is thus necessary to study the fate of illicit drug
90 residues under different sewer conditions in the presence of sewer biofilms.
91 Also, none of the stability studies mentioned above have directly assessed the transformation
92 of parent drugs to their metabolites, e.g. from cocaine to benzoyl ecgonine or from
93 methamphetamine to amphetamine, because of the interference of benzoyl ecgonine or
94 amphetamine already present in wastewater used in those studies. Since those metabolites, i.e.
95 benzoyl ecgonine and amphetamine, are themselves used as illicit drug residues in sewage
96 epidemiology, their formation during the residence time in the sewer system should be
97 evaluated.
98 This study investigated the transformation/degradation of some popular illicit drug residues
99 in laboratory-scale sewer reactors, either under rising main or gravity sewer conditions. A
100 control reactor without biofilms was also employed to determine the transformation in
101 wastewater alone. The selected illicit drug residues include cocaine (COC), its metabolite
102 benzoyl ecgonine (BE), methamphetamine (MA), MDMA and a metabolite of heroin, 6-
103 acetyl morphine (6-AM). These illicit drug residues are usually used to estimate the
104 consumption of COC, MA, MDMA and heroin, respectively, in sewage epidemiology. Batch
105 tests were carried out using different sewer reactors spiked with the selected illicit drug
106 markers. The use of deuterium labelled isotope compounds in this study helped determining
107 the direct transformations between related illicit drug residues, which were not evaluated
108 before. Concentrations of illicit drug residues were monitored at different time points during
109 a period of 12 hours after being spiked into the sewer reactors. The results obtained will help
110 to clarify the impact of sewer conditions including sewer biota and redox conditions to the
111 fate of illicit drug residues during their transport in the sewer system.
112

2. MATERIALS AND METHODS

2.1 Chemicals and reagents

Deuterium labelled COC, BE, MA, MDMA and 6-AM were monitored instead of the native compounds in order to trace their exclusive deuterium labelled degradation products (Table 1). All the deuterium labelled standards (COC-d3, BE-d3, MA-d8, MDMA-d5, 6-AM-d6) were purchased from Cerilliant (Texas, US). Working solutions of each deuterium labelled standards were prepared at a concentration of 5000 μg/L in methanol. All working solutions were stored at –20 °C until use. LCMS grade solvents (methanol, acetonitrile) were purchased from Merck, Germany. Deionised water was produced by a MilliQ system (Millipore, 0.22 μm filter, 18.2 mΩ cm^{-1}).

2.2 Laboratory-scale sewer reactors

The experiment was carried out using laboratory-scale sewer reactors, which have previously demonstrated to mimic the typical sewer conditions (Mohanakrishnan et al. 2009, Jiang et al. 2009, Guisasola et al. 2008, Jiang et al. 2011a). Three reactors were employed, namely a rising main (RM) sewer reactor, a gravity (GS) sewer reactor and a control (CR) sewer reactor without biofilms.

The air-tight rising main (RM) reactor was made of Perspex™ with a volume of 0.75 L (diameter of 80 mm and a height of 149 mm) (Jiang et al. 2010). Plastic carriers (Anox Kaldnes, Norway) of 1-cm diameter were clustered on four stainless-steel rods inside the reactor to provide additional surfaces for biofilm growth (Figure 1). The total volume of the carriers used for each reactor was about 15 mL (2% of the reactor volume). The total surface area on the reactor walls and carriers supporting biofiom growth is estimated to be 0.05 m^2. The gravity section (GS) reactor had the same dimensions but was only partially filled with

136 real wastewater, allowing a gas phase. This gas phase had free air exchange to the
137 atmosphere. A mixture of aerobic and anaerobic biofilm had been previously developed in
138 the GS reactor. The control reactor (CR) is identical to the GS reactor except that no biofilm
139 is allowed to develop through regular wall cleaning. Thus, CR reactor is basically a container
140 of wastewater similar to that used in other stability studies (Chen et al. 2012, Plósz et al. 2013,
141 van Nuijs et al. 2012).
142 Sewer biofilms in RM and GS reactors have been cultivated for 12 months using real
143 wastewater before the experiments. Domestic wastewater, collected weekly from a local
144 pumping station in Brisbane (Australia) was used as the feed. The sewage (pH around 7.5)
145 typically contained sulfide at concentrations of <3 mg-S/L, sulfate at 10-25 mg-S/L, total
146 COD and soluble COD at 450-600 mg/L and 260-450 mg/L, respectively, with the latter
147 including volatile fatty acids at 50-120 mg-COD/L. The sewage was stored at 4 °C and
148 heated up to 20 °C before being pumped into the reactors. The reactors were fed with sewage
149 through a peristaltic pump (Masterflex 7520-47) every 6 hours, a typical sewage hydraulic
150 retention time in sewers (Hvitved-Jacobsen 2002). Every feed pumping event lasted for 2
151 minutes, delivering one reactor volume (0.75 L) of sewage into each reactor. To ensure
152 homogeneous distribution in reactors, gentle mixing was provided with magnetic stirrers at
153 250 rpm (Heidolph MR3000).
154 Prior to the degradation experiments described in 2.3, batch tests were conducted to
155 determine biofilm activities, i.e. sulfate reduction and methanogenesis. The batch tests were
156 performed three times to confirm reactors were in semi-steady state, indicating by stable
157 sulfide production. The sulfate-reducing activity was measured as sulfide production rate, and
158 the methanogenic activity was measured as the methane production rate.
159 The batch tests were started with pumping fresh sewage into reactors, which lasted for 10
160 minutes to ensure a thorough replacement of liquid in reactors with fresh sewage. Wastewater

samples were taken at 0, 20, 40, and 60 minutes after feeding, for the analysis of dissolved inorganic sulfur and methane (procedures described above). Sulfide and methane production rates were calculated using linear regression of sulfide, and methane concentrations, respectively.

2.3 Batch tests for the transformation of illicit drug residues in sewer reactors

Two batch tests were conducted on each of the three reactors. The first batch test used COC-d3, MA-d8 and 6-AM-d6 while the second one used BE-d3 and MDMA-d8 in the spiking solution (Table 1). Three replicates were performed for each batch test.

For each replicate of the batch test, 10 L of fresh wastewater was heated up to 20^{o}C and its pH was adjusted to 7.5 using 1M NaOH and 1M HCl solution. The temperature and pH were selected to be comparable with OECD guideline No. 314 (OECD, 2008) and with other studies on the stability of illicit drug residues in wastewater mentioned previously. The temperature- and pH-adjusted wastewater was then pumped into the RM and GS reactors through a peristaltic pump (Masterflex 7520-47). This ensured that the liquid in each reactor was replenished with fresh sewage. The CR reactor was manually filled with fresh sewage from the top. Background samples were taken from all reactors after filling to measure the presence of illicit drug residues before spiking.

Working solutions of selected deuterium-labelled illicit drug and metabolites were prepared as described in section 2.1. The working solution was spiked into each of the three reactors to achieve initial concentrations of about 10 ng/mL in the wastewater. This relatively high initial concentration was used so that the concentration of illicit drug residues including the transformation products in the samples could be measured by the direct injection LCMSMS method described in section 2.5. This practice is similar to other studies on the degradation of

COC and BE (Castiglioni et al. 2011, Bijlsma et al. 2013) and is thought to not affect the kinetics of degradation of illicit drugs.

Continuous mixing was maintained for each reactor with magnetic stirrers at 250 rpm (Heidolph MR3000) during all the batch tests. The mixing enhanced surface aeration, producing aerobic/anaerobic condition in the GS reactor and aerobic condition in the CR (DO around 0.5 mg/L). Wastewater samples were then taken at time 0, 15, 30 min, 1, 2, 3, 6, 9 and 12 h after spiking the reactors. For each sample, aliquots of 1 mL were immediately filtered into 2-mL vials using 0.45 μm syringe filter (Phenomenex, Australia). Six μl of 2M HCl was added to each vial to adjust pH of the samples to around 2. The acidified samples were then frozen at – 20°C until analysis.

Wastewater samples were also taken, at time intervals of 0, 30 min, 1, 3, 6 and 12 h, to evaluate the biological activity of the reactors, with the sulfide and methane production rates as indicators. For the analyses of dissolved inorganic sulfide, 1.5 mL wastewater was filtered (0.22 μm membrane) into 0.5 mL preserving solution of sulfide anti-oxidant buffer (SAOB) (Jiang et al. 2010, Keller-Lehmann et al. 2006). For dissolved methane analysis, 5 mL sewage was filtered (0.22 μm membrane) and injected into vacuumed BD vacutainer® tubes using a hypodermic needle attached to a plastic syringe (Guisasola et al. 2008).

2.4 Chemical analysis of illicit drug residues, sulfide and methane in wastewater samples.

A chromatographic method originally developed and validated by Lai *et al.* (2011) was modified to analyse deuterium-labelled compounds in wastewater. Samples were injected into an LCMS system comprising of a Shimadzu Prominence UFLC system (Kyoto, Japan) connected to an ABSciex 5500QTRAP mass spectrometer, with a TurboIonSpray® source (ABSciex, Concord, Ontaria, Canada). The UFLC system consists of a Shimadzu LC-20AB

209 high-pressure pump, a SIL-20AHT autosampler and a CTO-20A column oven. An in-line
210 degasser (DGU-20A3) was placed prior to the solvent delivery system. Identification and
211 quantification of the target chemicals are performed with the mass spectrometer. The
212 acquisition is operated under multiple reaction monitoring (MRM) in positive ESI mode. All
213 data were collected using ABSciex Analyst software (version 1.5). Quantitation was
214 performed using MultiQuant version 2.1 software (ABSciex).
215 Since native illicit drugs cannot be used as internal standards due to their likely presence in
216 the sample matrix, external calibration curve was used. Six-point calibration curves (0.1, 0.5,
217 1, 5, 10, 50 ng/mL) using deuterium labelled standards were prepared. Solutions of
218 calibration standards are freshly prepared before analysis and analysed three times in each
219 batch of instrumental quantification. Procedural blanks, procedural recoveries and matrix
220 spike recoveries are analyzed in every batch of sample analysis. More information about the
221 quality control measures can be found in the Supplemental Materials.
222 Dissolved sulfide samples were analyzed within 24 h of sampling on an ion chromatograph
223 with an UV and conductivity detector (Dionex ICS-2000). For methane analysis, BD
224 vacutainer tubes were allowed to reach gas-liquid equilibrium overnight. Methane in the gas
225 phase was measured by gas chromatography (Shimadzu GC-9A) equipped with a flame
226 ionization detector. Concentrations of methane in sewage were calculated using mass balance
227 and Henry's law (Guisasola et al., 2008).
228

229 3. RESULTS AND DISCUSSION

230 *3.1 Biological activities in sewer reactors.*

231 The two types of biological sewer reactors, i.e. RM and GS, mimicked the different sewer
232 conditions. A control sewer reactor (CR) without biofilms reproduced the condition used in

previous studies (Bisceglia 2010, Chen et al. 2012, van Nuijs et al. 2012). The RM reactor was kept under anaerobic conditions. The wastewater biofilms attached to the RM reactor wall or plastic carriers looked like a dark green-brownish slime layer, with a depth between 500 to 1000 μm. The measured biomass (as volatile solids) of the biofilm in the whole reactor was 108.3±0.3 g/m^2. It was previously found that the mixed-culture biofilm out-layer was cocci-dominated, while the bottom layer was dominated by long filaments, and rod-shaped bacteria (Jiang and Yuan, 2013). The diversity of the biofilm population include a few species of sulfate-reducing bacteria detected using 16S rRNA-based DGGE (Mohanakrishnan et al., 2009).

The RM reactor provided a suitable habitat for sulfate-reducing bacteria and methanogenic archaea. The activity of sulfate-reducing bacteria and methanogenic archaea in the RM reactor was measured to be 4.3 ± 0.3 mgS/L-h and 18.9 ± 3.2 mg COD/L-h respectively. These biological rates were similar to previously reported values in sewers (Guisasola et al. 2008, Jiang et al. 2011a, Jiang et al. 2011b). In contrast, the GS reactor developed both aerobic and anaerobic microbial communities: the major part near the water surface is aerobic caused by oxygen diffusion through surface aeration. Due to oxygen consumption, biofilms further from the air-water interface are anaerobic. Activity tests in the GS reactor indicated that anaerobic activity is negligible in terms of sulfate reduction (0.17 ± 0.05 mgS/L-h) and methane generation (1.5 ± 0.15 mgCOD/L-h).

Figure 2 shows example profiles of both sulfide and methane for one batch test (described in section 2.3) in the RM, GS and CR reactors. In the RM reactor, both sulfide and methane increase linearly in the first 3 hours, reaching about 10 mg S/L and 55 mg COD/L. These levels were consistent to the measured background activities in the reactor, which implies that added illicit drug compounds had no discernible effects on the anaerobic activities in sewer biofilms. The RM reactor almost transformed all sulfate to sulfide, i.e. 18 mg S/L at the end

of the batch test. Dissolved methane in wastewater was close to saturation in 12 h, which was also observed in real sewers previously (Jiang et al. 2009, Guisasola et al. 2008, Foley et al. 2009).

In comparison to RM reactor, GS reactor showed barely any sulfide production in the whole 12-h batch test. There was about 10 mg COD/L of methane observed, which was then dissipated possibly through water-gas diffusion. Meanwhile, there was a continuous decrease of volatile fatty acids at a rate of about 30 mg COD/L-h (data not shown), indicating significant aerobic activities in the GS reactor. Similarly, no discernible biological activity was detected in the CR reactor (without sewer biofilms). The microorganisms existing originally in wastewater were not capable of conducting detectable sulfate reduction and methanogenesis. The results have shown the abundance of microorganisms, especially in RM reactor, compared to CR reactor, which could influence the fate of illicit drug residues in those reactors.

3.2 Transformation/degradation of illicit drug residues.

Since the illicit drug residues used in this study have low vapour pressure and are hydrophilic, their loss through volatilisation and adsorption during the 12-h test period is assumed to be negligible (Baker et al. 2012). Therefore, all losses of illicit drug residues during the test can be attributed to chemical degradation/transformation processes. Figure 3 shows the profiles of relevant illicit drug residues obtained from the batch tests. The concentrations of the marker compounds during the tests were expressed relative to the initial concentrations of those compounds in each replicate.

COC and BE

COC degrades relatively rapidly in wastewater. After 12 hours, 20% of COC was lost in the CR reactor while up to 60% was lost in the GS reactor. While the extent of loss of COC in the CR reactor is comparable to data reported by other studies (Chen et al. 2012, van Nuijs et

al. 2012, Baker and Kasprzyk-Hordern 2011, Bisceglia et al. 2010), it is clear that the loss in the reactors with biofilms are considerably higher, i.e. 25% and 40% higher for RM and GS reactors, respectively (Table 2). It is reasonable to assume that the microbial activity of the biofilms in the sewer reactors have accelerated the degradation rate of COC compared to that in wastewater alone. It should be noted that Plósz *et al.* (2013) have reported an extreme case where both COC and BE degraded more than 80% in a single replicate experiment about biotransformation of COC and its metabolites in wastewater (Table 2). Those data were considered not comparable with any other studies especially in term of BE rapid degradation in wastewater and hence were not used in our discussion.

As a consequence of COC transformation, BE was produced. Because the deuterium labelled COC was used in this study, the deuterium labelled BE measured in this batch test could be assumed to originate uniquely from the spiked deuterium labelled COC. It is the first time that the use of deuterium labelled chemicals confirmed the production of BE from the COC under sewer conditions.

After 12 hours, the average concentration of BE originated from COC ranges from 8% to 14% of the initial concentration of COC which is comparable with data from other stability studies using wastewater only as the medium (Gheorghe et al. 2008, van Nuijs et al. 2012). In CR reactor, the level of BE formed after 12h (14%) was close to the level of COC lost (19%) indicating that alkyl hydrolysis is the main transformation pathway in this sewer condition. Meanwhile, it is observed that the amounts of BE generated in RM and GS (8% and 14%, respectively) were much lower than the amounts of COC degraded (46% and 58%, respectively) during the same period (Fig 1a, b). Possibly, other transformation pathways have been adopted by sewer biofilms, forming different products that were not monitored in this study including the complete materialisation of COC by biofilm microorganisms.

Separately in the second batch, BE was observed to be a stable compound. Again the use of deuterium labelled BE has helped to ensure that no interference from background BE is present. BE is stable in all sewer conditions, making it a very good marker to measure the consumption of COC. However, care should be taken to take into account the generation of BE from COC available in the samples during the transport and storage as reported in the previous section.

MA and MDMA

Two popular amphetamine-like compounds, MA and MDMA, are relatively stable except for the case of MA in the GS reactor where about 12% of the initial mass was lost after 12 hours. Other studies usually found an increase (or constancy) of MA during storage (González-Mariño et al. 2010, Bisceglia 2010, van Nuijs et al. 2012, Baker and Kasprzyk-Hordern 2011) with only Chen *et al.* (2012) reporting a small reduction of MA (~5%) after 1 day at 20^{o}C. No deuterium labelled amphetamine, which is a transformation product of deuterium labelled MA, was detected in samples spiked with deuterium labelled MA even in the case of 12% loss of MA in the GS reactor.

6-AM

6-AM again demonstrated its susceptibility to degradation. On average, 30% of 6-AM was lost in the CR reactor and the presence of microbial activity of sewer biofilms increased the loss to around 90% after a period of 12 hours. The substantial losses of 6-AM in sewer reactors measured in this study have significantly surpassed all data reported in the literature on this compound. The instability of 6-AM has been attributed to the difficulty of measuring 6-AM in actual samples and therefore the difficulty of measuring heroin use through wastewater analysis.

3.3 Degradation kinetics of studied illicit drug residues

Linear regression (zero order) and pseudo first order regression to identify the degradation kinetics of illicit drug residues were applied to the data obtained from batch tests. Regression intercept was set through the start point, i.e. 100% or 0% at time 0 for marker compounds or the transformation products.

The degradation of COC fits well with the first order kinetics. Data of the regression is presented in Table 2. The half-life of COC in both types of sewer reactors is about 3 times shorter than that of the control. Also, the half-life in GS reactor is shorter than that in RM reactor, suggesting aerobic biofilms might be more active in degrading COC than anaerobic biofilms, but it is important to note that degradation in the RM reactor is still higher than in the CR reactor. Microbial activity in the biofilm of the sewer system has remarkably increased the hydrolysis of COC compared to the suspended microbes in the wastewater alone. The first order degradation rate of COC measured by Bisceglia (2010) is similar to that of the CR while it is much lower than those in GS and RM. It is not possible to compare with data from van Nuijs *et al.* (2012) since they applied the quadratic model to their data, which does not provide the rate or the half-life parameter.

Along with the degradation of COC, the generation of BE as a product of COC's alkyl ester hydrolysis can also be modelled. BE generation is well-fitted with the zero-order kinetics probably because COC, the precursor, is not a limiting factor in this experiment. However, as discussed in the previous section the extent of BE generation did not correspond to the loss of COC in the same reactor, especially in RM and GS reactors. The ratio between the amount of BE generated to the corresponding amount of COC loss among the reactors were also compared and it is interesting to see that this ratio is high in CR reactor while it is similarly low in RM and GS reactors. This result suggested that the activities of biofilm led to more

354 variety of transformed products or complete materialization than the simple one from the
355 alkyl ester hydrolysis.
356 The labile 6-AM quickly degraded following first order kinetics although the variation of
357 concentration in the CR made the fit less plausible (but still better than the zero order
358 kinetics). Most of the 6-AM was transformed to morphine (data not shown), which is
359 relatively stable (Bisceglia 2010, Chen et al. 2012). However, the formation of morphine
360 from 6-AM is less useful in sewer epidemiology since morphine can originate from many
361 licit sources and thus cannot be used as marker to estimate the consumption of heroin. Again,
362 in comparison with other stability studies, the rate of 6-AM degradation found in GS and RM
363 reactors are much higher while that of the CR is comparable. The biofilm has been proved to
364 play an important role in the degradation of illicit drug residues in the sewer system.
365 The application of the kinetic models also helped to confirm the stability of three illicit drug
366 residues, namely BE, MA and MDMA, except for the case of MA in GS reactors, which did
367 not fit well in any regression. Although the stability of MA and MDMA are reported in
368 previous studies, this is the first time BE can be confirmed to be stable on its own using the
369 specific deuterium labelled isotope. No plausible explanation is available for the degradation
370 of MA in the GS reactor since no deuterium labelled amphetamine (MA's metabolite) is
371 recorded.
372 To alleviate potential concerns over the impact of the high initial concentration on the
373 degradation kinetics of targeted compounds, some additional experiments were conducted
374 with lower initial concentrations for COC, 6-AM and MDMA. The results of the additional
375 experiment are similar to those described above and thus confirmed the finding of this study.
376 Details about the additional experiment can be found in the Supplemental Materials.
377

3.4 Impacts on sewage epidemiology

The abundance of microorganisms in actual sewer is much stronger than that in the wastewater alone due to the presence of the biofilm on the sewer walls. This study indicated that these microorganisms have significantly enhanced the degradation rate of COC, 6-AM compared to previous studies carried out in wastewater only (Gheorghe et al. 2008, Bisceglia 2010, Chen et al. 2012, van Nuijs et al. 2012, Baker and Kasprzyk-Hordern 2011). For other compounds such as the amphetamine-like stimulants (MA and MDMA) their degradation was found to be insignificant in the sewer system. And for BE, the principal COC metabolite, its stability and its formation from COC mean that the formation process will decide the level of BE in the sewer system.

The above results would influence on the selection of illicit drug residues to use as markers and the estimation of illicit drug consumption in sewer epidemiology. For example, to monitor COC use, it is recommended to use BE as the marker not COC itself since considerable loss of COC can occur in the sewer. Although there is formation of BE from COC in the sewer, it is unlikely to significantly influence the level of BE in the sewer since the level of BE found in wastewater is 2.5 - 5 folds that of COC (Lai et al. 2011, Castiglioni et al. 2011).

The drastic degradation of 6-AM under simulated sewer conditions of this study helps to explain the difficulty of measuring 6-AM in wastewater sample because the residence in some sewer systems make the concentration of 6-AM too low to measure. As 6-AM is used as the only specific marker for heroin (except heroin itself which is not commercially available as standard for chemical measurement), it is expected that new and more sensitive analytical techniques together with good sample preservation practice would help measure 6-AM in wastewater in the future. However, even when 6-AM can be measured, it is necessary to take its degradation during sewer residence into account in estimating heroin consumption.

403 It also means that knowledge about sewage residence time in the sewers is required for each
404 specific calculation so that the level of degradation can be estimated.
405 This study has also suggested that different sewer conditions (GS vs RM) have different
406 effects on specific illicit drug residues. For example, the degradation of COC (and formation
407 of BE) in this study is different in GS and RM reactors. Thus, more knowledge about the
408 sewer system, such as the ratio of GS and RM sewer in addition to the average residence time,
409 is required to minimize the uncertainty in sample collection process for sewer epidemiology.
410

411 ## *3.5 Limitations of the sewer reactor*

412 This study, to our knowledge, is the first one to evaluate the stability of popular illicit drug
413 residues under different sewer conditions. It was achieved by using laboratory-scale sewer
414 reactors to simulate the typical conditions in real sewers, especially the different biological
415 processes in different types of sewers. Using real wastewater as the feed, the capability of the
416 reactor to mimic real sewer has been demonstrated in terms of types of biofilm, microbial
417 populations and biological activity.
418 Biofilm exists universally in all sewer pipes and it is responsible for many biological
419 transformation processes (Hvitved-Jacobsen, 2002). Previous study also identified similar
420 microbial structure in the lab-scale sewer biofilm as those found in real sewers (Jiang and
421 Yuan, 2013; Mohanakrishnan et al., 2009). Through microbial activity tests, the primary
422 biological transformation rates, i.e. sulfate reduction and methane production, were
423 demonstrated to be similar to those occurring in real sewer pipes (section 3.1).
424 However, it must be admitted the lab-scale sewers only represent a certain type of real sewers
425 because they are designed with a fixed A/V ratio, and are operated at a fixed hydraulic
426 retention time. These factors should be considered while extending the results obtained in this
427 study to various sewer systems, with different pipe diameters and pumping patterns.

In this study, the lab-scale sewer reactors (RM and GS) have an A/V ratio of 70.9 m2/m^3. While it is similar to an A/V ratio of a sewer pipe with small diameter, it is higher than the average A/V ratio of an actual sewer system which comprises of small and large diameter pipes. And we understand that higher A/V ratio could facilitate more contact between illicit drugs and biofilms and hence lead to more degradation of illicit drugs in the sewer system. However, the chemical reaction processes in the sewer is determined by the combined effects of the A/V ratio and the hydraulic retention time of wastewater in the sewer (Hvitved-Jacobsen, 2002). Shorter hydraulic retention time would shorten the degradation processes including the biofilms-facilitated degradation of illicit drugs. Therefore, knowing the average A/V ratio and the average retention time of a sewer system would help better estimate the degradation rate of illicit drugs or other chemicals in this specific sewer system. That being said, further experiments with different A/V ratios should generate more thorough conclusions.

Another aspect of the sewer reactor that was not evaluated is its capability to mimic the flowing condition of the real gravity sewer which enhances the transfer of oxygen to the wastewater compartment. Improvement in experiment design can be made by installing the dissolved oxygen probe into this sewer reactor to continuously monitor the oxygen level in the wastewater along with other bioactivity tests.

4. CONCLUSIONS

This study evaluated the stability of typical illicit drug residues used in sewage epidemiology under different sewer conditions using unique deuterium labelled isotopes to avoid interference of natural illicit drugs in the wastewater matrix. The main conclusions are:

1. Compared to wastewater only, the simulated sewer conditions (rising main and gravity sewers) enhanced the degradation of some illicit drug residues, namely COC and 6-AM. This is likely due to the presence of sewer biofilms. Meanwhile, BE, MA, MDMA are stable in all sewer conditions.
2. Kinetic models of the degradation/transformation were selected for each illicit drug so that the level of in-sewer degradation or formation of those illicit drug residues can be estimated when the residence time and the type (RM or GS or mixed) of the sewer system is known.
3. The findings of this study suggest that information about specific sewer system is important in order to estimate accurately the extent of illicit drug degradation in this specific sewer system.
4. Further study about the effects of temperature and other environmental factors as well as the effect of A/V ratio in the sewer system is required to ensure a more accurate estimation of illicit drug consumption by sewer epidemiology approach.

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578

Table

Table 1. Structure of deuterium labelled illicit drug residues monitored in this study.

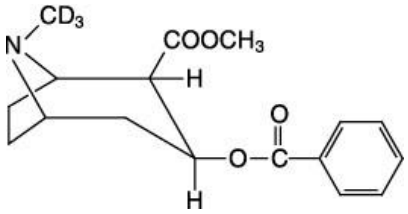
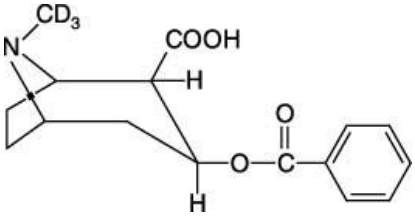
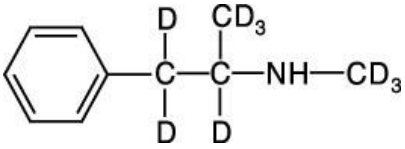
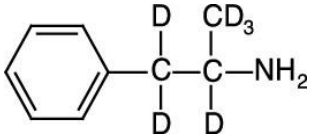
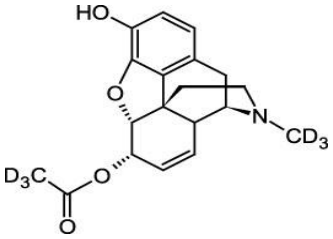
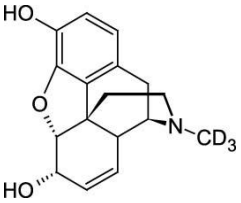
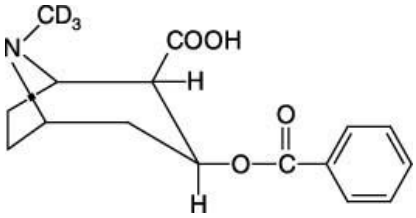
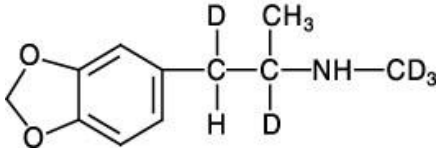
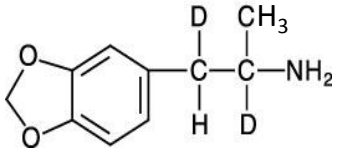
Spiked chemicals	Transformed products
 Cocaine D3 (COC)	 Benzoyl ecgonine D3 (BE)
 Methamphetamine D9 (MA)	 Amphetamine D6
 6 Acetylmorphine D6 (6-AM)	 Morphine D3
 Benzoyl ecgonine D3(BE)	
 MDMA D5 (MDMA)	 MDA D2 (not monitored)

Table 2. Comparing final results of different stability studies of selected illicit drug residues in wastewater. Negative values represent degradation; positive values represent formation.

	Chen *et al.* (2012)	Bisceglia (2010)	Baker and Kasprzyk-Hordern. (2011)	van Nuijs *et al.* (2012)	Plósz *et al.* (2013)	This study		
Experimental conditions	Wastewater pH = 7; 20 °C; 24 h.	Wastewater pH = 7.4; 23 °C; 12h.	Wastewater pH = 7.4; 19 °C; 24 h.	Wastewater pH = 7.5; 20 °C; 12h.	Wastewater pH = 7.4; 21 °C; 24h.	Waste-water; pH = 7.5; 20 °C; 12 h.	Gravity sewer; pH = 7.5; 20 °C; 12 h.	Rising main; pH = 7.5; 20 °C; 12 h.
COC	−9.3 ± 12.9	-50	−12.3 ± 2.8	−40	<−80[c]	-20	-60	-45
BE (+)[a]	not reported	10	7.4 ± 5.4	6	na	14	14	8
BE	−2.1 ± 10.0	na[b]	na	na	<−80[c]	0	0	0
MA	−4.6 ± 8.1	0	5.5 ± 1.9	2	na	0	5	0
MDMA	1.4 ± 3.1	0	2.8 ± 1.6	3	na	0	0	0
6-AM	−52.8 ± 15.1	-15	−41.5 ± 2.1	−20	na	-25	-88	-87

[a]BE (+): BE formed due to the hydrolysis of COC

[b]na: BE concentration measured in those studies is the combined concentration of both BE already present in the wastewater and BE generated by COC degradation

[c] value estimated from measured data presented in Fig. 2 (Plósz *et al.* (2013)

Table 3. Selection of kinetics models for unstable illicit drug residues in different sewer reactors. Model fitting with higher R^2 value will be selected.

	Control (wastewater only)				Kinetic model selected
	Linear regression		First-order kinetics		
	Slope (%/h)	R^2	Half life (h)	R^2	
COC	-1.39 ± 0.14	0.938	43.32	0.950	First
BE (+)	1.11 ± 0.02	0.999	4.13	0.919	Zero
MA	n.s.		n.s		
6-AM	-2.23 ± 0.38	0.832	25.6	0.848	First

	Gravity sewer (GS reactor)				Kinetic model selected
	Linear regression		First-order kinetics		
	Slope (%/h)	R^2	Half life (h)	R^2	
COC	-4.45 ± 0.47	0.928	10.05	0.975	First
BE (+)	1.03 ± 0.01	0.999	4.45	0.953	Zero
MA	-0.95 ± 0.26	0.645	41.86	0.238	Zero
6-AM	-6.82 ± 0.76	0.920	4.26	0.995	First

	Rising main (RM reactor)				Kinetic model selected
	Linear regression		First-order kinetics		
	Slope (%/h)	R^2	Half life (h)	R^2	
COC	-3.97 ± 0.30	0.962	13.07	0.986	First
BE (+)	0.58 ± 0.04	0.961	6.13	0.868	Zero
MA	n.s.		n.s.		
6-AM	-6.87 ± 0.66	0.939	4.23	0.989	First

BE (+): BE formed due to the hydrolysis of COC

n.s. not significantly deviated from zero

Figure

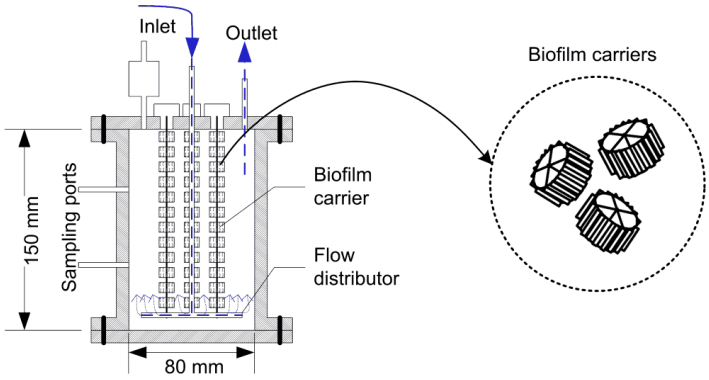


Figure 1. Sewer reactors with carriers to grow biofilms using wastewater.

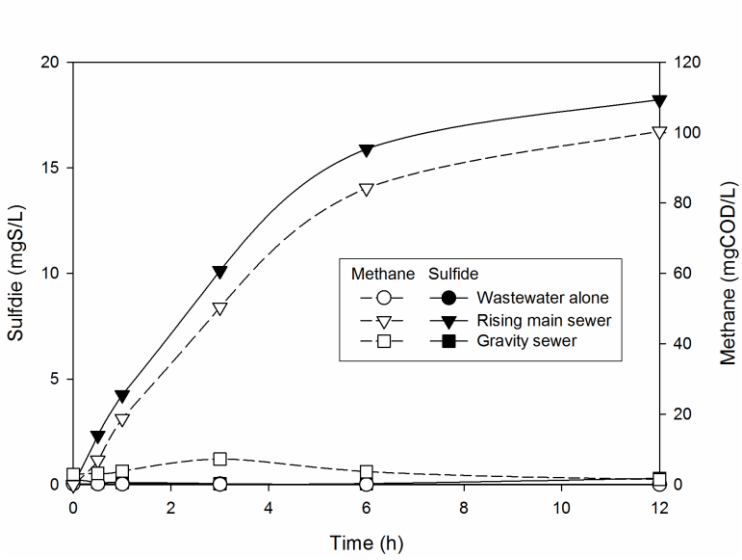


Figure 2. Sulfate reducing and methanogenic activities in rising main reactor, gravity sewer reactor and control reactor (wastewater alone).

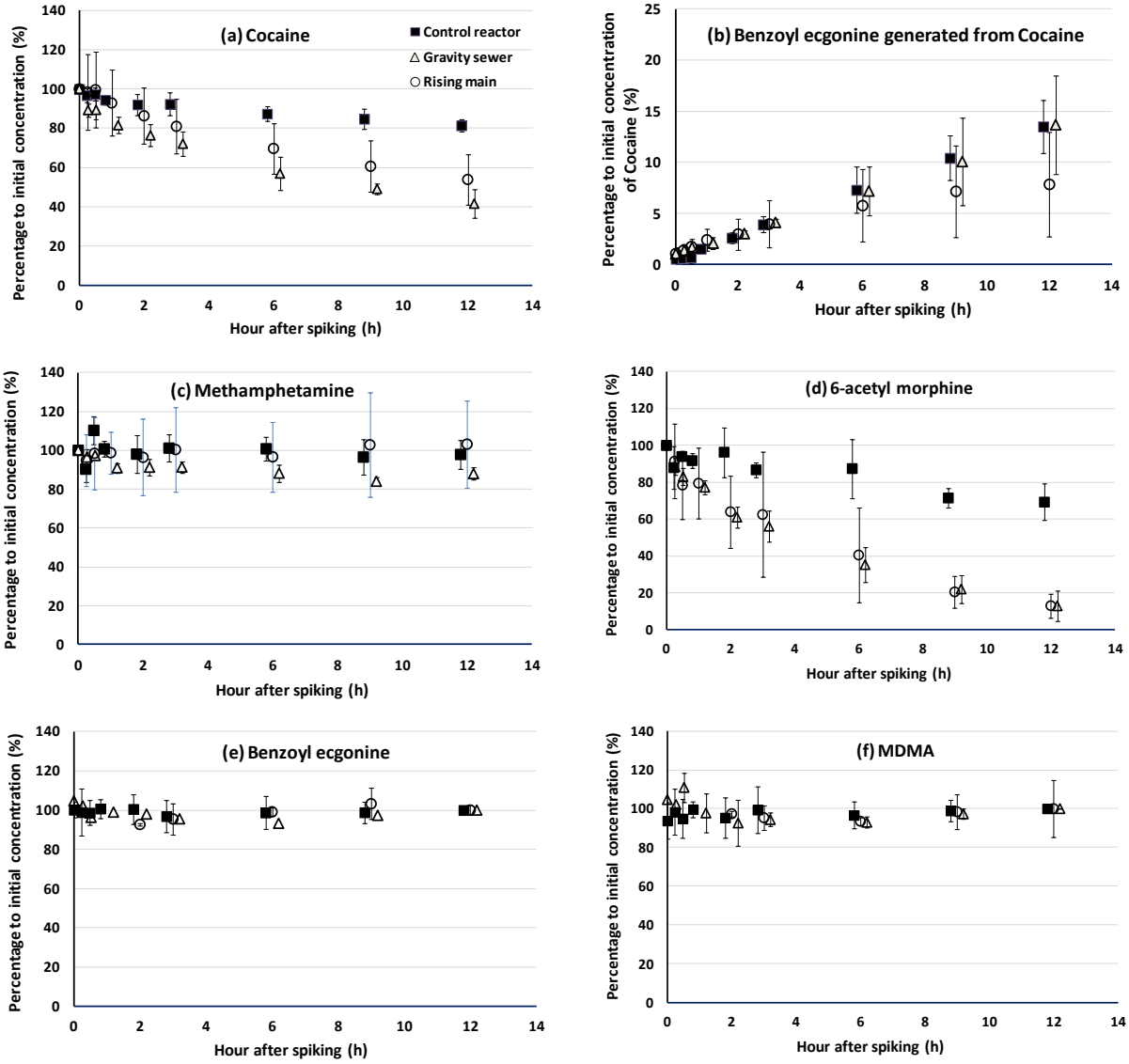


Figure 3. Degradation/formation profiles of selected illicit drug residues under different sewer conditions. Error bars represent the standard deviation of 3 replicates.