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Degradability of creatinine under sewer conditions affects its potential to be used as biomarker in sewage epidemiology

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ABSTRACT

Creatinine was proposed to be used as a population normalising factor in sewage epidemiology but its stability in the sewer system has not been assessed. This study thus aimed to evaluate the fate of creatinine under different sewer conditions using laboratory sewer reactors. The results showed that while creatinine was stable in wastewater only, it degraded quickly in reactors with the presence of sewer biofilms. The degradation followed first order kinetics with significantly higher rate in rising main condition than in gravity sewer condition. Additionally, daily loads of creatinine were determined in wastewater samples collected on Census day from 10 wastewater treatment plants around Australia. The measured loads of creatinine from those samples were much lower than expected and did not correlate with the populations across the sampled treatment plants. The results suggested that creatinine may not be a suitable biomarker for population normalisation purpose in sewage epidemiology, especially in sewer catchment with high percentage of rising mains.

1. Introduction

Sewage epidemiology (or wastewater analysis) has become a popular approach to estimate the consumption of illicit drugs at the population level and has been applied to different communities (Lai et al., 2011; Thomas et al., 2012; Banta-Green et al., 2009; Daughton, 2011; van Nuijs et al., 2011; Postigo et al., 2011; Baker et al., 2012). Until now, most studies have estimated the total mass of drug use in the catchment and then used the population number provided by the wastewater treatment plant (WWTP) to normalise the drug use (e.g. to mass used (g)/1000 people) in different catchments for comparison purposes. However, this population number estimated by the WWTP may differ from the actual population and the uncertainty incurred in estimating population size could be as high as 55%, the highest among uncertainties related to sewage epidemiology approach (Castiglioni et al., 2013).

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A number of researchers have recognised and attempted to address the issue of population variation in the sewer catchment during illicit drug consumption monitoring. The general approach is to find a chemical or a suite of chemicals in wastewater as surrogates to estimate the number of inhabitants in the catchment. Lai et al. (2011) have analysed different pharmaceuticals and an artificial sweetener to estimate the contributing population but recognised that the issues of compliance and demography (and thus the level of medicine use) can affect the certainty of the population estimation. van Nuijs et al. (2011) used the concentrations of phosphorous, nitrogen, biological oxygen demand and chemical oxygen demand to calculate the real-time number of inhabitants. However, these hydrochemical parameters are influenced by factors that cannot easily be controlled such as the composition of sewage (i.e. industrial, domestic, or mixed) which lead to large uncertainties in population estimation (Castiglioni et al., 2013). Castiglioni et al. (2013) also suggested that the estimation of population for a sewer catchment could be improved by using suitable biomarkers consumed or excreted in known amounts by a population such as creatinine and prescribed medicines.

Creatinine, a popular urinary biomarker, has been proposed as a per capita normalizing parameter to make illicit drug monitoring data comparable between sites (Daughton, 2001, 2012). The reason is that creatinine is widely used in clinical chemistry as a normalising parameter, and there is extensive published data on its excretion. In his recent review paper, Daughton (2012) has performed a desktop evaluation of creatinine as a potential biomarker for population estimation against a list of important attributes. While creatinine fulfils several criteria, an important attribute, the stability of creatinine in the sewer system, was not assessed due to a lack of available data.

Since the publication of Daughton's review, two new studies have used creatinine as a normalising parameter to compare per capita drug consumption estimates for different wastewater samples with expected changes of contributing population (Brewer et al., 2012; Burgard et al., 2013). However, in those studies, the stability of creatinine was only assessed in wastewater only as part of the sampling and storage stability test but not under conditions similar to the actual sewer system.

A sewer system typically consists of rising main sewers and gravity sewers. Rising main sewers are generally fully filled with wastewater and have anaerobic biofilms dominated on the pipe walls. Meanwhile, gravity sewers are only partially filled with wastewater and may sustain both aerobic and anaerobic biofilms/sediments (Hvitved-Jacobsen, 2013). Since biofilms are rich in microorganisms, which are capable of transforming/degrading various chemical compounds, it is hypothesized that creatinine can be transformed/degraded more strongly in sewers by those biofilms microbes than in wastewater alone where the microbial populations is scarer. Indeed, it has been reported that biofilms in rising main sewer produced substantially higher sulfide compared to suspended microorganisms in wastewater (Mohanakrishnan et al., 2009; Gutierrez et al., 2008). And Thai et al. (2014) also reported that sewer conditions enhanced the degradation of cocaine and 6 acetylmorphine compared to wastewater alone. Moreover, redox condition of the sewer, i.e. aerobic or anaerobic, can also influence biological transformation processes of chemicals including creatinine. It is thus necessary to study the fate of creatinine under different sewer conditions close to reality.

In this study, we attempted to address this problem by i) investigating the degradation of creatinine under different sewer conditions using laboratory-scale sewer reactors and; ii) looking for a correlation between the actual population in the sewer catchment (Census data with samples collected on Census day) with the mass load of creatinine in the sewer system. The results obtained will help to clarify the possibility of using creatinine as a biomarker for population estimation in a sewage catchment.

2. Materials and methods

2.1. Chemicals and reagents

Reagent grade creatinine was purchased from Sigma Aldrich (Castle Hill, Australia). Creatinine-d3 was purchased from Cambridge Isotope Laboratories (MA, USA).

LCMS grade solvents (methanol and acetonitrile) were purchased from Merck (Darmstradt, Germany). Deionised water was produced by a MilliQ system (Millipore, 0.22 μm filter, 18.2 MΩ cm^{-1}).

2.2. Analysis of creatinine

A chromatography method originally developed to analyse creatinine in serum by Hetu et al. (2010) was adapted and modified to analyse creatinine in wastewater. Creatinine in wastewater samples was measured by a liquid chromatography-tandem mass spectrometry (LC-MS/MS) system comprising of a Shimadzu Nexera LC system (Kyoto, Japan) connected to an ABSciex 5500QTRAP mass spectrometer (ABSciex, Concord, Ontaria, Canada). The LC system consists of a Shimadzu LC-20AB high-pressure pump, a SIL-20AHT autosampler and a CTO-20A column oven. An in-line degasser (DGU-20A3) was placed prior to the solvent delivery system. Separation was achieved using only a Phenomenex SecurityGuard strong cation exchange (SCX) cartridge (4.0 × 3.0 mm) (Phenomenex, CA, USA) using an ammonium acetate gradient at 0.6 mL/min. The gradient starts with 85% A: 15% B and holds for 40 s before ramping up to 100% B in 10 s and holding for 2 min before re-equilibrating at 15% B for a further 2 min. Mobile phases A and B both contain 75% methanol and 25% Milli Q water with 0.4% acetic acid and an ammonium acetate concentration of 1.0 mM for A and 10 mM for B.

The acquisition is operated under multiple reaction monitoring (MRM) in positive APCI mode. All data were collected using ABSciex Analyst software (version 2.1). Quantitation was performed using MultiQuant version 2.1 software (ABSciex).

Samples were prepared for analysis by adding 10 μL of internal standard (10 μg/mL creatinine-d3 in water) to 1 mL of filtered sample in the vial. The vials were then vortexed to mix well before analysis.