Using prescription and wastewater data to estimate the correction factors of atenolol, carbamazepine, and naproxen for wastewater-based epidemiology applications

Using prescription and wastewater data to estimate correction factors of atenolol, carbamazepine and naproxen for wastewater-based epidemiology applications

Jianfa Gao[1,2], Benjamin J. Tscharke[2], Phil M. Choi[2], Jake W. O'Brien[2], Tim Boogaerts[3], Hui Jiang[2], Mengting Yang[1], Samantha A. Hollingworth[4], Phong K. Thai[2,*]

1 College of Chemistry and Environmental Engineering, Shenzhen University, 1066 Xueyuan Avenue, Shenzhen, 518060, China

2 Queensland Alliance for Environmental Health Sciences, The University of Queensland, 20 Cornwall Street, Woolloongabba, 4102, Brisbane, Australia

3 Toxicological Centre, University of Antwerp, Universiteitsplein 1, 2610 Antwerp, Belgium

4 School of Pharmacy, The University of Queensland, 20 Cornwall Street, Woolloongabba, 4102, Brisbane, Australia

*Corresponding author: Dr. Phong Thai

Email: p.thai@uq.edu.au

Abstract:

Correction factor (CF) is a critical parameter in wastewater-based epidemiology (WBE) that significantly influences the accuracy of the final consumption estimates. However, most CFs have been derived from a few old pharmacokinetics studies and should be re-evaluated and refined to improve the accuracy of the WBE approach. This study aimed to review and estimate the CFs for atenolol, carbamazepine, and naproxen for WBE using the daily mass load of those pharmaceuticals in wastewater and their corresponding dispensed prescription data in Australia. Influent wastewater samples were collected from wastewater treatment plants serving approximately 24 % of the Australian population, and annual national dispensed prescription data. The estimated CFs for atenolol and carbamazepine are 1.37 (95% CI: 1.17-1.66) and 8.69 (95% CI: 7.66-10.03), respectively. Due to significant over-the-counter sales of naproxen, a reliable CF could not be estimated based on prescription statistics. Using an independent dataset of 186 and 149 wastewater samples collected in an urban catchment in 2011 and 2012, WBE results calculated using the new CFs matched well with dispensed data for atenolol and carbamazepine in the catchment area.

Keywords: Atenolol; Carbamazepine; Naproxen; Correction factor; Prescription data;

1. Introduction

Wastewater-based epidemiology (WBE) is expanding rapidly for applications evaluating chemical consumption and exposure, ranging from small communities to national and continental investigations. [1-3] It can provide temporal and geographical consumption and exposure pattern of both licit and illicit drugs, alcohol and tobacco, and industrial chemicals, which is useful for developing harm-reduction strategies and examining the effects of intervention actions. [4-6] In addition, WBE can also provide information on population well-being with monitoring of nutritional and disease biomarkers. [7, 8] Although much progress has been made in WBE over the last decade, there are remaining challenges in addressing the intrinsic uncertainties of the approach, such as those around sampling, biomarker stability, chemical analysis, and real-time population estimation. [9] Researchers have evaluated and, in turn, reduced these uncertainties thereby improving the accuracy of consumption estimations. For example, by comparing the results of different wastewater sampling strategies, flow-proportional sampling was recommended to be the best practice for representative sampling. [10] In addition, biomarker stability studies have been conducted in various configurations to evaluate and model the in-sample and in-sewer biomarker stability to improve the interpretation of consumption estimates. [11-15]

Correction factor (CF) is an important parameter to convert the mass of biomarkers measured in wastewater to the initial consumed mass by the population. They are developed by considering the mean percentage excretion of a given drug, in form of parent substance or a metabolite and their molecular mass ratio (parent drug/metabolite), as well as the potential degradation in sewer system. [16, 17] The CF is considered a source of uncertainty for back estimating how much of a substance is consumed. [18] For example, the CF of benzoylecgonine is estimated to contribute up to 26% of the total uncertainty for cocaine consumption estimation. [9] CFs used in WBE are mostly derived from the excretion data of pharmacokinetic studies, typically with a limited number of participants, and often being healthy Caucasians. [19, 20] To evaluate the accuracy of CFs derived from pharmacokinetic studies, comparison of the measured mass load of pharmaceuticals in

wastewater and the predicted mass load derived from prescription data was conducted in Belgium, results indicated up to one order of magnitude of deviations between the measured mass load and the predicted mass load. [21] Refinement of CFs has been performed in a few studies using pharmacokinetic data, sales data, and wastewater analysis results, and the refined CFs reportedly can improve the accuracy of WBE estimations. [21-25] However, previous studies have only evaluated the CFs of a limited number of licit (such as codeine, methadone, citalopram, and ramipril) and some illicit (such as cocaine and methamphetamine) drugs; [18, 22, 26] thus, the work should be extended to other compounds. Using WBE to evaluate population health, especially the consumption patterns of prescription pharmaceuticals, would require reliable CFs for prescription pharmaceuticals, which are important to monitor health and disease outcomes.

We chose three pharmaceuticals as the targets in this study due to their high detection frequency in wastewater, large consumption in the population and potential to be used as biomarkers in WBE. Atenolol is a beta-blocker primarily used to treat high blood pressure and heart-associated chest pain. [27] Carbamazepine is an anticonvulsant and antiepileptic pharmaceutical mostly used to prevent and control seizures and treat trigeminal neuralgia and some psychiatric disorders. [28] Naproxen is a commonly used nonsteroidal anti-inflammatory pharmaceutical to treat pain, menstrual cramps, and inflammatory diseases. [29] In Australia, atenolol, and carbamazepine are prescription-only (usually from a doctor) and are dispensed in community pharmacies, naproxen can be prescribed but it's also available over the counter (OTC) in pharmacies. Dispensing data for prescribed pharmaceuticals can be acquired from the Pharmaceutical Benefit Scheme (PBS). The PBS is a national formulary (schedule) of prescription pharmaceuticals subsidized by the Australian Government. Data on dispensing of PBS-listed pharmaceuticals are recorded for the whole country, so PBS data can provide detailed information about the dispensed pharmaceuticals, prescription location, and patient-related factors such as gender and age. [30] The PBS data covers all community prescribing but does not include pharmaceuticals used by hospital inpatients. [31]

While the PBS data can provide useful information on the supply side, WBE can provide estimates of consumption at finer temporal and spatial resolution.

Using the mass load of biomarkers in wastewater and dispensed prescription or sales data improves the applicability of CFs on a population scale. [17, 22] This approach has the advantage of having numerous individuals contributing to wastewater samples, which can help to diminish the influence of inter-individual variations in excretion. In addition, this CF calculation method can also integrate the formation and degradation of biomarkers in the sewer and the sample. The objective of this study is to calculate the CFs for atenolol, carbamazepine, and naproxen by juxtaposing their mass loads measured in wastewater samples collected from Australian WWTPs during Census week in 2016 with PBS-dispensed prescription data in the selected catchments in the same year. We focused on calculating the CF of the parent compounds due to their high in-sewer stability, low sorption potential to suspended solids (low log K_{ow} as in Table S1) and relatively high excretion. The calculated CFs were then applied to an independent WBE dataset of 335 daily samples from an urban catchment to examine their applicability.

2. Materials and methods

2.1 Chemicals and Reagents

Atenolol, carbamazepine, naproxen, atenolol-d7, and carbamazepine-d10 were purchased from Sigma Aldrich. The category, applications, log K_{ow} and water solubility of the three pharmaceuticals investigated are provided in Table S1. Analytical grade hydrochloric acid (32%) was purchased from Univar (Ingleburn, Australia). LCMS grade methanol was purchased from Merck (Germany). Deionized water was produced by a MilliQ system (Millipore, 0.22 μm filter, 18.2 $m\Omega \cdot cm^{-1}$).

2.2 Wastewater sampling and analysis

Influent wastewater samples were collected during the 2016 Census week from 31 wastewater treatment plants (WWTPs) across Australia; these WWTPs serve approximately 24% of the Australian population. Five to seven consecutive 24-h composite influent samples per WWTP were collected using autosamplers. Five samples were collected at locations where weekend sampling was not possible. Both time proportional (15 min sampling frequency as the typical setting) and flow proportional samplers (the settings were WWTP specific depending on the daily flow) were used. Detailed sample information is provided in Table S2. Samples were acidified on-site to pH 2 and frozen immediately after collection, couriered frozen overnight back to the laboratory. For validation purposes, we used data from 186 samples collected in 2011 and 149 samples collected in 2012 from an urban catchment in Queensland, Australia, with a population of ~240,000. [32] One mL filtered and acidified sample was spiked with 10 µL of deuterium-labeled internal standards mix (1 mg/L) before analysis. Samples were analyzed using liquid chromatography coupled with tandem mass spectrometry by direct injection of the filtered samples. [33] A Shimadzu Nexera HPLC system (Kyoto, Japan) coupled with a Sciex API 5500 mass spectrometer (Ontario, Canada) was used for quantification. A 2.6µm 50 x 2.0 mm Phenomenex Kinetek Biphenyl column (Torrance, CA, USA) was used for chromatographic separation. Detailed mass spectrometer parameters can be found in Table S3. The method validation results are presented in Table S4.

2.3 Data on dispensed pharmaceuticals

The data on dispensed use were acquired from the Department of Human Services, including information about year issued, PBS item code, dose, formulation (Table S5), prescription location (local governmental areas, LGAs and states or territories), gender, and age range between 2013 and 2017. The quantity of the selected pharmaceuticals dispensed to patients by gender and age group, and by formulation and dose are shown in Figures S1-S2. The annual consumption of active pharmaceuticals was calculated according to the mass (mg) in each of the formulations and the

quantity prescribed (e.g. 30 tablets per dispensed prescription for a month of treatment). The PBS does not cover the use of medicines by inpatients in public hospitals but this is likely to be a small proportion of total use. [30]

2.4 WWTP catchments, local government area (LGA), and population

Overall, 31 WWTPs across Australia were included in this study with an equivalent population of 5,567,069. Some WWTP catchment areas were represented by only one LGA, while several WWTP catchment areas contained multiple LGAs. In addition, some LGAs were serviced by multiple WWTPs. In the case where several LGA were within the WWTP catchment boundary, the geographic areas of the WWTPs and the LGA's were corresponded in geospatial software to determine the fraction of each LGA that was within the WWTP catchment boundary. The total prescribed drug was then summed according to the fraction of each LGA that was within the WWTP catchment boundary. Where multiple WWTP catchment areas were within one LGA area, the population-weighted average consumption estimate of each of the individual WWTP catchments was compared with the corresponding population normalized prescription data in the catchment.

The population of each catchment was refined using the Census mesh block information and the catchment boundaries using geospatial software (Arc GIS). [34] In this manner, the PBS and catchment data were compared at the highest possible spatial resolution. It should be noted that population movement between LGAs would affect the accuracy of the population used in the CF calculation, however, population movement influences the population estimate mainly in smaller populations in tourism-designated areas, with a negligible change expected for the other catchments.

2.5 Calculation of the new CFs

The daily mass load of the pharmaceuticals was calculated by multiplying the concentration measured in each sample by the corresponding daily flow, yielding a load of the pharmaceutical

excreted into the WWTP on that day. The annual dispensing data of each target pharmaceutical as described in section 2.3 was used as the consumed amount of pharmaceuticals in each WWTP catchment. In GraphPad Prism, the consumed amount of pharmaceuticals in each of the WWTP was plotted against the mass load of pharmaceuticals in wastewater (WBE) as showed in Fig. 3. A simple linear regression line was constructed for each compound with the line being forced to go through zero, and its 95% confidence bands displayed. The CF was the reciprocal of the slope of the linear regression line and the 95% confidence interval of CF was the reciprocal of the 95% confidence interval of the slope.

2.6 Application of the calculated CF

To check whether the calculated CFs can improve the accuracy of consumption estimates, separate temporal datasets in 2011 and 2012 were selected in one catchment in South East Queensland, Australia, and compared with PBS data. Specifically, the average daily WBE estimates were calculated for 2011 and 2012 from 186 daily wastewater samples in 2011 and 149 daily wastewater samples in 2012. Some of the catchment characteristics are provided in Table S6. Since we could not acquire the prescription data for 2011 and 2012, we compared the WBE results in 2011 and 2012 with the PBS records from 2013 to 2015. While this assumes similar consumption levels over the years, the purpose of the comparison was to determine the relative precision of the calculated CF. In addition, we do not expect large changes in consumption of these prescription pharmaceuticals over several years (Table S7). The estimate was also applied to reported data in literature to test the applicability of the CF in other countries.

3. Results and discussion

3.1 Per capita mass load of pharmaceuticals in wastewater

Unsurprisingly, there were strong linear correlations between the mass loads of pharmaceuticals in wastewater and the population of the wastewater catchments (R^2>0.95, Fig. 1). This indicated that the per-capita consumption of these pharmaceuticals was relatively similar across the 31

catchments. The per capita mass loads of atenolol, carbamazepine, and naproxen measured in wastewater were 360, 130, and 420 mg/day/1000 inh, respectively. It is noteworthy that the per capita mass load of atenolol across Australia was 6 times higher than Malè in the Maldives (58 mg/day/1000 inh) and slightly higher than five WWTPs in Belgium (64 - 222 mg/day/1000 inh) and Oslo in Norway (220 mg/day/1000 inh). However, carbamazepine in Australia was 18-67 times lower than the five WWTPs in Belgium (2900-8700 mg/day/1000 inh) and Oslo (2400 mg/day/1000 inh), but more than 3 times higher than Malè in the Maldives (38 mg/day/1000 inh) . [21, 35, 36] The climate between Australia, Belgium, Maldives, and Norway is very different, the possible in-sewer degradation of biomarkers may vary due to the temperature differences. In addition, the different prescription habits and/or disease prevalence among countries would also contribute to the observed per capita mass load differences.

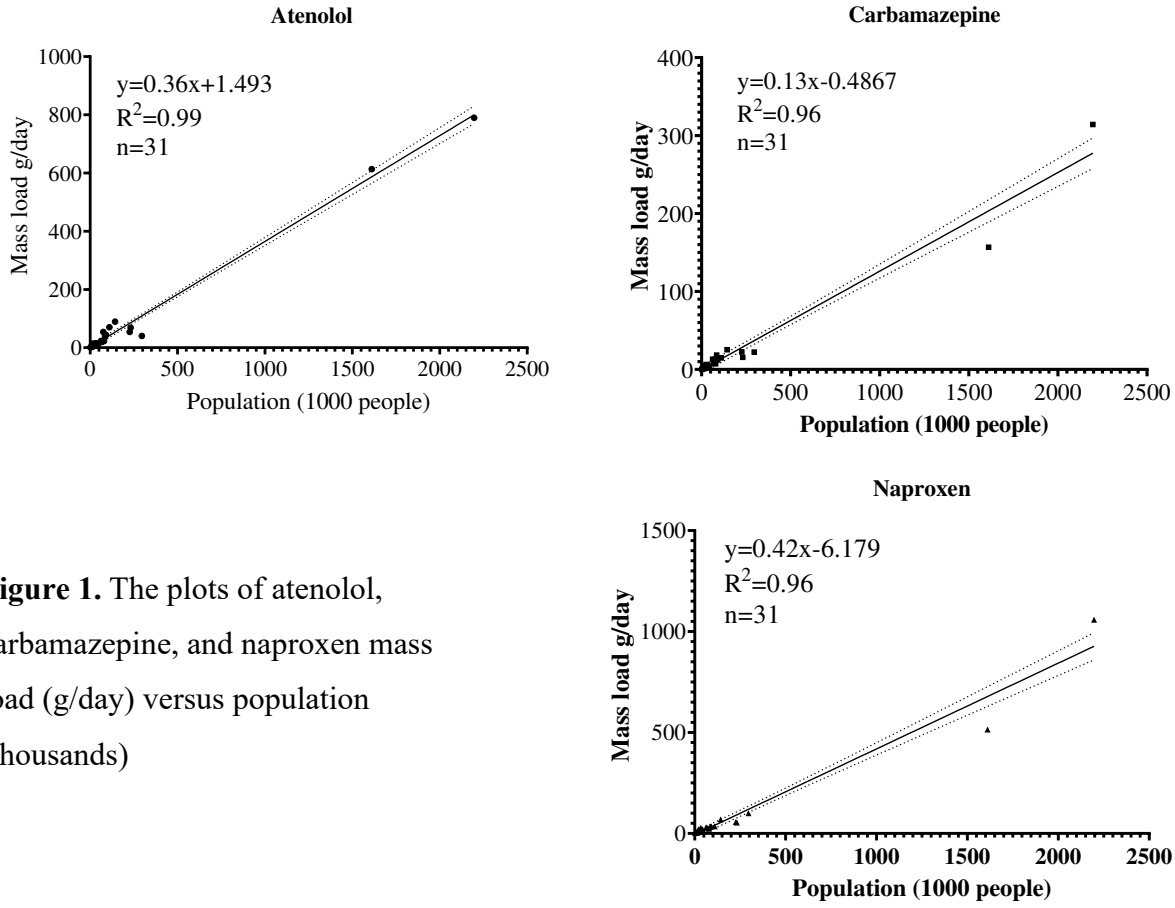


Figure 1. The plots of atenolol, carbamazepine, and naproxen mass load (g/day) versus population (thousands)

3.2 Pharmaceutical mass load vs dispensed data

Qualitatively, a simple comparison of the per capita dispensed data and the population normalized mass loads of target pharmaceuticals detected in wastewater at a state-level demonstrated that the two datasets follow similar trends (Fig. 2). For example, Queensland (QLD) and Victoria (VIC) had higher per capita PBS dispensed use (consumption) of atenolol than New South Wales (NSW), and the population normalized mass load of atenolol in wastewater samples collected from these two states were also higher. It is because the large population and aggregated WBE data may have helped to reduce the variation[37]. The figure demonstrates that both WBE and dispensed data can be used to monitor the geographical consumption patterns of pharmaceuticals on a large scale. Dispensed (PBS) data can provide more detail about the profile of patients (such as gender and age distribution) (Fig. S1 and S2); while WBE can provide daily consumption profiles and estimate the pharmaceutical adherence at the population level if accurate CFs can be used.

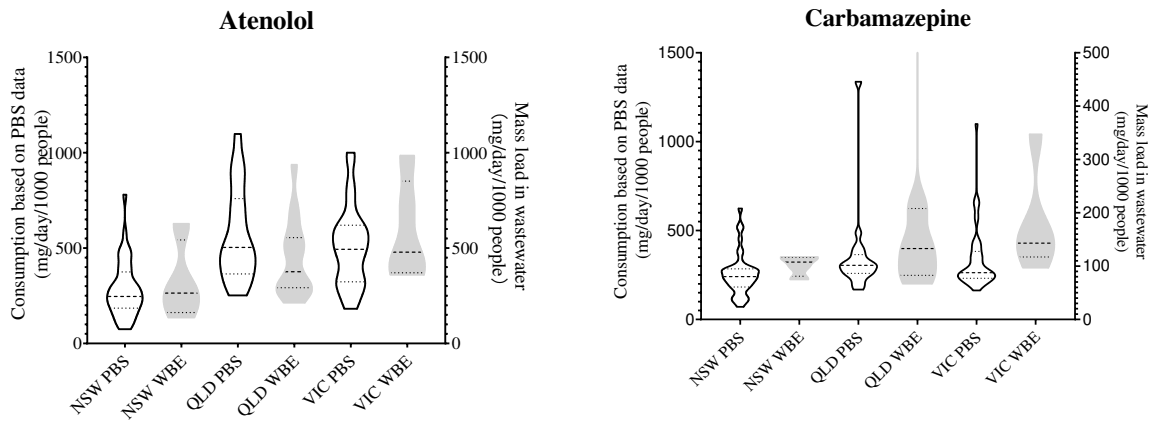
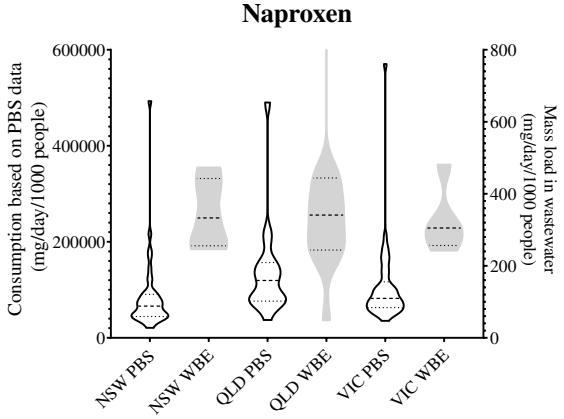


Figure 2. Per capita mass loads of atenolol, carbamazepine, and naproxen (g/day) and the average daily consumption-based in different States. The Violin plots show the frequency distribution of the data, the thick dash line is the median value and the light dash lines are the 25% and 75% percentile



3.3 Derivation of the CFs

Plots of the daily mass loads of atenolol, carbamazepine, and naproxen in wastewater and the corresponding daily consumption in the 31 WWTP catchments are shown in Fig. 3. The newly derived CF values are shown in Table 1 with the associated 95% confidence interval.

The R^2 values of the linear regression were relatively low, especially for naproxen. Several factors can contribute to the poor correlations which are discussed in section 3.5. Nevertheless, for the case of naproxen, the lack of over-the-counter sales data would strongly affect the correlation of mass load in wastewater and dispense data in our study.

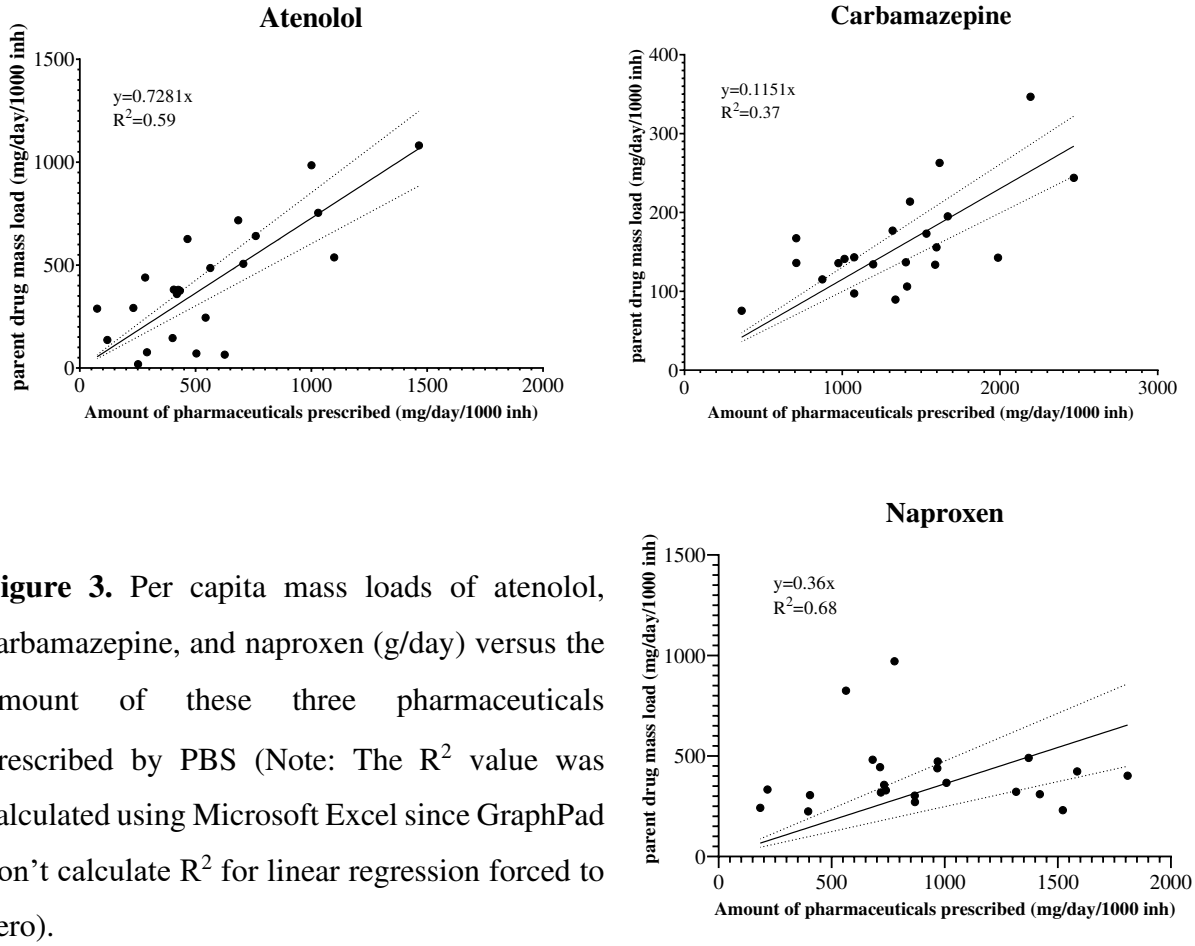


Figure 3. Per capita mass loads of atenolol, carbamazepine, and naproxen (g/day) versus the amount of these three pharmaceuticals prescribed by PBS (Note: The R^2 value was calculated using Microsoft Excel since GraphPad don't calculate R^2 for linear regression forced to zero).

Table 1. Correction factors (CFs) of atenolol, carbamazepine, and naproxen calculated in this study, and CF used in previous studies

	Atenolol	Carbamazepine	Naproxen
CF Mean (95% CI)	1.37 (1.17-1.66)	8.69 (7.66-10.03)	>2.63 (2.11-4.03)
CF used in previous studies	1.11[38], 1.20[39, 40], 2.22[21], 2.7[21, 35, 38-40]	6.67[39, 40], 7.14[35], 10[38], 20[21],	1.43[38], 10[39]
CF calculated using the method from Gracia-Lor et al., 2016 using pharmacokinetic data	1.75	83	1.11

3.4 Comparison with CFs derived from pharmacokinetic studies

3.4.1 Atenolol

The CF of atenolol is 1.37 (Table 1), which is slightly greater than the CF derived from pharmacokinetic studies of 1.22 or 1.15 (Table S8, reciprocal of the excretion factor) [41, 42], but smaller than 2.22 and 2.7 as previously used. [35, 43] Our calculated CF is also slightly smaller than the 1.75 CF that used the weighted mean of pharmacokinetic studies as reported previously. [24] With six healthy male volunteers it was found that some stereoselective metabolism of racemic atenolol was observed (d-atenolol and l-atenolol excretion rate of 22% and 19%, respectively) . [19] This translates to CF's of 4.54 and 5.26, respectively, which is much higher than our calculated CF. The route of administration (i.e. whether atenolol is taken orally or intravenously), can affect the excretion rate from 50% to 85%. [44] However, in Australia, atenolol is predominantly prescribed as a 50 mg tablet with negligible amounts of oral liquid and without intravenous solution (Table S5). In addition, some studies suggest that there could be 5-48% of atenolol could be excreted in feces. [45, 46] However, this would not affect the overall CF determined with our method since atenolol is quite hydrophilic, and the atenolol excreted in feces would preferentially partition into the water phase and be quantified during wastewater

255 analysis. Therefore, lateral WBE studies should use CF considering not only the excretion in
256 the urine but also in the feces.

257 **3.4.2 Carbamazepine**

258 The carbamazepine CF of 8.69 calculated in this study is much lower than 200 or 40 based on
259 the excretion fraction of 0.5% or 2-3% reported in previous pharmacokinetic studies (Table
260 S8). [47, 48] Carbamazepine undergoes extensive metabolism after its consumption, and therefore
261 the excretion of the parent compound is limited. However, carbamazepine-N-glucuronide is
262 also excreted following carbamazepine consumption [49], and carbamazepine-N-glucuronide
263 would likely undergo in-sewer/in-sample deconjugation and release free carbamazepine. [50-52]
264 This may be a cause of the discrepancy between the CFs used in the literature and the calculated
265 CF in our study. Our calculated CF of 8.69 is also much smaller than 83 as calculated using the
266 available pharmacokinetic data by weighing the excretion factor of each study by the number
267 of participants, and this further support that carbamazepine conjugates would undergo
268 deconjugation in the sewers. In addition, it was reported that approximately 28% of
269 carbamazepine and its metabolites are excreted in faeces[28], and carbamazepine is potentially
270 excreted with feces and has a certain amount absorbed in the suspended solids in wastewater,
271 which can subsequently contribute to the CF uncertainty. [53]

272 It was also found that the excretion profile of carbamazepine from healthy volunteers can be
273 different to epileptic patients who regularly use carbamazepine for treatment. [54] Also worth
274 noting is that the metabolism and excretion of carbamazepine among epilepsy patients are dose-
275 dependent. [55] In Australia the dose formulations of carbamazepine vary from 100 mg to 400
276 mg tablets, with modified release forms available (Table S5). The CF calculation method in
277 this study would integrate such variance and provide an overall CF for consumption estimation
278 on a large population scale which accounts for some of the complexity surrounding
279 administration, doses, and metabolic variance between individuals as discussed above.

3.4.3 Naproxen

The calculated naproxen CF in this study is 2.63, which is likely an underestimation because of the lack of OTC consumption data. This CF value is different from CF of 1.43 based on the sum of naproxen and conjugated forms with a total excretion factor of ~70%. The CF is also higher than the 1.11 derived using the method proposed by Gracia-Lor et al. (2016). With 100 mg intravenous injection on healthy volunteers, Runkel et al. (1974) reported naproxen is excreted as 10% unchanged with another 60% conjugated,[56] which was used by Riva et al. (2015) that assumed complete deconjugation for an excretion factor of 70% in their calculation. Upton et al. (1980) found less than 1% unchanged and approximately 60% in the conjugated form in 12-hour urine samples from four healthy volunteers taking 500 mg naproxen orally. [57] Kasprzyk-Hordern et al. (2009) used <1% unchanged with 66-92% in the conjugated form in their calculations, also assuming complete deconjugation. [38] Therefore, according to available pharmacokinetic studies, considerable amounts of naproxen are excreted in conjugated form, and most studies assume in-sewer biochemical processes completely deconjugate naproxen, which may not necessarily be the case. Factors such as age and alcohol consumption are also understood to impact the metabolism and excretion of naproxen [58, 59]. Therefore, catchments with older populations or substantially higher alcohol consumption may influence the derived CF. The dose of naproxen may also affect the excretion factor considering naproxen dose formulations in Australia vary from 250 mg to 1 g tablets with negligible amounts of 25 mg/ mL oral liquid (Table S5).

3.5 Application of the calculated CFs

The WBE estimates based on the newly calculated CF matched relatively well with the dispensed data in the urban catchment for atenolol and carbamazepine (Table 2) despite the considerable daily variation of pharmaceutical mass loads in the catchment (Fig. S3). However, the new CF for naproxen did not provide a good match as the other two pharmaceuticals. It

again indicates that the lack of OTC sales of naproxen increases the uncertainty of naproxen CF and makes it less suitable for WBE applications. We acknowledge the limitations of comparing WBE and prescription data in different years. The PBS data showed that the annual use of the three pharmaceuticals did not fluctuate significantly (coefficient of variation of 9%, 6%, and 6% for atenolol, carbamazepine, and naproxen annual total masses consumed 2013-2017). These fluctuations should be considered when interpreting the data, as the data were collected in different years: WBE 2011-2012 and the PBS 2013-2015.

We also noted that the WBE estimates and the dispensed data are poorly matched to literature from other countries using either their existing CF or our calculated CF; WBE can overestimate or underestimate the pharmaceutical consumption compared with dispensed prescription data (Table 3). For example, atenolol consumption was overestimated and underestimated by WBE in France in the same WWTP by the same research group. [39, 40] Overall, the matches using the old literature CF (1.11-2.7 in different studies) are better than using our calculated CF for atenolol, and the literature CF fall into the 95% CI of our calculated CF, this indicates that atenolol CF may vary among catchments within the range of 1.10-2.42. Our calculated CF is similar to the one used by Kasprzyk-Horden et al. (2009), and provided better matches between WBE and prescription data in France and Belgium. The limited number of wastewater samples in these publications may have contributed to the poor matches between WBE estimates and annual prescription/sales data as a previous study has found out that up to 56 stratified random samples are required to obtain reliable annual estimates of illicit drug loads. [37] Besides, those studies reported results from one or two catchments and used the national average prescription data for comparison, where there could be significant spatial differences in consumption adding another layer of uncertainty. Therefore, it is important to have adequate data to evaluate the applicability of our calculated CF in other countries.

330 **Table 2** WBE estimates using calculated CFs and PBS data in an urban catchment

	WBE estimates g/day in 2011 (186 days)	WBE estimates g/day in 2012 (149 days)	PBS 2013 g/day	PBS 2014 g/day	PBS 2015 g/day
Atenolol	147±33	160±38	148	136	130
Carbamazepine	233±41	229±47	206	228	240
Naproxen	313±70	297±85	212	186	189

331 PBS: The Pharmaceutical Benefit Schedule (PBS) is a national formulary of prescription
332 pharmaceuticals subsidized by the Australian Government. The PBS covers community use of
333 medicines but does not include those used by hospital inpatients. There can be seasonal
334 variations because of the effect of the Safety Net (consumers get their medicines dispensed
335 while they may be eligible for reduced prices within a calendar year). There may be a time lag
336 from the date of dispensing to consumption; the actual consumption could be lower than
337 dispensed PBS volumes if the patient does consume the full course of medicines (reduced
338 adherence).

Table 3 Comparison of WBE estimates using old and new EF/CF in the literature

Country	Catchment population	# of samples	Prescription/sales data (mg/day/1000 inh)	Old estimate (mg/day/1000 inh)	Estimate using new CF (mg/day/1000 inh)	Match Old vs New
Atenolol						
Norway[35]	~58,000	7	2396*	582	295	24% vs 12%
UK[38]	111,000 and 30,000	~20	2000	5900	7281	295% vs 364%
France[39]	~70,000	13	761*	887	1013	116% vs 133%
France[40]	~70,000	83	761*	319	353	42% vs 48%
Belgium[21]	59400, 63000, 58500, 43200, 54900	5	758/728/649/906/1073	149/64/189/222/107	92/40/117/137/66	9-29% vs 5-18%
Carbamazepine						
Norway[35]	~58,000	7	10879*	16679	23351	153% vs 215%
UK[38]	111,000 and 30,000	~20	2500	4700	4700	188% vs 188%
France[39]	~70,000	13	1390*	99	158	7% vs 11%
France[40]	~70,000	83	1390*	366	512	26% vs 37%
Belgium[21]	59400, 63000, 58500, 43200, 54900	5	1040/2040/1449/1339/1397	2923/4261/8739/3859/3556	1329/1937/3972/1754/1616	209-603% vs 95-274%
Naproxen						
France[39]	~70,000	13	1550*	895	224	58% vs 14%
UK[38]	111,000 and 30,000	~20	2500	700	1225	28% vs 49%

* : sales data

3.6 Factors affecting the derivation of CFs in this study

Apart from the lack of OTC data that critically affects the calculation of the naproxen CF with our approach, other factors could contribute to the uncertainty of the CF.

3.6.1 Seasonal variation of pharmaceutical consumption

We have shown previously that the level of atenolol and naproxen consumption varied according to the ambient temperature, i.e. seasons, while carbamazepine consumption was stable throughout the year. Atenolol was consumed more during winter but naproxen was consumed more in summer. [32]

Our approach used five to seven daily samples from each catchment in August (winter in Australia), which may not be fully representative of the annual average daily consumption. We obtained the average daily dispensed amount by dividing the annual consumption by the total number of days in a year. Therefore, the seasonal consumption variation could contribute to the poor correlation between the mass load of pharmaceuticals and the corresponding prescription dispensing data (see section 3.2).

3.6.2 Incomplete use and direct disposal of unused pharmaceuticals to the sewers

The poor correlation between pharmaceutical mass loads in wastewater and the dispensed prescription data can also be attributed to poor adherence and incomplete use of the prescribed pharmaceuticals, possible direct disposal of unused pharmaceuticals to the sewers, and hospital use. Previous studies have found that some of the pharmaceuticals kept by patients may not be fully consumed. [60, 61] If there were considerable amounts of pharmaceuticals that remain unused in Australia, then the PBS data may overestimate actual consumption and subsequently overestimate the CF. Also, if the unused pharmaceuticals are disposed directly into the toilet and sewers, this would increase the mass load of parent compounds in the wastewater samples

and would underestimate the CF. Therefore, further studies should focus on calculating the CF of stable metabolites instead of parent compounds.

3.6.3 Demographics of the catchment population

The metabolism and excretion of pharmaceuticals are affected by age [62], gender [63], and ethnicity [64]. The WWTP Census sampling catchment populations have diverse demographic characteristics such as age distribution [34]. Therefore, depending on the age distribution and ethnic group compositions, the excretion factor and CF can vary between catchments, so the CF calculated in this study is mainly for estimation of the selected pharmaceuticals in large populations. For geographical comparison of smaller populations using WBE, catchment-specific CF may need to be considered if demographic characteristics such as age distribution are very different.

3.6.4 Dosage, form, and co-consumption of substances

The excretion factor of pharmaceuticals can vary by dose and formulation (i.e. tablet, capsule, oral liquid, or intravenous solution) [65, 66] and the co-consumption of substances such as alcohol, tobacco, and caffeine. [67] All the three pharmaceuticals investigated in this study were available in multiple dose formulations in the Australian market (Table S2, Fig. S1-3), so the CFs calculated in this study are the overall CFs of all dose formulations and dose regimes. Besides, there are considerable regional differences in alcohol and tobacco consumption in the Australian population [4], therefore, the CFs for a particular catchment with substantially higher or lower alcohol and tobacco consumption would deviate from the CFs we calculated. Previous studies have derived CFs by conducting a meta-analysis of all pharmacokinetic studies and taking the route of administration and number of subjects into account. Such CFs would minimize the uncertainty of excretion variations due to dose formulations and different number of participants among traditional pharmacokinetic studies. [9, 24] However, the CFs calculated with such method does not include the possible sorption/degradation and incomplete use of

389 pharmaceuticals. Therefore, it is recommended to derive CFs using our method where both
390 WBE and prescription data are available.

391 **3.6.5 Sorption and degradation of biomarkers**

392 Although the three investigated biomarkers are relatively hydrophilic with Log K_{ow} range from
393 0.16 to 3.18 (Table S1), and the previous study has indicated that sorption of all the three
394 biomarkers to suspended solids are small in wastewater[68], but the acidification preservation of
395 samples may change the charge state of biomarkers and promote the sorption to suspended
396 particles and filters. Therefore, loss of biomarkers due to sorption would contribute to the
397 overall uncertainty of our calculated CFs, especially for naproxen with a pKa value of 4.84 will
398 be present as the fully protonated form which could have higher sorption potential. The poor
399 correlation between naproxen mass load in wastewater and the PBS data as in section 3.3 may
400 also attribute to the sorption issue of naproxen in acidified wastewater. Furthermore, in-sewer
401 and in-sample loss of biomarkers can also affect the accuracy of the calculated CF. Naproxen
402 was found to have some level of degradation with high area/volume ratios and long hydraulic
403 retention time in sewer reactors, despite its stability in pilot sewers. [12, 69] Atenolol and
404 carbamazepine were found to be stable under laboratory and pilot-scale sewer conditions.
405 However, these studies were conducted at near room temperature (~20 ℃) and controllable
406 environment, during the sampling period in this study (August) there could be considerable
407 differences in wastewater temperatures across Australia (and the sewer microorganisms and
408 bioactivity would be expected to vary largely) where the substantial biomarker losses can vary.
409 [70]

410 **3.7 Limitations and future perspective**

411 The CFs reported in our study are the combination of several variables: the drug excretion
412 following ingestion, the in-sewer sorption and degradation, and the proportion of dispensed

413 drug which is consumed (compliance). Due to the nature of the CF calculation and the CF
414 validation method, we acknowledge there are some limitations of our method. Firstly, we
415 calculated the CF of the parent drugs for atenolol, carbamazepine, and naproxen. While
416 analyzing metabolites would be more specific for identification purposes (and eliminate the
417 influence of the disposed drug), most WBE studies have focussed on these pharmaceuticals as
418 the parent drug, and so further studies should expand this method to more prescription
419 pharmaceuticals and calculate the CF of both parent compound and their metabolites. Secondly,
420 we applied the CF using WBE data from different years to prescription data: 2011-2012 and
421 2013-2015, respectively. Unfortunately, these were the only years available for comparison but
422 may introduce some uncertainty of the indirect comparison. The prescription statistics of the
423 three drugs did not vary much year-to-year and so this would have had a minor effect.
424 Validating the CF in another country would also be beneficial in the future. Thirdly, the
425 prescription data used is the best available dataset that can reflect the consumption of
426 prescription pharmaceuticals. However, it may not include the total amount of drug dispensed
427 due to the collection and recording method of PBS data, which may underestimate the CF to
428 some extent. Therefore, more accurate prescription dispensing/sales data is preferable for
429 further studies using our method.

430 In summary, we observed considerable temporal and spatial variations in the consumption of
431 atenolol, carbamazepine, and naproxen in Australia. Using the mass load of pharmaceuticals
432 in wastewater and the dispensed data, we calculated the CFs for atenolol, carbamazepine, and
433 naproxen. The newly calculated CFs of atenolol and carbamazepine were validated to an
434 independent WBE dataset consisting of >300 daily samples from an urban catchment in
435 Queensland and WBE gave results with 111% (atenolol) and 103% (carbamazepine) of the
436 expected value calculated from prescription data. It was not successful for naproxen, probably
437 due to the lack of OTC data. CFs can vary due to multiple factors, but the method in this study

integrates some of the variations and provides suitable CFs for WBE studies in large populations.

Acknowledgments

The Queensland Alliance for Environmental Health Sciences and the University of Queensland gratefully acknowledges the financial support of the Queensland Department of Health. The authors acknowledge and express our thanks to the SewAus team for their assistance in analyzing and collecting the samples as well as the personnel of all WWTPs for the sampling and information provided.

Supporting Information

Additional information about the chemical properties of investigated pharmaceuticals, wastewater samples, LC-MS/MS parameters for analysis, the PBS classification of investigated pharmaceuticals, Catchment characteristics of the urban catchment used for CF verification, Quantity of atenolol, carbamazepine, and naproxen prescribed in Australia from 2014 to 2017, pharmacokinetic information of the three pharmaceuticals, the quantity of atenolol, carbamazepine and naproxen prescribed in the Australian population in 2016 categorized by gender, the quantity of atenolol, carbamazepine and naproxen prescribed in the Australian population categorized by age group, and the mass load of atenolol, carbamazepine and naproxen in the urban catchment of Queensland 2011-2012 is provided.

Reference

1. EMCDDA, E. M. C. f. D. a. D. A. *Wastewater analysis and drugs — a European multi-city study*; 2018. http://www.emcdda.europa.eu/topics/pods/waste-water-analysis
2. Ort, C.; van Nuijs, A. L.; 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), 1338-52.
3. Postigo, C.; a, M. L. d. A.; Barceló, D., Evaluation of drugs of abuse use and trends in a prison through wastewater analysis. *Environment International* **2011,** *37*, (1), 49-55.
4. Lai, F. Y.; Gartner, C.; Hall, W.; Carter, S.; O'Brien, J.; Tscharke, B. J.; Been, F.; Gerber, C.; White, J.; Thai, P., Measuring spatial and temporal trends of nicotine and alcohol consumption in Australia using wastewater-based epidemiology. *Addiction* **2018**, 1-10.
5. Gonzalez-Marino, I.; Baz-Lomba, J. A.; Alygizakis, N. A.; Andres-Costa, M. J.; Bade, R.; Bannwarth, A.; Barron, L. P.; Been, F.; Benaglia, L.; Berset, J. D.; Bijlsma, L.; Bodik, I.; Brenner, A.; Brock,

A. L.; Burgard, D. A.; Castrignano, E.; Celma, A.; Christophoridis, C. E.; Covaci, A.; Delemont, O.; de Voogt, P.; Devault, D. A.; Dias, M. J.; Emke, E.; Esseiva, P.; Fatta-Kassinos, D.; Fedorova, G.; Fytianos, K.; Gerber, C.; Grabic, R.; Gracia-Lor, E.; Gruner, S.; Gunnar, T.; Hapeshi, E.; Heath, E.; Helm, B.; Hernandez, F.; Kankaanpaa, A.; Karolak, S.; Kasprzyk-Hordern, B.; Krizman-Matasic, I.; Lai, F. Y.; Lechowicz, W.; Lopes, A.; Lopez de Alda, M.; Lopez-Garcia, E.; Love, A. S. C.; Mastroianni, N.; McEneff, G. L.; Montes, R.; Munro, K.; Nefau, T.; Oberacher, H.; O'Brien, J. W.; Oertel, R.; Olafsdottir, K.; Pico, Y.; Plosz, B. G.; Polesel, F.; Postigo, C.; Quintana, J. B.; Ramin, P.; Reid, M. J.; Rice, J.; Rodil, R.; Salgueiro-Gonzalez, N.; Schubert, S.; Senta, I.; Simoes, S. M.; Sremacki, M. M.; Styszko, K.; Terzic, S.; Thomaidis, N. S.; Thomas, K. V.; Tscharke, B. J.; Udrisard, R.; van Nuijs, A. L. N.; Yargeau, V.; Zuccato, E.; Castiglioni, S.; Ort, C., Spatio-temporal assessment of illicit drug use at large scale: evidence from 7 years of international wastewater monitoring. *Addiction* **2020,** *115*, (1), 109-120.

6. González-Mariño, I.; Rodil, R.; Barrio, I.; Cela, R.; Quintana, J. B., Wastewater-Based Epidemiology as a New Tool for Estimating Population Exposure to Phthalate Plasticizers. *Environmental science & technology* **2017,** *51*, (7), 3902-3910.

7. Daughton, C. G., Monitoring wastewater for assessing community health: Sewage Chemical-Information Mining (SCIM). *Science of The Total Environment* **2018**, 748-764.

8. Choi, P. M.; O'Brien, J. W.; Li, J.; Jiang, G.; Thomas, K. V.; Mueller, J. F., Population histamine burden assessed using wastewater-based epidemiology: The association of 1,4methylimidazole acetic acid and fexofenadine. *Environment International* **2018,** *120*, 172-180.

9. Castiglioni, S.; Bijlsma, L.; Covaci, A.; Emke, E.; Hernández, F. l.; Reid, M.; Ort, C.; Kevin V. Thomas, A. L. N. V. N., Pim de Voogt, Ettore Zuccato, Evaluation of uncertainties associated with the determination of community drug use through the measurement of sewage drug biomarkers. *Environmental Science & Technology* **2013,** *47*, (3), 1452-1469.

10. Ort, C.; Lawrence, M. G.; Reungoat, J.; Mueller, J. F., Sampling for PPCPs in Wastewater Systems: Comparison of Different Sampling Modes and Optimization Strategies. *Environmental Science & Technology* **2010,** *44*, (16), 6289-6296.

11. Li, J.; Gao, J.; Thai, P. K.; Shypanski, A.; Nieradzik, L.; Mueller, J. F.; Yuan, Z.; Jiang, G., Experimental Investigation and Modeling of the Transformation of Illicit Drugs in a Pilot-Scale Sewer System. *Environmental Science & Technology* **2019,** *53*, (8), 4556-4565.

12. Gao, J.; Li, J.; Jiang, G.; Shypanski, A. H.; Nieradzik, L. M.; Yuan, Z.; Mueller, J. F.; Ort, C.; Thai, P. K., Systematic evaluation of biomarker stability in pilot scale sewer pipes. *Water Research* **2019,** *151*, 447-455.

13. Ramin, P.; Brock, A. L.; Causanilles, A.; Valverde Pérez, B.; Emke, E.; de Voogt, P.; Polesel, F.; Plosz, B. G., Transformation and sorption of illicit drug biomarkers in sewer biofilms. *Environmental Science & Technology* **2017,** *51*, 10572-10584.

14. 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 Research* **2017,** *122*, 655-668.

15. Choi, P. M.; Li, J.; Gao, J.; O'Brien, J. W.; Thomas, K. V.; Thai, P. K.; Jiang, G.; Mueller, J. F., Considerations for assessing stability of wastewater-based epidemiology biomarkers using biofilm-free and sewer reactor tests. *Sci Total Environ* **2019,** *709*, 1-7.

16. Castiglioni, S.; Thomas, K. V.; Kasprzyk-Hordern, B.; Vandam, L.; Griffiths, P., Testing wastewater to detect illicit drugs: State of the art, potential and research needs. *Science of The Total Environment* **2014,** *487*, 613-620.

17. Thai, P. K.; O'Brien, J. W.; Tscharke, B. J.; Mueller, J. F., Analyzing Wastewater Samples Collected during Census To Determine the Correction Factors of Drugs for Wastewater-Based Epidemiology: The Case of Codeine and Methadone. *Environmental Science & Technology Letters* **2019,** *6*, (5), 265-269.

18. Riva, F.; Castiglioni, S.; Pacciani, C.; Zuccato, E., Testing urban wastewater to assess compliance with prescription data through wastewater-based epidemiology: First case study in Italy. *Science of The Total Environment* **2020,** *739*, 139741.

19. Boyd, R. A.; Chin, S. K.; Don-Pedro, O.; Williams, R. L.; Giacomini, K. M., The pharmacokinetics of the enantiomers of atenolol. *Clinical Pharmacology Therapeutics* **1989,** *45*, (4), 403-410.

20. Bernus, I.; Dickinson, R.; Hooper, W.; Eadie, M., Early stage autoinduction of carbamazepine metabolism in humans. *European Journal of Clinical Pharmacology* **1994,** *47*, (4), 355-360.

21. van Nuijs, A. L.; Covaci, A.; Beyers, H.; Bervoets, L.; Blust, R.; Verpooten, G.; Neels, H.; Jorens, P. G., Do concentrations of pharmaceuticals in sewage reflect prescription figures? *Environmental Science and Pollution Research* **2015,** *22*, (12), 9110-9118.

22. 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. *Environment International* **2016,** *94*, 307-14.
23. Khan, U.; Nicell, J. A., Refined sewer epidemiology mass balances and their application to heroin, cocaine and ecstasy. *Environment International* **2011,** *37*, (7), 1236-1252.
24. Gracia-Lor, E.; Zuccato, E.; Castiglioni, S., Refining correction factors for back-calculation of illicit drug use. *Science of The Total Environment* **2016,** *573*, 1648-1659.
25. Khan, U.; Nicell, J. A., Sewer epidemiology mass balances for assessing the illicit use of methamphetamine, amphetamine and tetrahydrocannabinol. *Sci Total Environ* **2012,** *421-422*, 144-62.
26. Khan, U.; Nicell, J. A., Refined sewer epidemiology mass balances and their application to heroin, cocaine and ecstasy. *Environ Int* **2011,** *37*, (7), 1236-52.
27. Baselt, R. C., *Disposition of toxic drugs and chemicals in man*. Eleventh Edition ed.; 2017.
28. Morselli, P. L.; Frigerio, A., Metabolism and pharmacokinetics of carbamazepine. *Drug Metab Rev* **1975,** *4*, (4), 97-113.
29. Todd, P. A.; Clissold, S. P., Naproxen. *Drugs* **1990,** *40*, (1), 91-137.
30. Mellish, L.; Karanges, E. A.; Litchfield, M. J.; Schaffer, A. L.; Blanch, B.; Daniels, B. J.; Segrave, A.; Pearson, S. A., The Australian Pharmaceutical Benefits Scheme data collection: a practical guide for researchers. *BMC Res Notes* **2015,** *8*, 634.
31. PBS Australian Statistics on Medicines 2015. http://www.pbs.gov.au/info/statistics/asm/asm-2015 (2020.5.5),
32. Phung, D.; Mueller, J.; Lai, F. Y.; O'Brien, J.; Dang, N.; Morawska, L.; Thai, P. K., Can wastewater-based epidemiology be used to evaluate the health impact of temperature?–An exploratory study in an Australian population. *Environmental Research* **2017,** *156*, 113-119.
33. Gao, J.; O'Brien, J.; Du, P.; Li, X.; Ort, C.; Mueller, J. F.; Thai, P. K., Measuring selected PPCPs in wastewater to estimate the population in different cities in China. *Science of the Total Environment* **2016,** *568*, 164-70.
34. Tscharke, B. J.; O'Brien, J. W.; Ort, C.; Grant, S.; Gerber, C.; Bade, R.; Thai, P. K.; Thomas, K. V.; Mueller, J. F., Harnessing the Power of the Census: Characterizing Wastewater Treatment Plant Catchment Populations for Wastewater-Based Epidemiology. *Environmental Science & Technology* **2019,** *53*, (17), 10303-10311.
35. Baz-Lomba, J. A.; Salvatore, S.; Gracia-Lor, E.; Bade, R.; Castiglioni, S.; Castrignanò, E.; Causanilles, A.; Hernandez, F.; Kasprzyk-Hordern, B.; Kinyua, J., Comparison of pharmaceutical, illicit drug, alcohol, nicotine and caffeine levels in wastewater with sale, seizure and consumption data for 8 European cities. *BMC public health* **2016,** *16*, (1), 1035.
36. Fallati, L.; Castiglioni, S.; Galli, P.; Riva, F.; Gracia-Lor, E.; Gonzalez-Marino, I.; Rousis, N. I.; Shifah, M.; Messa, M. C.; Strepparava, M. G.; Vai, M.; Zuccato, E., Use of legal and illegal substances in Male (Republic of Maldives) assessed by wastewater analysis. *Sci Total Environ* **2020,** *698*, 134207.
37. Ort, C.; Eppler, J. M.; Scheidegger, A.; Rieckermann, J.; Kinzig, M.; Sorgel, F., Challenges of surveying wastewater drug loads of small populations and generalizable aspects on optimizing monitoring design. *Addiction* **2014,** *109*, (3), 472-81.
38. Kasprzyk-Hordern, B.; Dinsdale, R. M.; Guwy, A. J., Illicit drugs and pharmaceuticals in the environment--forensic applications of environmental data. Part 1: Estimation of the usage of drugs in local communities. *Environmental Pollution* **2009,** *157*, (6), 1773-7.
39. Thiebault, T.; Boussafir, M.; Le Milbeau, C., Occurrence and removal efficiency of pharmaceuticals in an urban wastewater treatment plant: mass balance, fate and consumption assessment. *Journal of environmental chemical engineering* **2017,** *5*, (3), 2894-2902.
40. Thiebault, T.; Fougère, L.; Destandau, E.; Réty, M.; Jacob, J., Temporal dynamics of human-excreted pollutants in wastewater treatment plant influents: Toward a better knowledge of mass load fluctuations. *Science of the Total Environment* **2017,** *596*, 246-255.
41. Tabacova, S. A.; Kimmel, C. A., Atenolol: pharmacokinetic/dynamic aspects of comparative developmental toxicity. *Reproductive Toxicology* **2002,** *16*, 1-7.
42. Riva, F.; Zuccato, E.; Castiglioni, S., Prioritization and analysis of pharmaceuticals for human use contaminating the aquatic ecosystem in Italy. *Journal of pharmaceutical biomedical analysis* **2015,** *106*, 71-78.
43. Petrie, B.; Barden, R.; Kasprzyk-Hordern, B., A review on emerging contaminants in wastewaters and the environment: current knowledge, understudied areas and recommendations for future monitoring. *Water research* **2015,** *72*, 3-27.
44. Fitzgerald, J.; Ruffin, R.; Smedstad, K.; Roberts, R., Studies on the pharmacokinetics and

588 pharmacodynamics of atenolol in man. *European journal of clinical pharmacology* **1978,** *13*, (2), 81-89.
589 45. Reeves, P. R.; McAinsh, J.; McIntosh, D. A. D.; Winrow, M. J., Matabolism of atenolol in man.
590 *Xenobiotica* **1978**, (8), 313-320.
591 46. Lienert, J.; Güdel, K.; Escher, B. I., Screening Method for Ecotoxicological Hazard Assessment
592 of 42 Pharmaceuticals Considering Human Metabolism and Excretory Routes. *Environmental Science*
593 *& Technology* **2007,** *41*, (12), 4471-4478.
594 47. Faigle, J.; Feldmann, K., Carbamazepine: Biotransformation. In *Antiepileptic Drugs*, D.M.
595 Woodbury, J. K. P., R.P. Schmidt, Ed. Raven Press, New York: 1982; pp 507-519.
596 48. Eichelbaum, M.; Köthe, K. W.; Hoffmann, F.; von Unruh, G. E., Kinetics and metabolism of
597 carbamazepine during combined antiepileptic drug therapy. *Clinical Pharmacology & Therapeutics*
598 **1979,** *26*, (3), 366-371.
599 49. Lynn, R.; Smith, R.; Thompson, R.; Deinzer, M.; Griffin, D.; Gerber, N., Characterization of
600 glucuronide metabolites of carbamazepine in human urine by gas chromatography and mass
601 spectrometry. *Drug Metabolism Disposition* **1978,** *6*, (4), 494-501.
602 50. Gao, J.; Li, J.; Jiang, G.; Yuan, Z.; Eaglesham, G.; Covaci, A.; Mueller, J. F.; Thai, P. K., Stability of
603 alcohol and tobacco consumption biomarkers in a real rising main sewer. *Water Research* **2018,** *138*,
604 19-26.
605 51. Jelic, A.; Rodriguez-Mozaz, S.; Barcelo, D.; Gutierrez, O., Impact of in-sewer transformation on
606 43 pharmaceuticals in a pressurized sewer under anaerobic conditions. *Water Research* **2015,** *68*, 98-
607 108.
608 52. Gao, J.; Banks, A.; Li, J.; Jiang, G.; Lai, F. Y.; Mueller, J. F.; Thai, P. K., Evaluation of in-sewer
609 transformation of selected illicit drugs and pharmaceutical biomarkers. *Science of The Total*
610 *Environment* **2017,** *609*, 1172-1181.
611 53. Bahlmann, A.; Brack, W.; Schneider, R. J.; Krauss, M., Carbamazepine and its metabolites in
612 wastewater: Analytical pitfalls and occurrence in Germany and Portugal. *Water Res* **2014,** *57*, 104-14.
613 54. Eichelbaum, M.; Tomson, T.; Tybring, G.; Bertilsson, L., Carbamazepine metabolism in man.
614 *Clinical pharmacokinetics* **1985,** *10*, (1), 80-90.
615 55. Bernus, I.; Dickinson, R. G.; Hooper, W. D.; Eadie, M., Dose-dependent metabolism of
616 carbamazepine in humans. *Epilepsy research* **1996,** *24*, (3), 163-172.
617 56. Runkel, R.; Forchielli, E.; Sevelius, H.; Chaplin, M.; Segre, E., Nonlinear plasma level response
618 to high doses of naproxen. *Clinical Pharmacology & Therapeutics* **1974,** *15*, (3), 261-266.
619 57. Upton, R. A.; Buskin, J. N.; Williams, R. L.; Holford, N. H. G.; Riegelman, S., Negligible excretion
620 of unchanged ketoprofen, naproxen, and probenecid in urine. *Journal of pharmaceutical sciences* **1980,**
621 *69*, (11), 1254-1257.
622 58. Upton, R.; Williams, R.; Kelly, J.; Jones, R., Naproxen pharmacokinetics in the elderly. *British*
623 *journal of clinical pharmacology*
624 **1984,** *18*, (2), 207-214.
625 59. Williams, R.; Upton, R.; Cello, J.; Jones, R.; Blitstein, M.; Kelly, J.; Nierenburg, D., Naproxen
626 disposition in patients with alcoholic cirrhosis. *European journal of clinical pharmacology*
627 **1984,** *27*, (3), 291-296.
628 60. Guirguis, K., Medications collected for disposal by outreach pharmacists in Australia. *Pharm*
629 *World Sci* **2010,** *32*, (1), 52-8.
630 61. Bettington, E.; Spinks, J.; Kelly, F.; Gallardo-Godoy, A.; Nghiem, S.; Wheeler, A. J., When is a
631 medicine unwanted, how is it disposed, and how might safe disposal be promoted? Insights from the
632 Australian population. *Australian Health Review* **2018,** *42*, (6), 709-717.
633 62. Shi, S.; Klotz, U., Age-related changes in pharmacokinetics. *Current drug metabolism* **2011,** *12*,
634 (7), 601-610.
635 63. Kato, R., Sex-related differences in drug metabolism. *Drug metabolism reviews* **1975,** *3*, (1), 1-
636 32.
637 64. Yasuda, S.; Zhang, L.; Huang, S. M., The role of ethnicity in variability in response to drugs:
638 focus on clinical pharmacology studies. *Clinical Pharmacology & Therapeutics* **2008,** *84*, (3), 417-423.
639 65. Henderson, E. S.; Adamson, R. H.; Oliverio, V. T., The Metabolic Fate of Tritiated Methotrexate.
640 *Cancer Research* **1965,** *25*, (7 Part 1), 1018-1024.
641 66. Riess, W.; Stierlin, H.; Degen, P.; Faigle, J. W.; Gerardin, A.; Moppert, J.; Sallmann, A.; Schmid,
642 K.; Schweizer, A.; Sulc, M.; Theobald, W.; Wagner, J., Pharmacokinetics and Metabolism of the Anti-
643 Inflammatory Agent Voltaren. *Scandinavian Journal of Rheumatology* **1978,** *7*, (sup22), 17-29.
644 67. Vestal, R. E.; Norris, A. H.; Tobin, J. D.; Cohen, B. H.; Shock, N. W.; Andres, R., Antipyrine
645 metabolism in man: influence of age, alcohol, caffeine, and smoking. *Clinical Pharmacology &*

Therapeutics **1975,** *18*, (4), 425-432.
68. Aminot, Y.; Fuster, L.; Pardon, P.; Le Menach, K.; Budzinski, H., Suspended solids moderate the degradation and sorption of waste water-derived pharmaceuticals in estuarine waters. *Science of The Total Environment* **2018,** *612*, 39-48.
69. O'Brien, J. W.; Banks, A. P.; 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. *Environmental Science & Technology* **2017,** *51*, (7), 3816-3823.
70. Hart, O. E.; Halden, R. U., Modeling wastewater temperature and attenuation of sewage-borne biomarkers globally. *Water Res* **2020,** *172*, 115473.