In-sewer stability of selected analgesics and their metabolites

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In-sewer stability of selected analgesics and their metabolites

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ABSTRACT

Understanding the in-sewer stability of analgesic biomarkers is important for interpreting wastewater-based epidemiology (WBE) data to estimate community-wide analgesic drugs consumption. The in-sewer stability of a suite of 19 analgesics and their metabolites was assessed using lab-scale sewer reactors. Target biomarkers were spiked into wastewater circulating in simulated gravity, rising main and control (no biofilm) sewer reactors. In-sewer transformation was observed over a hydraulic retention time of 12 h. All investigated biomarkers were stable under control reactor conditions. In gravity sewer conditions, diclofenac, desmetramadol, ibuprofen carboxylic acid, ketoprofen, lidocaine and tapentadol were highly stable (0–20% transformation in 12 h). Valdecoxib, parecoxib, etoricoxib, indomethacin, naltrexone, naloxone, piroxicam, ketoprofen, lidocaine, tapentadol, oxymorphone, hydrocodone, meperidine, hydromorphone were considered as moderately stable biomarkers (20–50% transformation in 12 h). Celecoxib and sulindac were considered unstable biomarkers (>50% transformation in 12 h). Ketoprofen, lidocaine, tapentadol, meperidine, hydromorphone were transformed to 0–20% whereas diclofenac, desmetramadol, ibuprofen carboxylic acid, valdecoxib, parecoxib, etoricoxib, indomethacin, naltrexone, piroxicam were transformed up to 20–50% in 12 h in rising main reactor (RMR). These biomarkers were considered as highly stable and stable biomarkers in RMR, respectively. Sulindac, celecoxib, naloxone, oxymorphone and hydrocodone were transformed more than 50% in 12 h and considered as unstable biomarkers in RMR. This study provides the information for a better understanding of the in-sewer loss of the analgesics before using them in WBE biomarkers for estimating drug loads at the population level.

1. Introduction

Physical pain is almost an unavoidable part of human life. It is one of the major reasons patients seek medical assistance (Fishman 2007). Treatment of pain is complex but pain medications, known as analgesics, are considered effective and quick treatment options to relieve pain. Analgesics comprise mainly non-steroidal anti-inflammatory drugs (NSAIDs) and opioids (Brower 2000). While analgesics act to relieve body pain, misuse and overdose of analgesics, and specifically opioids, can pose a health burden and additional mortality to our communities (Berterame et al., 2016).

Assessing the prevalence of pain in a community or population can be challenging. Individual pain assessment tools provide the scope to measure pain (Relland et al., 2019) but are often limited to an individual or personal scale. We recently proposed a new theoretical method through the wastewater-based measurement of the population treated

pain burden (Ahmed et al., 2020b). A theoretical basis for quantifying analgesic drugs in wastewater followed by normalisation of the relative drug potency to estimate community pain burden was presented (Ahmed et al., 2020b). The proposed method would allow us to estimate analgesics and rank the population treated pain burden area within and between populations.

Wastewater-based epidemiology (WBE) entails the systematic sampling and analysis of chemical markers in wastewater to estimate human consumption of or exposure to chemicals through the analysis of wastewater (Daughton 2018; Zuccato et al., 2008). WBE has been successfully applied to estimate illicit drug consumption (Ort et al., 2014b; Sodré et al., 2018; Thomas et al., 2012) and lifestyle-related factors, such as alcohol (Reid et al., 2011) and tobacco use (Zheng et al., 2017). WBE can also provide information about the health, diet and diseases associated with a community (Ahmed et al., 2020a; Ahmed et al., 2020c; Choi et al., 2018a; Choi et al., 2018b; Ryu et al., 2016). WBE relies on the

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quantification of specific biomarkers in wastewater that ideally are: 1) excreted through urine in consistent amounts with an adequate concentration once diluted in wastewater, 2) sufficiently stable in wastewater during the transport process and 3) unique to human metabolism (Chen et al., 2014; Gracia-Lor et al., 2017; McCall et al., 2016). Consumed analgesics can remain as the parent compound or transform into a metabolite through human metabolic pathways before excretion in urine into the sewer system.

Sewer networks typically contain a mixture of gravity sewers, pump stations, rising mains and other structures. Wastewater contains soluble and particulate organics which can work as primary substrates for microbial growth on the sewerage infrastructure surface which is generally termed as biofilms (Romaní et al., 2016). Different wastewater characteristics or parameters (e.g. dissolved oxygen, nutrient, pH, temperature) control the microbial growth and transformation of chemicals in the sewer network (Sharma et al., 2013). Gravity sewers are partially filled with wastewater where both aerobic and anaerobic microbes can exist whereas rising mains are filled with wastewater, typically deprived of oxygen, hence anaerobic microbes are largely predominant (Hvit-ved-Jacobsen et al., 2013). Several studies have already reported the in-sewer stability of a limited number of opioids or NSAIDs in lab-scale reactors and showed their applicability as a WBE biomarkers (Gao et al., 2017; O'Brien et al., 2017; Ramin et al., 2017). However, there is a lack of information on the stability of different analgesic drugs, particularly NSAIDs, and this needs to be addressed to get accurate background information of their suitability as WBE biomarkers.

In this study, we examined the stability of 19 NSAIDs and opioids biomarkers in the lab-scale sewer reactors simulating real sewers. This is to date the first in-sewer stability assessment experiment of a broad range of analgesics and metabolites, to evaluate their suitability as biomarkers for WBE. Minimal in-sewer biomarker loss increases the confidence in WBE estimation of analgesics consumption by the population.

2. Materials and methods

2.1. Chemicals and reagents

Nineteen analgesics and their metabolites were selected due to their common use as analgesics and potential for abuse. These are diclofenac, desmetramadol, ibuprofen carboxylic acid, valdecoxib, parecoxib, celecoxib, etoricoxib, indomethacin, sulindac, naltrexone, naloxone, piroxicam, ketoprofen, lidocaine, tapentadol, oxymorphone, hydrocodone, meperidine, and hydromorphone. Detailed description of the biomarkers is shown in the SI, Table S1. All reference standards and deuterated label internal standards (IS) were supplied from Novachem (New South Wales, Australia) and PM separations (Queensland, Australia). Methanol (analytical grade) was purchased from Merck Pty Ltd. (Highway Bayswater, Australia). MilliQ system (0.22 µm filtered at 18.2 MΩ cm^{-1}, Millipore, Bedford, USA) was used for producing MilliQ water.

2.2. Instrumentation

All compounds were analysed using a validated method (Ahmed et al., 2021) with a Shimadzu Nexera X2 UHPLC system coupled to a Sciex 6500+ QTRAP mass spectrometer (Ontario, Canada). An Ultra AQ C18 (100 × 2.1 mm × 3 µm) column with a guard column (Restek, USA CAT #27,475) was used for chromatographic separation at a flow rate of 0.50 mL/min. The injection volume was used as 2 µL. Mobile phase A was 99% water with 0.1% formic acid and mobile phase B was 99.9% methanol with 0.1% formic acid. The mobile phase gradient was as follows: $t = 1.0$:12% B, $t = 8.20$: 80% B, $t = 8.50$: 98% B, $t = 9.5$: 0.65% B, $t = 10.90$: 98% B, $t = 11.00$: 12% B, $t = 13$: 0.5% B, $t = 14$: STOP. The mass spectrometry parameters and method validation results are listed in the SI (Table S2, S3, S4). Data were attained and processed using

Analyst 1.7.1 (Sciex) and MultiQuant 3.0.2, respectively.

2.3. Lab-scale sewer reactors

Representative lab-scale sewer reactors were used to assess the transformation of selected analgesics and their metabolites under realistic sewer conditions. These lab-scale sewer reactors comprised a rising main reactor (RMR), a gravity sewer reactor (GSR), and a control reactor (CR) (Li et al., 2018). The diameter of RMR was 80 mm and was made of Perspex™ with a volume of 0.75 L. Four stainless-steel rods (1-cm diameter) inside the reactor were used to facilitate the biofilm growth. The GSR was partially filled with real wastewater with similar dimension of RMR. The GSR had a mixture of biofilm (aerobic and anaerobic). RMR and GSR have been established and operating over years under anaerobic and aerobic conditions, respectively (Banks et al., 2018; Jiang et al., 2009).

Mature biofilms were cultivated on the inner surfaces of the reactor walls, with a calculated biofilm-area-to-wastewater-volume (A/V) ratio of 72.5 m^2/m^3 in RMR and 50 m^2/m^3 in GSR. Biofilms in both the RMR and the GSR showed strong biological activities, as indicated by their sulfidogenic and methanogenic activities (see Section 3.1 for results). Domestic sewage was used to feed the reactors and was pumped in with a peristaltic pump (Masterflex 7520–47) every 6 h. A magnetic stirrer was used for continuous mixing (250 rpm) inside each reactor to confirm homogeneous distribution. Typical sewage parameters are shown in the SI, Section 1. Due to regular cleaning and a lack of plastic carriers which facilitate biofilm growth, the CR has no biofilm formation and contains wastewater only.

Triplicate batch tests were conducted to estimate the transformation of the analgesic compounds and their biomarkers. A chemical mix of target biomarkers (10 µg/L) were spiked into the reactors. Before spiking the target biomarkers, wastewater was warmed to room temperature and then mixed rapidly before feeding to the drained reactors. Wastewater samples were collected at multiple time points during each 12 hrs batch test (0, 0.5, 1, 2, 4, 6, 8 and 12 hrs), filtered, and then stored at −80 °C until further analysis. Before instrumental analysis, samples were defrosted to room temperature and spiked with internal standards (10 µg/L), then immediately analysed by direct injection. Due to the high spiked levels, direct injection analysis was sufficient.

2.4. Acceptable criteria to select the stability of compounds

In this study, we grouped the biomarkers in the following criteria to assess their stability in the sewer reactor.

a **Highly stable biomarkers:** 0–20% transformation within 12 h.
b **Moderately stable biomarkers:** 20–50% transformation within 12 h.
c **Unstable biomarkers:** >50% transformation within 12 h.

2.5. Quality assurance and quality control (QA/QC)

A nine-point calibration curve was used (0.1 to 100 µg/L, linearity R^2 > 0.99) with isotopically labelled internal standards (10 µg/L) for quantification. For every ten samples analysed, a calibration point, a duplicate sample, a pooled wastewater sample, a native spiked pooled wastewater sample and a method blank were analysed to confirm and monitor the instrument and analytical method performance. The results of QA/QC are listed in supplementary information (SI, Table S5)

2.6. Data processing

At time 0, the concentration of chemicals was considered as the initial concentration (100%). For each 12 h experiment, all concentrations were normalised to a percentage relative to the initial concentration. To evaluate the degradation profile under sewer conditions, zero-

Table 1

List of sulfate-reducing and methanogenic activities in sewer reactors in the present study and other similar studies.

Study	Type of sewer	Sulfide generating activity in mgS/L/h	Methanogenic activity in mgCOD/L/h
Present study	RMR reactor	4.7	23.1
	GSR reactor	1.7	4.4
(He et al., 2021)	RMR reactor	6.1	25.6
	GSR reactor	1.4	2.6
(O'Brien et al., 2017; Banks et al., 2018)	RMR reactor	7.2	29.7
	GSR reactor	4.2	14.8
(Thai et al., 2014b; Thai et al., 2014a)	RMR reactor	4.3	18.9
	GSR reactor	0.2	1.5
(Gao et al., 2017)	RMR reactor	5.6	12.1
(Li et al., 2018)	RMR reactor	4.8	12.4

order or first-order kinetics models were applied and the best fit kinetic model was identified based on the highest correlation (r) value. Half-life in pseudo first-order model was computed in pseudo first-order model. Analyses were carried out using GraphPad Prism 9 (GraphPad Software Inc) and Matlab (version 2020b).

3. Results and discussion

3.1. Biological activities in the sewer reactors

Wastewater pH was stable during the batch tests (RMR pH = 7.06 ± 0.1, GSR pH = 7.13 ± 0.1 and CR pH = 7.50 ± 0.2). Biological activity, as described by methane and sulfide production rates were monitored in the simulated RMR and GSR. Stronger activities of sulfate-reducing bacteria and methanogens were found in the RMR reactor. The production of dissolved sulfide and methane was lower in the GSR reactors. This is likely because of the presence of oxygen and the transfer of hydrogen sulfide and methane from the bulk wastewater phase to air in GSR. Limited biological activity was observed in CR in the absence of biofilms as indicated by the little consumption of sulfate or SCOD. The

results of the biological activities are comparable with previous studies (Table 1) (Gao et al., 2017; Thai et al., 2014a; Thai et al., 2014b).

3.2. Sewer reactor data analysis

To assess biomarker stability under the different sewer conditions, regression models (zero or first order) were fitted to the biomarker data to establish best fit (Table 2). We used the regression with the highest R^2 value except the linear regression had a slope not significantly different from zero (indicates the stability over the test period). It indicated that relatively large deviation between the observed data and the fitted regression model could be attributed to the complexity of the reactors, including the potential variations in biological activities and wastewater conditions.

3.2.1. Transformation of biomarkers in the control reactor

In the CR reactor, the transformation profile is best described by a zero-order model. All the studied biomarkers were highly stable in the CR. (Table 3 & Fig 1). However, ibuprofen carboxylic acid, naloxone and ketoprofen showed slight formation behaviour in the CR. Low rates of transformation are expected in the CR as there is no biofilm present and microbial activity is likely to be low (Thai et al., 2014a). In a previous study with the same reactor, O' Brien et al. (O'Brien et al., 2019) outlined that the increased gas-liquid transfer causing the higher dissolved oxygen (DO) level in CR which may be responsible for the transformation of certain biomarkers in CR (Table 3). All the investigated biomarkers were highly stable for at least 12 h in the CR (Fig. 1).

3.2.2. Transformation of biomarkers in gravity sewer reactor (GSR)

In the GSR reactor, the transformation profile was best described by a first-order model except ibuprofen carboxylic acid, lidocaine and tapentadol (Table 2). Ibuprofen carboxylic acid, lidocaine and tapentadol followed the stable transformation over the experimental time. Based on our stability criteria, where 0–20% transformation over 12 h is considered highly stable and diclofenac, desmetramadol, ibuprofen carboxylic acid, ketoprofen, lidocaine and tapentadol in GSR meet these criteria (Table 3 and Fig 1). Valdecoxib, parecoxib, etoricoxib, indomethacin, naltrexone, naloxone, piroxicam, ketoprofen, lidocaine, tapentadol, oxymorphone, hydrocodone, meperidine, hydromorphone were transformed up to 20–50% in 12 h and classified as moderately stable biomarkers. Celecoxib and sulindac were considered unstable biomarkers in GSR with >50% transformation over 12 h (Table 3 and Fig 1).

Table 2

Regressions model of biomarkers degradation in sewer reactor.

Biomarkers	CR R^2	Best fit model	K value	GSR R^2	Best fit model	K value	RMR R^2	Best fit model	K value
Diclofenac		n. s	0.01132	0.50	First order	0.01476	0.70	First order	0.03243
Desmetramadol		n. s	0.01515	0.43	First order	0.01600	0.85	First order	0.01600
Ibuprofen carboxylic acid		n. s	−0.0004229		n. s	0.001302		n. s	0.02446
Valdecoxib		n. s	0.003772	0.64	First order	0.04732	0.54	First order	0.1045
Parecoxib		n. s	0.006518	0.47	First order	0.03407	0.74	First order	0.05630
Celecoxib		n. s	0.01337	0.62	First order	0.06653	0.52	First order	0.04714
Etoricoxib		n. s	−0.0003481	0.52	First order	0.03777	0.74	First order	0.09152
Indomethacin		n. s	0.009781		n. s	0.04055	0.85	First order	0.04509
Sulindac		n. s	0.01181	0.90	First order	0.08575	0.94	First order	0.2000
Naltrexone		n. s	0.005403	0.56	First order	0.03574	0.63	First order	0.04794
Naloxone		n. s	−0.0006439		n. s	0.03260	0.52	First order	0.09281
Piroxicam		n. s	0.007292		n. s	0.02050	0.51	First order	0.03967
Ketoprofen		n. s	−0.01615		n. s	0.009019		n. s	0.01587
Lidocaine		n. s	−0.002656		n. s	0.01200		n. s	−0.004793
Tapentadol		n. s	9.686e-005		n. s	−0.004409		n. s	0.005998
Oxymorphone		n. s	−0.005292	0.5	First order	0.04001	0.54	First order	0.05906
Hydrocodone		n. s	1.114e-005		n. s	0.02911	0.50	First order	0.07956
Meperidine		n. s	−0.003908	0.32	First order	0.01639	0.50	First order	0.02384
Hydromorphone		n. s	−0.003389	0.26	First order	0.01598		n. s	0.01603

n. s = slope not significantly different from zero. CR = control reactor, GSR = gravity sewer reactor, RMR= rising main reactor.

Table 3
Stability of biomarkers in sewer reactor conditions.

| Biomarkers	Time to 10% transformation (h)			Time to 20% transformation (h)			Time to 50% transformation (h)		
	CR	GSR	RMR	CR	GSR	RMR	CR	GSR	RMR
Diclofenac	>12	7.1	3.2	>12	>12	6.9	>12	>12	>12
Desmetramadol	>12	6.6	3.6	>12	>12	7.7	>12	>12	>12
Ibuprofen carboxylic acid	>12	>12	4.3	>12	>12	9.1	>12	>12	>12
Valdecoxib	>12	2.2	1.1	>12	4.7	2.1	>12	>12	>12
Parecoxib	>12	3.1	1.9	>12	6.5	6.9	>12	>12	>12
Celecoxib	>12	1.6	1.7	>12	3.4	3.6	>12	10.4	11.2
Etoricoxib	>12	2.8	1.5	>12	5.9	2.4	>12	>12	>12
Indomethacin	>12	2.6	2.3	>12	4.9	4.9	>12	>12	>12
Sulindac	>12	1.2	0.5	>12	2.6	1.1	>12	8.0	3.5
Naltrexone	>12	2.9	2.2	>12	6.2	4.7	>12	>12	>12
Naloxone	>12	3.3	1.1	>12	6.8	2.4	>12	>12	7.5
Piroxicam	>12	5.1	2.7	>12	11.9	5.6	>12	>12	>12
Ketoprofen	>12	>12	6.6	>12	>12	>12	>12	>12	>12
Lidocaine	>12	>12	>12	>12	>12	>12	>12	>12	>12
Tapentadol	>12	>12	>12	>12	>12	>12	>12	>12	>12
Oxymorphone	>12	2.6	1.8	>12	5.6	3.8	>12	>12	11.7
Hydrocodone	>12	3.2	1.1	>12	6.9	2.4	>12	>12	7.5
Meperidine	>12	6.4	6.6	>12	>12	>12	>12	>12	>12
Hydromorphone	>12	6.6	6.6	>12	>12	>12	>12	>12	>12
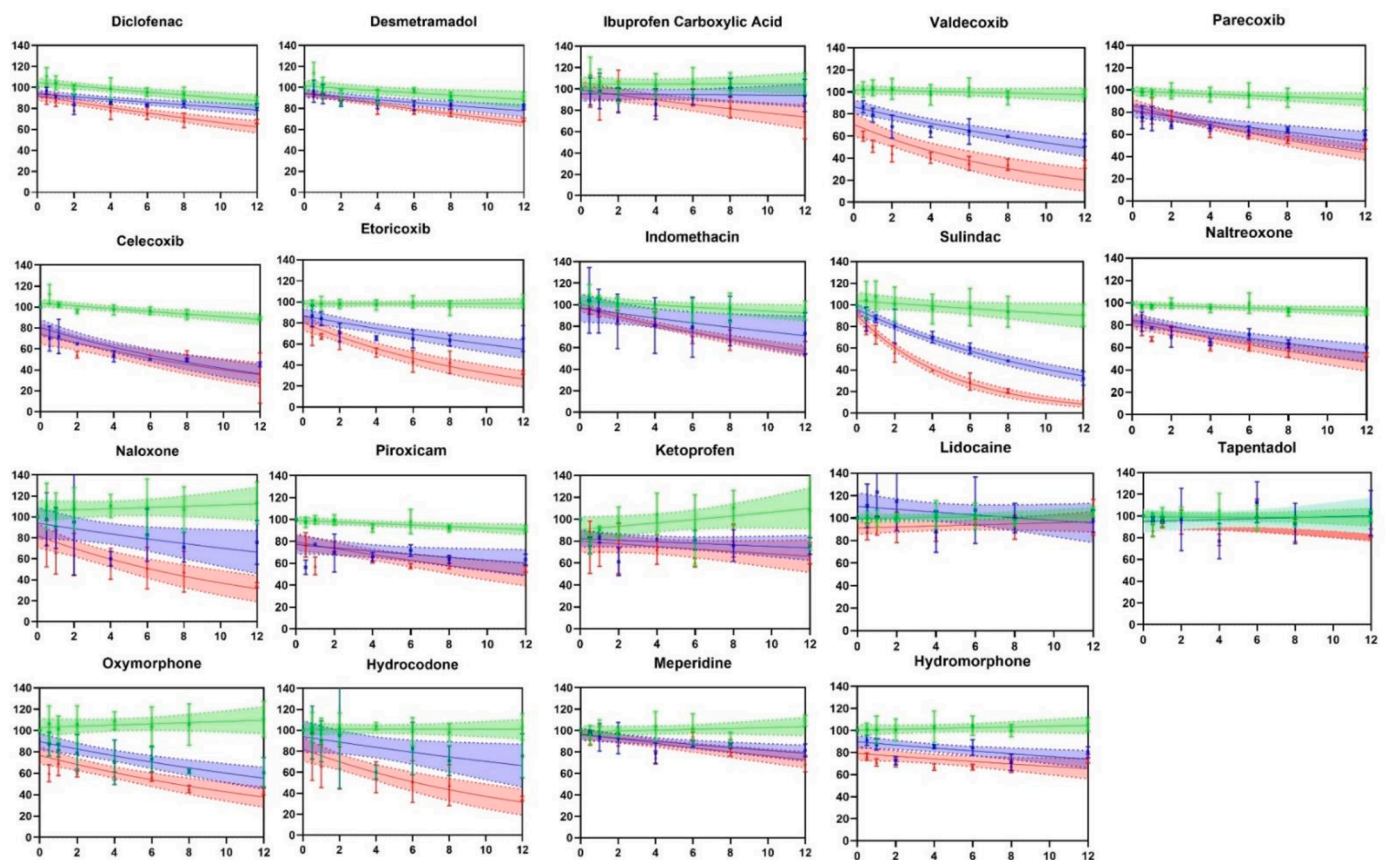
Fig. 1. Transformation profile of studied analgesics and their metabolites under different sewer conditions (Green: control reactor, Blue: gravity sewer, Red: rising main). X – axis: time (hours) after spiking. Y – axis: percentage (%) of concentration at t = 0. Concentrations for each sample are from triplicate sewer reactor experiments. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.2.3. Transformation of biomarkers in the rising main reactor (RMR)

Lidocaine and tapentadol were stable over the 12 h experimental period. Ketoprofen, lidocaine, tapentadol, meperidine, hydromorphone were transformed by 0–20% over the experimental period in RMR (Table 3 and Fig 1). Diclofenac, desmetramadol, ibuprofen carboxylic acid, valdecoxib, parecoxib, etoricoxib, indomethacin, naltrexone, piroxicam were transformed up to 20–50% in 12 h. These biomarkers were considered moderately stable biomarkers in RMR. Sulindac, celecoxib,

naloxone, oxymorphone and hydrocodone were transformed more than 50% in 12 h and considered as unstable biomarkers in the RMR (Table 3 and Fig 1).

3.3. Implication for wastewater-based epidemiology

The primary goal of WBE is to quantify community-wide chemical consumption or exposure. The method is subject to several uncertainties

associated with different steps. Detailed understanding of biomarker stability under different sewer conditions can help improve the accuracy of final estimates and assist in interpretation. In this study, diclofenac, desmetramadol, ibuprofen carboxylic acid, piroxicam, ketoprofen, lidocaine, tapentadol, meperidine and hydromorphone were stable ($\leq$ 20% transformation within 12 h) considering all conditions and the type of sewers.

However, the type of sewer reactor and hydraulic retention time (HRT) could play a crucial part in interpretation of our results. It is noted that most wastewater catchments mainly comprise gravity sewer with the median in-sewer residence time of approximately 4 h (Ort et al., 2014a). Based on this study, we could say only celecoxib and sulindac had 20% loss in 4 h before entering the network and passing the sampling location at the WWTP.

Biofilms in both GSR and RMR increased the transformation of the biomarkers, hence, to estimate the analgesic consumption through WBE, we need to also consider the associated uncertainties (sewer infrastructures, population characteristics, etc.). Further knowledge about the sorption and transformation behaviour of chemicals could help to develop a systematic model that can reduce the uncertainties in back-estimating consumption using WBE.

4. Limitations

There are several limitations that need to be acknowledged in this study. In a real world scenario, the surface area to volume ratio (A/V) of RM and GS is different compared to lab-scale sewer reactors (Gao et al., 2017). It is also noted that for certain biomarkers the GS and RM sewer reactors may overestimate the degradation profile compared to pilot sewers functioning with GSR and RMR mode (Choi et al., 2020). In this study, we only measured the free and non-conjugated forms of the analgesic analytes. Also, there may be residual concentrations that transform to the biomarkers in the sewer reactors but at the levels it was spiked we believe this would have negligible impact. As opioids typically have a relatively high log K_{ow}, they may be prone to sorption to particulate matter and biofilms (Baker et al., 2012; Subedi and Kannan 2014).

5. Conclusions

This study examined the potential for a panel of analgesic biomarkers to be used for WBE. The transformation of analgesics and their metabolites are sewer-specific and depend on the proportion of GSR and RMR in the catchment area. Diclofenac, desmetramadol, ibuprofen carboxylic acid, piroxicam, ketoprofen, lidocaine, tapentadol, meperidine and hydromorphone in GSR and ketoprofen, lidocaine, tapentadol, meperidine and hydromorphone in RMR were shown to be stable biomarkers, respectively. In the future, a comprehensive in-sewer study including mathematical modelling could help to reduce the uncertainties in overall estimation.

Declaration of Competing Interest

The authors have no conflict of interest to declare.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.watres.2021.117647.

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