THE UNIVERSITY OF QUEENSLAND

AUSTRALIA

Refining the uncertainties and expansion of wastewater-based epidemiology for assessing population exposure to chemicals

Jake William O'Brien

Bachelor of Science (Hons I)

A thesis submitted for the degree of Doctor of Philosophy at

The University of Queensland in 2016

School of Medicine

Abstract

Systematic sampling and analysis of wastewater is an increasingly used tool to complement more traditional techniques for assessing consumption of licit and illicit chemical substances in the population. The use of wastewater sampling and analysis contributes to a broader field that is referred to as wastewater-based epidemiology (WBE). Both spatial and temporal analysis can be conducted quantitatively, quickly and cost effectively using the WBE approach.

In brief, per capita exposure to a given chemical within a population is estimated by measuring the concentration of that chemical or a metabolite in a representative wastewater sample multiplied by the volume of wastewater represented by the sample, divided by the population size from which the sample originates and correcting by factors such as the excretion factor of the metabolite or chemical, molecular weight change and potential stability of the chemical within the sewer. It has previously been determined that the population size is the largest uncertainty for WBE estimates.

The aim of this thesis it to therefore identify useful markers that allow population estimation for a given wastewater sample and apply this technique including to assess per capita exposure/release of a group of chemicals that have not been examined in previous WBE studies.

The approach for this thesis was to systematically collect samples on a day when the population is well defined. For this we collected samples on the 2011 Census Day in Australia from 10 wastewater catchments ranging in size from approximately 1,500 to 500,000 people. By providing catchment maps to the Australian Bureau of Statistics, the accurate population size for each catchment was determined.

The most obvious choice of a potentially useful population markers are endogenous chemicals such as creatinine. Therefore, in Chapter 2, creatinine was assessed as a population marker. It was found that there was no correlation between the mass load of creatinine in wastewater and the population. Using laboratory-scale sewer reactors with conditions representative of both gravity sewers and rising mains, it was found that creatinine, while stable in collected samples, is unstable under sewer conditions. We therefore conclude that creatinine is not suitable for predicting differences in population size particularly when different sewer systems are compared.

In Chapter 3, a method was developed to quantify 96 chemicals in wastewater influent and applied to the census wastewater samples to identify potential population size markers. Thirteen chemicals including acesulfame, caffeine, and pharmaceuticals and personal care products were detected in all samples and found to have a good correlation ($R^2 > 0.8$) between mass load and population size. A Bayesian inference model was developed which incorporated these potential population size markers to provide a population size estimate. The model was validated using a leave-one-out approach for all sites and comparing the population size estimate from the model with the accurate census population data. It was shown that for small catchments, the uncertainty of the estimate as measured by the width of the posterior was 1.1 to 2.4 times narrower than the width of the posterior using only the WWTP operator population size estimates. For large WWTP catchments, the width of the posterior using the population size model was between 5 and 40 times narrower than the WWTP operator population size estimates. Additionally, it was found that the posterior width of the model was improved with addition of more chemicals in the model.

In Chapter 4, using laboratory-scale sewer reactors, the impact of in-sewer degradation of the population markers identified in Chapter 3 was evaluated. It was found that five of the fourteen markers were stable under all conditions over the 12 hour study period. Those which were unstable ranged from little degradation over the study period and only under certain conditions to rapid degradation regardless of sewer conditions. Additionally we assessed whether or not the degradation of these chemicals impacted the population size estimation model by excluding the unstable compounds from the model. We found that the uncertainty of the estimate did not decrease through exclusion of the unstable chemicals.

In Chapter 5 we assessed whether WBE can be expanded to chemicals other than those previously assessed (i.e. illicit drugs, alcohol and tobacco). For this a method was developed to analyse organophosphorous flame retardants (PFRs) in wastewater influent. Using the samples collected during census, it was estimated that 2.1 mg $person^{-1}$ day^{-1} enter Australian wastewater. In addition we found a good correlation ($r^2 \geq 0.87$) between the population size and mass load for each of the four main contributors (TBOEP, TCIPP, TDCIPP and TCEP).

Overall, this thesis demonstrates that the uncertainty of WBE estimates can be improved through identifying population markers, and developing and calibrating a population model. Additionally, we found that WBE is not limited to assessing exposure/consumption of the chemicals previously assessed (i.e. alcohol, illicit drugs and tobacco) and can be expanded to other chemical groups.

Declaration by author

This thesis is composed of my original work, and contains no material previously published or written by another person except where due reference has been made in the text. I have clearly stated the contribution by others to jointly-authored works that I have included in my thesis.

I have clearly stated the contribution of others to my thesis as a whole, including statistical assistance, survey design, data analysis, significant technical procedures, professional editorial advice, and any other original research work used or reported in my thesis. The content of my thesis is the result of work I have carried out since the commencement of my research higher degree candidature and does not include a substantial part of work that has been submitted to qualify for the award of any other degree or diploma in any university or other tertiary institution. I have clearly stated which parts of my thesis, if any, have been submitted to qualify for another award.

I acknowledge that an electronic copy of my thesis must be lodged with the University Library and, subject to the policy and procedures of The University of Queensland, the thesis be made available for research and study in accordance with the Copyright Act 1968 unless a period of embargo has been approved by the Dean of the Graduate School.

I acknowledge that copyright of all material contained in my thesis resides with the copyright holder(s) of that material. Where appropriate I have obtained copyright permission from the copyright holder to reproduce material in this thesis.

Publications during candidature

Peer-reviewed Papers:

Thai, P.K., **O'Brien, J.,** Jiang, G., Gernjak, W., Yuan, Z., Eaglesham, G., Mueller, J.F., 2014. Degradability of creatinine under sewer conditions affects its potential to be used as biomarker in sewage epidemiology. Water Research. 55, 272-279.

O'Brien, J.W., Thai, P.K., Eaglesham, G., Ort, C., Scheidegger, A., Carter, S., Lai, F.Y., Mueller, J.F., 2014. A model to estimate the population contributing to the wastewater using samples collected on census day. Environmental Science & Technology. 48, 517-525.

Bruno, R., Hall, W., Kirkbride, K.P., Lai, F.Y., **O'Brien, J.W.,** Prichard, J., Thai, P.K., Mueller, J.F., 2014. Commentary on Ort et al. (2014): What next to deliver on the promise of large scale sewage-based drug epidemiology? Addiction. 109, 1353-1354.

Gao, J., **O'Brien, J.W.,** Lai, F.Y., van Nuijs, A.L., He, J., Mueller, J.F., Xu, J., Thai, P.K., 2015. Could wastewater analysis be a useful tool for China? - A review. Journal of environmental sciences. 27C, 70-79.

O'Brien, J.W., Thai, P.K., Brandsma, S.H., Leonards, P.E., Ort, C., Mueller, J.F., 2015. Wastewater analysis of Census day samples to investigate per capita input of organophosphorus flame retardants and plasticizers into wastewater. Chemosphere. 138, 328-334.

Lai, F.Y., Anuj, S., Bruno, R., Carter, S., Gartner, C., Hall, W., Kirkbride, K.P., Mueller, J.F., **O'Brien, J.W.,** Prichard, J., Thai, P.K., Ort, C., 2014. Systematic and Day-to-Day Effects of Chemical-Derived Population Estimates on Wastewater-Based Drug Epidemiology. Environmental Science & Technology. 49, 999−1008.

Lai, F.Y., Thai, P.K., **O'Brien, J.,** Gartner, C., Bruno, R., Kele, B., Ort, C., Prichard, J., Kirkbride, P., Hall, W., Carter, S., Mueller, J.F., 2013. Using quantitative wastewater analysis to measure daily usage of conventional and emerging illicit drugs at an annual music festival. Drug and Alcohol Review. 32(6), 594-602.

Thai, P.K., Lai, F.Y., Edirisinghe, M., Hall, W., Bruno, R., **O'Brien, J.W.,** Prichard, J., Kirkbride, K.P., Mueller, J.F., 2016. Monitoring temporal changes in use of two cathinones in a large urban catchment in Queensland, Australia. Science of the Total Environment. 545-546, 250-255.

Lai, F.Y., **O'Brien, J.W.,** Thai, P.K., Hall, W., Chan, C., Mueller, J.F. 2016. Trends in methamphetamine residues in wastewater in metropolitan and regional cities in South East Queensland 2009-2015. The Medical Journal of Australia. 204 (4), 151-153.

Humphries, M.A., Bruno, R., Lai, F.Y., Thai, P.K., Holland, B.R., **O'Brien, J.W.,** Ort, C., Mueller, J.F., 2016. Evaluation of monitoring schemes for wastewater-based epidemiology to identify drug use trends using cocaine, methamphetamine, MDMA and methadone. Environmental Science & Technology. 50 (9), 4760-4768.

Ryu, Y., Barcelo, D., Barron, L. P., Bijlsma, L., Castiglioni, S., de Voogt, P., Emke, E., Hernandez, F., Lai, F. Y., Lopes, A., de Alda, M. L., Mastroianni, N., Munro, K., **O'Brien, J.,** Ort, C., Plosz, B. G., Reid, M. J., Yargeau, V., Thomas, K. V., 2016. Comparative measurement and quantitative risk assessment of alcohol consumption through wastewater-based epidemiology: An international study in 20 cities. Science of the Total Environment. 565, 977-983.

Lai, F.Y., **O'Brien, J.W.,** Thai, P.K., Hall, W., Chan, G., Bruno, R., Ort, C., Prichard, J., Carter, S., Anuj, S., Kirkbride, K.P., Gartner, C., Humphries, M., Mueller, J.F. 2016. Cocaine, MDMA and methamphetamine residues in wastewater: consumption trends (2009-2015) in South East Queensland, Australia. Science of the Total Environment. 568, 803-809.

Lai, F.Y., **O'Brien, J.W.,** Bruno, R., Hall, W., Prichard, J., Kirkbride, K.P, Gartner, C., Thai, P., Carter, S., Lloyd., B., Burns, L., Mueller, J.F., 2016. Spatial variations in the consumption of illicit stimulant drugs across Australia: a nationwide application of wastewater-based epidemiology. Science of the Total Environment. 568, 810-818.

Gao, J., **O'Brien, J.W.,** 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. 568, 164-170.

Thai, P.K., Lai, F.Y., Bruno, R., van Dyken, E., Hall, W., **O'Brien, J.W.,** 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. 94, 307-314.

Conference Presentations (Oral):

O'Brien, J.W., Thai, P.K., Eaglesham, G.K., Ort, C., Scheidegger, A., Lai, F.Y., Mueller, J.F. 2013. A better method for estimating the number of contributing people to a wastewater treatment plant. Lisbon. Testing the waters: first international multidisciplinary conference on detecting illicit drugs in wastewater.

O'Brien, J.W., Banks, A., Eaglesham, G.K., Jiang, G., Lai, F.Y., Mueller, J.F., Scheidegger, A., Ort, C., Thai, P.K. 2015. Degradation of population markers under sewer conditions may affect the prediction certainty/reliability of population estimates. Ascona. Testing the waters II: 2nd International Conference on "Wastewater-based drug epidemiology".

Mueller, J.F., Lai, F.Y., Thai, P.K, **O'Brien, J.W.,** Ort, C., Gartner, C., Prichard, J., Bruno, R., van Dyken, E., Humphries, M., Carter, S., Wilkins, C., Kirkbride, P., Hall, W. 2015. Ascona. Testing the waters II: 2nd International Conference on "Wastewater-based drug epidemiology".

Lai, F.Y., Hall, W., Wilkins, C., Gartner, C., Carter, S., Anuj, S., **O'Brien, J.W.,** Thai, P.K, Bruno, R., Ort, C., Prichard, J., Kirkbride, P., Mueller, J.F. Application of wastewater-based epidemiology on monitoring illicit drug, alcohol and nicotine use in Australia and New Zealand populations. 2015. Ascona. Testing the waters II: 2nd International Conference on "Wastewater-based drug epidemiology".

Conference Presentations (Poster):

van Dyken, E., Hall, W., Lai, F.Y., Gartner, C., Carter, S., **O'Brien, J.W.,** Thai, P.K., Bruno, R., Kirkbride, P., Mueller, J.F., Prichard, J. Can we use sewage epidemiology to evaluate mandatory drug testing in fUK prisons? 2015. Ascona. Testing the waters II: 2nd International Conference on "Wastewater-based drug epidemiology".

Thai, P.K., van Dyken, E., Banks, A., Lai, F.Y., **O'Brien, J.W.,** Bruno, R., Hall, W., Prichard, J., Mueller, J.F. Refining the excretion factors of methadone and codeine for applications in sewer epidemiology. 2015. Ascona. Testing the waters II: 2nd International Conference on "Wastewater-based drug epidemiology".

Publications included in this thesis

Thai, P.K., **O'Brien, J.,** Jiang, G., Gernjak, W., Yuan, Z., Eaglesham, G., Mueller, J.F., 2014. Degradability of creatinine under sewer conditions affects its potential to be used as biomarker in sewage epidemiology. Water Res 55, 272-279. – incorporated as Chapter 2.

Contributor	Statement of contribution
Thai P.K.	Designed experiments (30%) Wrote the paper (40%)
O'Brien J.W. (Candidate)	Designed experiments (20%); Development of analytical method (50%); Wrote and edited the paper (20%); Data

	interpretation (30%); editing the paper; revising and responding to reviewers' comments (10%)
Jiang, G.	Experimental setup and chemical analysis for wastewater characteristics (20%); Statistical analysis of data in tables 2 and 3 (10%)
Gernjak, W.	Designed experiments (10%) and revised the manuscript (5%)
Yuan, Z.	Designed experiments (10%) and revised the manuscript (5%)
Eaglesham, G.K.	Development of analytical method (50%);
Mueller, J.F.	Designed experiments (10%) and revised the manuscript (10%)

O'Brien, J.W., Thai, P.K., Eaglesham, G., Ort, C., Scheidegger, A., Carter, S., Lai, F.Y., Mueller, J.F., 2014. A model to estimate the population contributing to the wastewater using samples collected on census day. Environ. Sci. Technol. 48, 517-525. – incorporated as Chapter 3.

Contributor	Statement of contribution
O'Brien J.W. (Candidate)	Study design (70%); Development of analytical method (50%); Sample collection (100%); Sample preparation and analysis (90%); Wrote and edited the paper (80%); Revising and responding to reviewers' comments (60%); Data analysis and statistics (35%);
Thai, P.K.	Wrote and edited the paper (10%); Revising and responding to reviewers' comments (10%);
Eaglesham, G.K.	Development of analytical method (50%); Study design (10%); Sample preparation and analysis (10%);
Ort, C.	Wrote and edited the paper (10%); Data analysis and statistics (35%); Revising and responding to reviewers' comments (10%);
Scheidegger, A.	Data analysis and statistics (35%);

Carter, S.	Provided analytical standards and assisted with method development
Lai, F.Y.	Editing the paper
Mueller, J.F.	Study design (20%); Wrote and edited the paper (10%); Revising and responding to reviewers' comments (10%);

O'Brien, J.W., Thai, P.K., Brandsma, S.H., Leonards, P.E., Ort, C., Mueller, J.F., 2015. Wastewater analysis of Census day samples to investigate per capita input of organophosphorus flame retardants and plasticizers into wastewater. Chemosphere 138, 328-334. – incorporated as Chapter 5.

Contributor	Statement of contribution
O'Brien J.W. (Candidate)	Study design (70%); Development of analytical method (50%); Sample collection (100%); Sample preparation and analysis (90%); Wrote and edited the paper (80%); Revising and responding to reviewers' comments (70%); Data analysis and statistics (70%);
Thai, P.K.	Study design (10%); Wrote and edited the paper (10%); Revising and responding to reviewers' comments (10%);
Brandsma, S.H.	Development of analytical method (50%); Study design (10%); Sample preparation and analysis (10%); Revising and responding to reviewers' comments (10%);
Leonards, P.E.	Wrote and edited the paper (10%); Study design (10%)
Ort, C.	Data analysis and statistics (35%);
Mueller, J.F.	Study design (20%); Wrote and edited the paper (10%); Revising and responding to reviewers' comments (10%);

Contributions by others to the thesis

The overall project conception was financially supported by Jochen Mueller and Phong Thai. They also provided continuous mentoring on the project. Instrumental training, expert analytical advice, data interpretation and project ideas were provided by Geoff Eaglesham. Data interpretation and statistical analysis advice were provided by Christoph Ort, Andreas Scheidegger and Andrew Novic. Elements of degradation/transformation study designs were provided by Guangming Jiang, Wolfgang Gernjak and Zhiguo Yuan. Elements of sample collection, sample preparation for analysis and data interpretation was provided by Andrew Banks. Steve Carter provided some analytical advice and chemical standards for the project. Additional analytical advice was provided by Foon Yin Lai.

For the secondment to the Free University of Amsterdam, project and financial support was supported through the INTERFLAME project which is part of the European Union Seventh Framework Programme FP7/2007-2013 under Grant Agreement 295138. Elements of this secondment including study design and instrumental training were supported by Pim Leonards, Sicco Brandsma, Jacob de Boer, Jacco Koekkoek and Martin van Velzen.

Staff and students on the National Research Centre for Environmental Toxicology (Entox) provided advice on elements of the manuscripts, proof reading, preparation of samples and preparing analytical standards. Numerous wastewater treatment plant operators provided samples and details about their specific sewer catchments and the samples collected for the project. The Australian Bureau of Statistics provided population data for the various sewer catchments.

Statement of parts of the thesis submitted to qualify for the award of another degree

None.

Acknowledgements

This project was only possible due to many people with great minds and different skills who were willing to share their expertise with me. Firstly I would like to thank Professor Jochen Mueller for not just his support but for creating challenges which made me work hard and drive my interest to learn new things. Jochen has also taught me that you can never have too many friends and being an expert in everything is unnecessary when experts are only a phone call away. I have been very fortunate to have a supervisor who is both critical and encouraging, teaches you to question things and think about the bigger picture. Jochen has also let me take responsibility of my own projects and let me contribute to other people's projects. I am very fortunate to have Jochen as a mentor, colleague and friend.

Secondly I would like to thank Dr Phong Thai for teaching me that you should question every hypothesis – then find the quickest and easiest way to prove or disprove it. Phong has encouraged me all the way and has been a role model researcher who has worked hard to help me and others progress as researchers and always shares his ideas for new projects. I also appreciate Phong teaching me that regardless of how early it is, it is never too early to ask for a beer.

Thirdly, although not an official supervisor, I have to thank Geoff Eaglesham for sharing his wealth of analytical chemistry knowledge. Geoff also provides me with new research ideas, solutions to problems and given me the necessary skills to continue in analytical chemistry with confidence so I can answer my own questions. Fourthly or 'Third Runner-up' as ES&T would put it, I would like to thank my other unofficial supervisor Dr Christoph Ort for giving up his time to teach me statistical analysis far beyond what I thought I could achieve and for encouraging me to program for myself. Chris also has shown me ways to turn complex datasets into amazing graphs so that others can really understand the big picture. Chris has also shown me how to turn a good dataset into a great paper. Additionally Chris was willing to share his broad network of researchers with me such as Andreas Scheidegger, the SCORE coordinators and the SEWPROF team. These people continually advance the wastewater-based epidemiology field and continue to encourage me to stay active within it.

I would also like to thank the Entox team, past and present, who not only help me with my projects but also let me work on theirs so we can all learn new skills, progress things quickly and share in the rewards. There is never a bad day at work with these guys around because none of them are just colleagues but are also really good friends. These include Alex, Alfred, Amy, Andrew B, Andrew N, Anna, Beate, Birgitte, Bob, Caroline, Chang, Charlotte, Cheng, Christie, Christine, Clare, Daniel, Dudsadee, Elissa, Fanny, Fisher, Foon, Greta, Gulsah, Hao, Hue, Jack G, Jack N, Jack T, Janet, Javier, Jenny, Jess, Kristie, Kristy, Laurie, Leanne, Linus, Li Yan, Liesbeth, Lucie, Loren, Louise, Maria-Jose, Marjolijn, Matti, Mike, Natalia, Paxy, Peta, Que, Rachel, Sarah, Sarit, Sasikumar, Sharon, Shiva, Soumini, Summer, Suzanne, Svenja, Veronika, Vincent, Wasa, Wiebke and Yiqin. I hope we can be colleagues for many years to come as that way it will never feel like I'm working.

As part of my PhD I was fortunate enough to work at the IVM within the Free University of Amsterdam. This was my first experience working abroad and Pim, Sicco, Martin, Jacco, Jacob and the many staff and students of the IVM made this experience incredible. Not only did they teach me new things but also shared their amazing country and culture with me. I hope that we can collaborate for many more years.

This thesis would not have been possible without the help of the staff at the many wastewater treatment plants I have collected samples from, particularly those at Wetalla and the extended Kele family (and friends). These guys not only helped with providing access to samples, but also helped collect them, collate flow data, provide information about the samples (sometimes too much), shared great stories and raised many great questions. I really appreciate you for doing this specifically the Kele family for giving myself and others free tickets and exceptional accommodation at the best festival I've been to.

Finally I would like to thank my parents, sisters, family, friends, band members, trivia team and girlfriend Bianca for their ongoing support. All of you have heard more than

enough bad “poo jokes” to last a lifetime but have still managed to support me with patience and understanding. For this I will be forever grateful.

The instrumental support for the LC-MS/MS and LC-QToF/MS used during my PhD were provided through ARC LIEF grants LE110100032 and LE140100129.
I was also fortunate enough to receive an APA PhD scholarship and a Top-Up Scholarship courtesy to Jochen Mueller. Thank you!

Keywords

wastewater-based epidemiology, chemical analysis, population, health, exposure, uncertainty, consumption

Australian and New Zealand Standard Research Classifications (ANZSRC)

ANZSRC code:	111705, Public Health and Health Services, Environmental and Occupational Health and Safety 50%
ANZSRC code:	030105, Analytical Chemistry, instrumental methods, 40%
ANZSRC code:	039901, Chemical sciences, environmental chemistry, 10%

Fields of Research (FoR) Classification

FoR code:	1117, Public Health and Health Services, 50%
FoR code:	0301, Analytical Chemistry, 40%
FoR code:	0399, Chemical sciences, other chemical sciences, 10%

Thesis Structure

Chapter 1: Introduction and aims

1.1 Wastewater-based epidemiology: an emerging tool for assessing chemicals used by large populations of people

1.2 Back-calculating chemical consumption/exposure from wastewater analysis

1.3 Limitations and uncertainties of wastewater-based epidemiology

1.4 Aims

Chapter 2: Assessment of creatinine as a population marker

Chapter 3: Assessment of pharmaceuticals and personal care products as population markers and the development of a population model

Chapter 4: Impact of in-sewer degradation of pharmaceutical and personal care product (PPCP) population markers on a population model

Chapter 5: Wastewater analysis of Census Day samples to investigate per capita input of organophosphorus flame retardants and plasticizers into wastewater

Chapter 6: Conclusion: key findings, final discussion and future research

Bibliography

Appendix

Appendix 1: Supplemental Material: Degradability of creatinine under sewer conditions affects its potential to be used as a biomarker in sewage epidemiology

Appendix 2: Supplemental Information – A model to estimate the population contributing to the wastewater using samples collected on census day

Appendix 3: Supporting Information – Impact of in-sewer degradation of pharmaceutical and personal care products (PPCPs) population markers on a population model

Appendix 4: Supporting Information – Wastewater analysis of census day samples to investigate per capita input of organophosphorous flame retardants and plasticizers into wastewater

Appendix 5: Measuring selected PPCPs in wastewater to estimate the population in different cities in China

Appendix 6: Systematic and Day-to-Day Effects of Chemical-Derived Population Estimates on Wastewater-Based Drug Epidemiology

List of Tables

List of Figures

List of Abbreviations

Abbreviation	Definition
ABS	Australian Bureau of Statistics
A/V	Area to volume
BOD	Biological oxygen demand
CE	Collision energy
COD	Chemical oxygen demand
COP	Coprostanol
CR	Control reactor
CV	Coefficent of variation
CXP	Collision exit potential
DO	Dissolved oxygen
DP	Declustering potential
EI	Electron Ionisation
EP	Entrance potential
EPS	Equilibrium passive sampler
ESI	Electrospray ionisation
GC-MS	Gas chromatography-mass spectrometry
GS	Gravity sewer
LC-MS/MS	Liquid chromatography-tandem mass spectrometry
LOD	Level of detection
LOQ	Level of quantification
MRM	Multiple reaction monitoring
N	Nitrogen
P	Phosphorus
PBS	Pharmaceutical benefit scheme
PFR	Organophosphorus flame retardant
PNDC	Population normalised drug consumption
POCIS	Polar organic chemical integrative sampler
PPCP	Pharmaceutical and personal care product
PTFE	Polytetrafluoroethylene
QA	Quality assurance

QC	Quality control
RM	Rising main
RSD	Relative standard deviation
S	Sulphur
SCX	Strong cation exchange
SD	Standard deviation
SIM	Select ion monitoring
sMRM	Scheduled multiple reaction monitoring
SPE	Solid phase extraction
SPMD	Semi-permeable membrane device
STP	Sewage treatment plant
WBE	Wastewater-based epidemiology
WWA	Wastewater analysis
WWTP	Wastewater treatment plant

Chapter 1: Introduction and aims

1.1. Wastewater-based epidemiology: an emerging tool for assessing chemicals used by large populations of people

Finding links between environmental stressors and human health is typically a complex undertaking. Identifying such links in a timely manner is crucial if the goal is to prevent or reduce exposure before widespread health effects occur. Measuring exposure to environmental stressors is often costly and is usually limited to small cohort studies which may not be representative of the larger community. However, a relatively new approach for assessing exposure at the community level by systematic sampling and quantitatively analysing markers of chemical exposure within raw wastewater has gained in popularity in the last decade. This field has been termed wastewater analysis (WWA) or wastewater-based epidemiology (WBE). Wastewater-based epidemiology is based upon the assumption that traces of the chemicals we consume or are exposed to are excreted either unchanged or as a mixture of unchanged and metabolites in both our urine and/or faeces and as a result end up in wastewater (Castiglioni et al. 2014). As wastewater networks are municipal, the wastewater itself contains chemical residues which are representative of whole communities. Therefore analysis of wastewater is representative of whole communities and can be used as a non-invasive tool to monitor chemical consumption and exposure. This non-invasive approach has great potential particularly for assessing consumption of chemicals which typically have clandestine usage such as illicit drugs (Daughton 2001). Additionally, as samples are representative of whole communities, it can be used to assess chemical consumption differences between cities (van Nuijs et al. 2009b) and even countries (Ort et al. 2014b). The main advantage of WBE, particularly for estimating illicit drug consumption, is it provides evidence-based, near-real-time and objective estimates of illicit drug consumption in a given wastewater catchment with shorter study times and reduced costs compared to traditional methods such as surveys. The approach

can also be used during special events such as festivals (Lai et al. 2013c), over long periods of times to identify changes, and also to cross compare between locations. Initially applied to measure illicit drug consumption in Milan (Italy), Lugano (Switzerland) and London (United Kingdom), the first results indicated that the estimates from WBE were in line with national annual prevalence data hence showed much promise (Zuccato et al. 2008). Since then, WBE for estimating illicit drug consumption has been applied in multiple cities in Australia (Lai et al. 2015, Lai et al. 2013a, Lai et al. 2013b, Lai et al. 2016a, Lai et al. 2016b, Lai et al. 2016c, Lai et al. 2011, Lai et al. 2013c), Canada (Metcalfe et al. 2010), Europe (Ort et al. 2014b) and the United States (Banta-Green et al. 2009).

Wastewater-based epidemiology has recently received global attention in regards to illicit drug surveillance (Thomas and Reid 2011). Of particular note is that the European Monitoring Centre for Drug and Drug Addiction has now included illicit drug consumption estimates from WBE analysis in their Perspective on Drugs report (EMCDDA 2016). Further developing WBE techniques and applying them to other chemical groups should produce improved assessments of human exposure to chemicals. This will encapsulate the short term goals of determining which chemicals and chemical groups we can measure as well as detecting per capita regional differences, which can then be applied to the long term goal of monitoring per capita exposure changes.

As the main focus of WBE has been on measuring illicit drug consumption, there are certain compounds which have been more thoroughly investigated than others. The most commonly investigated chemicals using WBE are shown in Table 1.

Table 1 Most common chemicals and their target residues assessed using the wastewater-based epidemiology approach.

Chemical Group	Consumed chemical	Target residue	Reference
Alcohol	Ethanol	ethyl sulphate, ethyl glucuronide	(Reid et al. 2011)
Illicit drugs	cocaine	benzoylecgonine, cocaine	(Lai et al. 2011, van Nuijs et al. 2011c)
	methamphetamine	amphetamine, methamphetamine	

	MDMA	MDMA	
	THC	THC-COOH	
Tobacco	nicotine	cotinine, nicotine, *trans*-3'-hydroxycotinine	(Harman et al. 2011)

1.2. Back-calculating chemical consumption/exposure from wastewater analysis

Wastewater-based epidemiology relies on the principle that when substances are ingested they are metabolised and then excreted as the parent compound and/or their metabolites and that these ultimately enter the urban sewer networks. Therefore, by measuring concentrations of target compounds (C_i) in wastewater samples which are representative of whole days, multiplying by the daily flow (F), correcting for excretion factors ($\frac{R_i}{E_i}$) and dividing by the population (P) the amount of a chemical consumed on a per capita basis is calculated:

$$Daily\ chemical\ consumption_i \left(\frac{\frac{mg}{day}}{1000\ people} \right) = \frac{C_i \times F \times \frac{R_i}{E_i}}{P}$$

To confirm consumption of a given chemical using the WBE approach, the target residue is usually a human specific metabolite of the parent compound excreted primarily in the urine. Therefore pharmacokinetic data is required for each chemical which outlines the average excretion and the ratio of the molar mass of the parent compound to that of the metabolite. Table 2 outlines the pharmacokinetic parameters used when back-calculating consumption of the most common chemicals in WBE studies.

1.3. Limitations and uncertainties of wastewater-based epidemiology

Generation of WBE data involves many steps and each step has its own uncertainty which affects the overall quality of the final data. The uncertainty for each parameter is expressed as a percentage (relative standard deviation, RSD). This approach allows for Gaussian error propagation to be used to calculate the total uncertainty for

the consumption measurement of a given chemical using the WBE approach (Lai et al. 2011). The uncertainties are related to the sampling, stability of the markers, the analytical measurement, reliability of back-calculation of consumption data such as excretion, flow measurement, and the population size. Only some of these uncertainties have been investigated and few techniques have been identified and introduced to reduce these uncertainties.

The common uncertainties associated with back-calculating the consumption of chemicals using the wastewater-based epidemiology approach as outlined by Castiglioni et al. is shown in Figure 1.

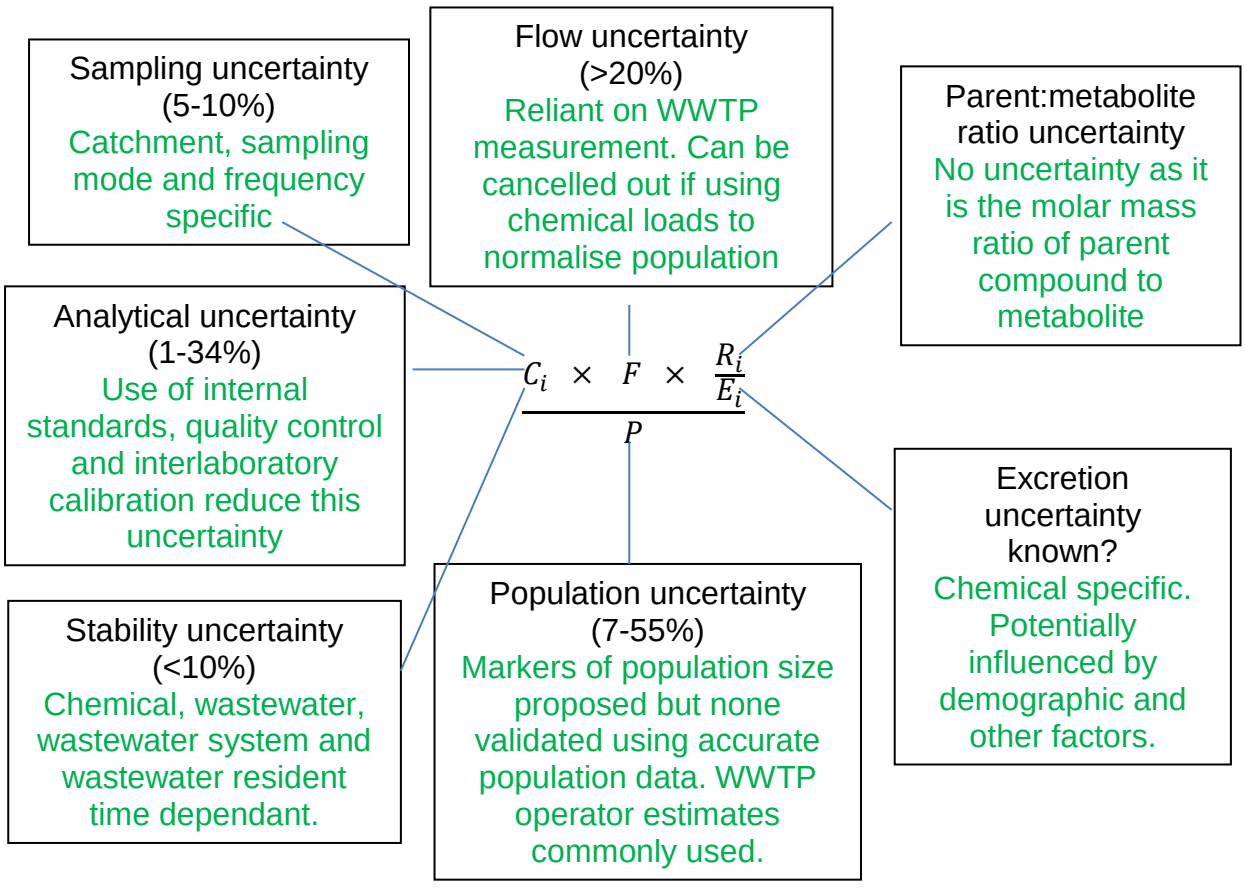


Figure 1 Uncertainties associated with back-calculating the consumption of chemicals using the wastewater-based epidemiology approach as outlined by Castiglioni et al. (Castiglioni et al. 2013).

1.3.1. Sampling Uncertainty

The first uncertainty of WBE and one which can be controlled is the sampling uncertainty. Collected samples should be representative of the wastewater released

within a catchment over a given period (typically 24 hours). Ort et al. (Ort et al. 2010b) thoroughly evaluated the sampling mode and frequency required in order to reduce sampling artifacts and found that they can range from non-significant to more than 100%. To control the sampling uncertainty they outlined that continuous flow proportional sampling is best in order to capture the most representative sample and avoid over interpretation of results. However, Castiglioni et al. noted that the continuous flow proportional sampling method is rarely adopted but the sampling uncertainty based on other sampling methods such as frequent flow or volume proportional sampling for large catchments still manage to keep the random sampling uncertainty below 10% (Castiglioni et al. 2013).
An additional consideration to the sampling uncertainty is that conventional sampling methods focus only on representatively collecting chemicals associated with the dissolved phase and thus chemicals which partition to the particulate matter in wastewater are not representatively sampled.

1.3.2. Analytical Uncertainty

The emergence of WBE has primarily been driven by environmental and analytical chemists and thus the analytical uncertainties have been investigated thoroughly and protocols have been established to reduce these uncertainties. Many but not all WBE analytical methods employ solid-phase extraction (SPE) to concentrate target residues in order to measure them with certainty (van Nuijs et al. 2014). Most analytical analyses are conducted using liquid chromatography coupled to mass spectrometry. These methods are optimized for each specific target residue with an emphasis on separating potentially interfering chemicals from the target analytes both in terms of chromatographic separation and mass spectra such as by using multiple reaction monitoring or high-resolution mass spectrometry. This is particularly important as wastewater is a complex matrix containing an enormous range of chemicals (Grabic et al. 2012). Using such a method also allows for labelled analogues of target residues such as deuterated or carbon labelled standards, which are measured simultaneously, to both identify and reduce impacts of matrix effects and in turn improve upon the accuracy of the analytical method (Castiglioni et al. 2013). Even using such methods, the analytical uncertainty remains between 6 and 26% (Castiglioni et al. 2013).

1.3.3. Uncertainty associated with chemical loss in the wastewater and in the sewer system

Chemical stability is an important uncertainty to consider for WBE as it is not just limited to the stability of the target residues in the collected samples but also the loss of the chemicals within the sewer. For some chemicals, the stability improves by chilling or freezing the collected samples, changing the conditions of the sample such as by acidifying or adding a preservative such as sodium metabisulphite, or by stabilizing the chemical by exchanging it onto a solid phase immediately after collection. While the stability of chemicals in collected samples can be controlled to some extent (Chen et al. 2012a), the transformation processes during in-sewer transport cannot.

There are two major processes in sewers which determine chemical stability and fate. The first is the mass transfer between phases i.e. sorption and uptake by organisms. The second is the alteration of the structure of the chemicals themselves such as via metabolism, reduction or oxidation of the chemicals (McCall et al. 2015). Different sewer types also favour certain microbial conditions. For instance, rising mains sewers favour anaerobic activity whereas gravity sewers favour aerobic activity (Hvitved-Jacobsen et al. 2013). For chemicals which do degrade or are transformed, the extent of degradation is also dependent on the exposure to such microbial or environmental conditions. Therefore degradation/transformation within sewers for some chemicals is entirely dependent on the specific sewer catchment itself and also the point at which it enters the sewer catchment. It is therefore necessary to conduct degradation/transformation studies on target residues to understand their fate in the sewer in addition to experiments to assess their stability in collected wastewater samples and how to preserve those which do degrade/transform.

1.3.4. Correction factor uncertainties

The back-calculation of chemical consumption by people within the specified catchment relies upon multiple correction factors. These correction factors include the stability of the target residues in the wastewater and the metabolism of the

parent compound in humans. However, most WBE, as outlined by the equation above only take into account the pharmacokinetic correction factors of parent chemical to metabolite ratio, and excretion factors. These factors however are chemical specific and as such have their own chemical specific uncertainties. For some chemicals some methods for back-calculating consumption have been relatively well investigated. As an example, the metabolism of cocaine in humans is reasonably well understood, producing a major metabolite, benzoylecgonine, which has an average excretion rate of 35% and a molar mass ratio (parent to metabolite) of 1.1. The excretion profile is also known for the main routes of cocaine administration which include intravenous (injection), intranasal (snorting), ingestion and inhalation (of smoke). A table of the ratio of the molar pass of the parent compound to its metabolite and the average excretion for the most commonly assessed illicit drugs using the WBE approach is shown in Table 2.
Even for a chemical such as cocaine with known pharmacokinetics, the uncertainty of back-calculation as assessed using best practice requirements outlined by Castiglioni et al. is approximately 26% (Castiglioni et al. 2013). For other chemicals there is limited data, particularly for emerging compounds such as novel psychoactive substances or chemicals with non-intentional consumption such as flame retardants and thus the uncertainty remains unknown.

Table 2 Pharmacokinetic parameters from the literature required for back-calculating chemical consumption using the wastewater-based epidemiology approach for some example chemicals. Table adopted from (Lai 2013) and (Castiglioni et al. 2015).

Consumed chemical	Target residue	ratio of the molar mass of the parent compound to its metabolite (R_i)	Average excretion (E_i) (%)
cocaine	benzoylecgonine, cocaine	1.1 1.0	35[1,2,3,a] 7.5[1,2,3,a]
methamphetamine	amphetamine, methamphetamine	1.1 1.0	5.5[4,5,6,b] 39[4,5,6,b]
THC	THC-COOH	0.9	0.6[6,7,c]
nicotine	cotinine, nicotine, *trans*-3'-hydroxycotinine	0.9 1.1 0.8	30[8,9,10] 13[8,9,10] 44[8,9,10]
[1](Ambre et al. 1984); [2](Ambre et al. 1988); [3](Cone et al. 2003); [4](Baselt 2008); [5](Khan and Nicell 2012); [6](Postigo et al. 2010); [7](Zuccato et al. 2008); [8](Hukkanen et al. 2005); [9](Byrd et al. 1992); [10](Randall and Baselt 2004); [a]routes of administration include injection, snorting and smoking (n = 26); [b]based on oral administration (n = 32); [c]based on smoking			

1.3.1. Uncertainty associated with flow

So far there has been limited research conducted on the uncertainty associated with flow measurements. In most cases flow measurements are provided by WWTP operators and there is limited potential for the researchers to validate the accuracy of the measurement and or calibrate the flow meters. Measuring flow in sewers is a complex task and the uncertainty is generally considered around 20% even under optimal conditions (Ort et al. 2010b). Well maintained flow meters in rising main (pressurised) sewers are considered to more accurately measure the flow than flow meters in open channel, partially filled, gravity fed sewers. However, without considerable investment into the calibration of any flow meter, the uncertainty of the flow measurement remains unknown. One option to reduce the uncertainty of the flow is to normalise loads of chemicals in the wastewater against other chemicals in the same sample to cancel out the errors in the flow measurement (Lai et al. 2011).

This however has its own associated uncertainties such as the homogeneity of consumption of the chemicals used for normalisation and the stability of these chemicals in the sewer.

1.3.2. Uncertainty associated with population

As outlined in the review of WBE uncertainties by Castiglioni et al., the major uncertainty of WBE studies is that of the catchment population size which even using best practice requirements still remains between 7 and 55%.
There are considered to be two population types per catchment. These are the *de jure* population (the people who reside in the catchment) and the *de facto* population (the people who are present in a catchment during a given period which includes people who are working there, holidaying there, and excludes the usual residents who are not present during that period).
For WBE, the *de facto* population is the most important as it reflects the population who contributed to the wastewater during the sampling period.
The most common approach to estimate the *de facto* population include estimates based on hydrochemical parameters (e.g. biological oxygen demand (BOD), chemical oxygen demand (COD), nitrogen (N) and phosphorous (P)), census data, and design capacities of the WWTPs. One difficulty of using the most recent census data is that populations are dynamic and the census data may become quickly outdated.
Hydrochemical parameters may be more reflective of the *de facto* population, but there are multiple factors which influence the load of these in the wastewater
These include input from industry as well as processes occurring within the sewer such as chemical processes and microbial activity. The hydrochemical population estimates have an additional uncertainty as they require accurate flow data of the wastewater influent in order to be calculated. Castiglioni et al. concluded that the current best practice to reduce the uncertainty of the population estimate is to consult experts from each individual WWTP to identify the major industrial sources of hydrochemical parameters. By doing this, population estimates can be calculated using the hydrochemical parameter/s which are least influenced by industry.
As an alternative approach to using the common methods such as hydrochemical parameters or the most recent census data, Lai et al. proposed the measurement of

high use human specific markers such as pharmaceuticals (e.g. atenolol, carbamazepine and hydrochlorothiazide) and an artificial sweetener (acesulfame) (Lai et al. 2011). By correcting the measured mass load by excretion data for each of these chemicals and comparing against national audit data, Lai et al. showed that for the catchment investigated, atenolol was the best population marker. This is because its use in the general population is relatively high (1 - 3% in Australia), it is required to be consumed regularly, it is thought to be relatively stable in the sewer as it shows little removal during wastewater treatment, and the back-calculated consumed load strongly agreed with the population estimate provided by the WWTP staff.

While atenolol was found to be a good population marker for the specific catchment investigated, it may not necessarily be a good marker for other catchments such as where the age demographic is younger (the demographic for atenolol consumers is greater than 65 years of age, ABS, 2006). Therefore, other markers of population size which are more representative of the population need to be identified. Preferably these markers would be human specific endogenous markers.

Daughton recognised the shortcomings of the commonly parameters used for assessing population size and proposed what he considered to be ideal attributes for chemicals in wastewater to have in or to be used as population markers (Table 3) (Daughton 2012a). In this assessment he proposed to use endogenous markers such as creatinine and coprostanol to be used as population markers.

Table 3 Ideal attributes for chemicals in wastewater biomarkers targeted for estimating population as published in Daughton (2012a)

Attribute	Example	Potential problem
Must be excreted into sewage	Excretion via urine rather than faeces poses fewer sampling and analytical challenges	Excretion via faeces creates a non-homogenous sample stream and requires more comprehensive sample preparation
Uniqueness to human metabolism	Coprostanol (CoP) is produced in significant quantities primarily by higher vertebrates that synthesize cholesterol	Input possible from other animals where industrial/agricultural sewage is mixed with human sewage or runoff carrying animal faeces into combined sewers (both would serve to confound data)
Exogenous sources are minimal	Low occurrence in raw or cooked foods	Creatinine occurs in meats and can be created from creatine during cooking (can confound data)

Attribute	Example	Potential problem
Minimal intra-individual variance in daily excretion	Daily levels excreted by an individual vary minimally over time	Stress or disease can affect the excretion of all biomarkers
Minimal inter-individual variance in daily excretion	Per capita daily excretion across a population varies minimally	A wide spectrum of physiological variables can dictate the excretion of biomarkers (age, gender, genetics, stress)
Daily per capita excretion in sewage is independent of extraneous variables	Minimal effect from season, weather, geographic locale, water-use restrictions, medication usage, diet	Diet can influence excretion variance for both creatinine and CoP
Occurrence levels independent of design and usage sewerage system	Length of sewerage distribution pipes and residence time of sewage in pipes	Time-dependent degradation by microorganisms during sewage transit can lead to variable reductions in biomarker levels
Minimal degradation of biomarker in flowing sewage (levels persist in sewage)	Slow degradation allows sampling raw sewage further downstream, permitting better mixing of influent "pulses"; ensures minimal losses during transit through sewer connections of varied lengths and residence times	Sewage pulses with widely varying biomarker levels (compounded by changing pulse frequency) greatly increase the required frequency of sampling [a]
Levels in raw sewage well above method detection limit (MDL)	Few analytical interferences; easier implementation of a routine method	Isobaric biomarker isomers often become common interferences with methods using mass spectrometry
Minimal potential for exogenous interference from other sources	Exogenous sources include residues of target analyte on analyst's hands	Residues of target analytes can sometimes be excreted as sweat (e.g., drugs) or remain from prior dermal contact or direct application (e.g., personal care products) (Daughton and Ruhoy 2009)
Homogenous distribution; biomarker preferably partitions to aqueous phase	Minimal partitioning to dissolved or suspended solids or sludge	Partitioning to solids increases the complexity of sampling and sample preparation
Minimal degradation of analyte in sampled sewage (levels persist in sewage)	Refractory to microbial degradation or to further physicochemical degradation during sample shipment or storage	Preservatives may be required to inhibit microbial degradation in stored or shipped samples
Minimal de novo, ex vivo formation of analyte in sewage	Minimal formation by microbial activity during sewage transit and during sewage treatment	Sampling of raw sewage as early as possible in influent stream may be necessary
Minimal sample clean-up and sample preparation	Requires minimal pre-concentration to meet MDL	Excretion of biomarker in the form of conjugates may require additional hydrolysis step
Analytical determination uses instrumentation routinely available; analytical methodology amenable to standardization	Conventional GC/MS, LC/MS, or immunoassay	Innovative "research grade" methodologies are too costly or complex for wide implementation
Minimal capital investment in instrumentation; minimal analyst time	Allows for high-frequency sampling	"Research grade" methodologies are too costly for wide implementation

Attribute	Example	Potential problem
High sample through-put	Amenable to automation; reduces cost	Analyst intervention reduces timeliness of results
Potential for in-stream continuous sampling or monitoring	Equilibrium passive samplers (EPS) allow for passive, time-integrated sampling;[b] in-stream sensors facilitate real-time data	Discrete sampling gives biased results because of stream heterogeneity and sewage pulses
Minimal occupational hazards for technicians	Minimal hazards from samples, and from analytical reagents or reactions	Handling raw sewage poses risks associated with pathogen exposure

[a] The challenges associated with obtaining representative samples from an STP are discussed by Ort et al. (2010b).
[b] Examples of EPS (Zabiegała et al., 2010) include polar organic chemical integrative samplers (POCIS) and semipermeable membrane devices (SPMD).

Creatinine was proposed due to its wide use in clinical chemistry to normalise urine samples as it is a continually produced nitrogenous waste product which is cleared by the kidney. However, in order for creatinine to be used as a population marker further assessment would be required in terms of stability in wastewater and within the sewer network. Additionally, it was noted that even though creatinine has been extensively used to normalise urine samples since the 1950s, there is considerable debate surrounding the high intra- and inter-day excretion variance and thus calibration of the mass load in wastewater against a true population size would be required.

Unlike creatinine which may have high intra- and inter-day excretion variance, Daughton proposed coprostanol as its variance is comparatively low and is considered to be stable over time (de Leon et al. 1987). Coprostanol is the major human metabolite of cholesterol accounting for 60% of the sterol content in faeces. It is fully excreted as it is fully saturated hence poorly absorbed from the gut. However, a large uncertainty remains around the use of coprostanol as a population marker as it is poorly water soluble and thus its partitioning to organic matter is likely (Takada and Eganhouse 1998).

As outlined above, multiple population markers have been proposed but none have been validated since no reliable population data were available (such as calibration against samples collected during a census). Additionally, their persistence in wastewater and within the sewer is also unknown. With such limited research on the uncertainties of population size and measuring population changes, the

establishment of more reliable markers for population size should be the focus in order to reduce the overall uncertainty of WBE.

1.4. Aims

The overall aim of this thesis is to evaluate and propose an approach to reduce the uncertainty of population size estimates for WBE studies. This was to be achieved first by identifying and evaluating markers of population size (Chapters 2 and 3). Chapter 3 also proposed a model for assessing population size using a suite of markers that could reduce the population size uncertainty (Appendix 5 and 6 are additional publications which apply the population model developed in Chapter 3). Subsequently, the uncertainty of the model itself was assessed in Chapter 4 through the assessment of the impact of in-sewer degradation on the selected population markers.

Chapter 5 explored the applicability of WBE for measuring exposure of emerging chemicals (in this case organophosphorous flame retardants for which there is limited exposure data in Australia). In addition to this aim, this study contains the first application of the population model developed in Chapter 3 to estimate the population size for a new WWTP catchment.

Chapter 2: Assessment of creatinine as a population marker

As outlined above, defining the population size is an essential element for wastewater-based epidemiology to estimate the per capita consumption, chemical exposure and to facilitate cross comparison of sites (Castiglioni et al. 2014). Attempts at measuring the population contributing to the wastewater have been made (e.g. measuring different hydrochemical parameters (Andreottola G. 1994)) but in most wastewater-based epidemiology studies, design capacity of the WWTP or census estimates are used and there may be high variability between these estimates (Castiglioni et al. 2014). Daughton proposed that the wastewater itself may contain chemical markers which have the potential to quantify the population contributing to a wastewater sample. Furthermore,
For this chapter, creatinine, an endogenous compound arising from the breakdown of creatine phosphate in muscles was investigated as a primary population marker. In previous assessments of population markers, head-counts have been estimated. The novelty of this study was accessing an accurate head-count via wastewater samples collected from 10 sites across Australia on Census Day 2011. As these samples are related to an accurate population, they provide the most accurate assessment of population size markers to date.

The following publication is incorporated as Chapter 2:

Thai, P.K., **O'Brien, J.W.**, Jiang, G., Gernjak, W., Yuan, Z., Eaglesham, G., Mueller, J.F., 2014. Degradability of creatinine under sewer conditions affects its potential to be used as biomarker in sewage epidemiology. Water Res 55, 272-279.

Peer–reviewed and published by Water Research
Accepted author manuscript

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

Phong K. Thai[1 *], Jake O'Brien[1], Guangming Jiang[2], Wolfgang Gernjak[2], Zhiguo Yuan[2], Geoff Eaglesham[1], and Jochen F. Mueller[1]

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

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

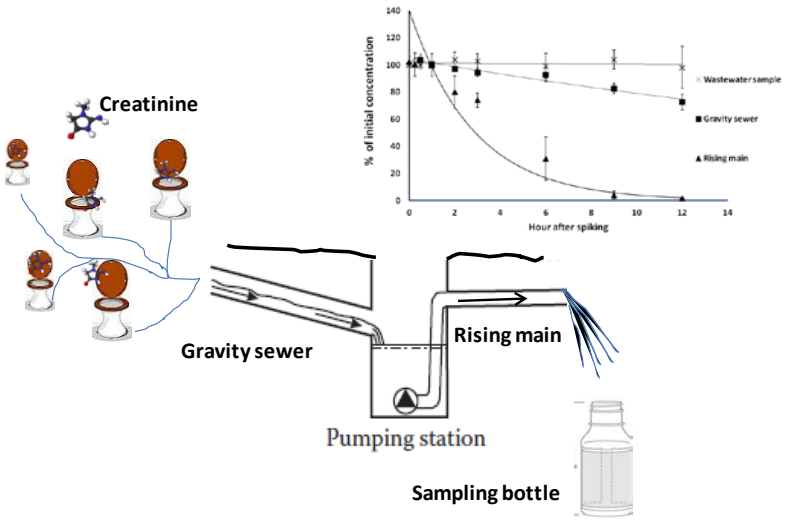
*** Corresponding Author. Tel.: +61 (07) 3274 9060. Fax: +61 (07) 3274 9003, E-mail address:** p.thai@uq.edu.au

2. 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 rates in rising main conditions than in gravity sewer conditions. 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 candidate to be a biomarker for population normalisation purpose in sewage epidemiology, especially in sewer catchments with high percentage of rising mains.

Keywords: stability, sewer reactor, degradation, sewer biofilms, wastewater analysis

2. Graphical Abstract



2.1 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 (Baker et al. 2012, Banta-Green et al. 2009, Daughton 2011, Lai et al. 2011, Postigo et al. 2011, Thomas et al. 2012, van Nuijs et al. 2011b). 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).

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, Daughton 2012a). 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 (Hvited-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 (Gutierrez et al. 2008, Mohanakrishnan et al. 2009). And Thai et al. (2013) 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 in the sewer system 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.

2.2 Materials and Methods

2.2.1 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}).

2.2.2 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 an 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 x 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 seconds before ramping up to 100% B in 10 seconds and holding for 2 minutes before re-equilibrating at 15% B for a further 2 minutes. 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.
Calibration curves (5, 10, 25, 50, 100, 250, 500, 1000 ng/mL) were run three times in each batch of instrumental quantification and the calibration curves were linear within

the range. The limit of detection (LOD) for creatinine is 3 ng/mL based on the signal to noise of 3. The corresponding limit of quantification (LOQ) is 10 ng/mL based on the signal to noise of 10.

The repeatability and reproducibility of the method were evaluated using both a wastewater sample (mean concentration of 38 ng/mL) and a spiked wastewater sample (200 ng/mL) with 10 replicates. The relative standard deviations were 3.5% and 1.7%, respectively. Recovery was calculated by subtracting the absolute average of the wastewater sample from the spiked wastewater sample and showed an average recovery of 112% (108 – 116%). The chromatograms of standard and real sample were shown in the Supplemental Materials (Figure S- 1).

Procedural blanks, procedural recoveries and matrix spike recoveries were analyzed in every batch of samples and were considered acceptable as no positive detection was observed for the blank samples and the recoveries of spiked samples are within the recorded range.

2.2.3 Laboratory-scale sewer reactors

The use of laboratory-scale sewer reactors to investigate the biotransformation and degradation of various chemicals has been described previously (Jiang et al. 2009, Thai et al. 2014a). The experiment employed well-controlled laboratory-scale reactors to simulate typical sewer conditions (Figure 1), which have been reported in many studies about in-sewer processes and the control of activities of sewer biofilms. (Gutierrez et al. 2008, Jiang et al. 2011a, Jiang et al. 2010, Jiang et al. 2013, Mohanakrishnan et al. 2009) In this study, three different sewer reactors were employed, namely a rising main (RM) sewer reactor, a gravity sewer (GS) reactor and a control (CR) sewer reactor without biofilms. Detailed description of the reactors can be found in Thai et al. (2013).

Sewer biofilms in RM and GS reactors have been cultivated for 12 months using real wastewater before the experiments. Domestic wastewater, collected weekly from a local pumping station in Brisbane (Australia) was used as the feed. The sewage (pH around 7.5) typically contained sulfide at concentrations of <3 mg-S/L, sulfate at 10-25 mg-S/L, total COD and soluble COD at 450-600 mg/L and 260-450 mg/L, respectively, with the latter including volatile fatty acids at 50-120 mg-COD/L. The sewage was stored at 4 °C and heated up to 20 °C before being pumped into the

reactors. The reactors were fed with sewage through a peristaltic pump (Masterflex 7520-47) every 6 hours, a typical sewage hydraulic retention time in sewers (Hvited-Jacobsen, 2013). Every feed pumping event lasted for 2 minutes, delivering one reactor volume (0.75 L) of sewage into each reactor. To ensure homogeneous distribution in reactors, gentle mixing was provided with magnetic stirrers at 250 rpm (Heidolph MR3000).

Prior to the degradation tests for creatinine described in Section 2.4, batch tests were conducted to determine the biological activities of sewer biofilms, i.e. sulfate reduction and methanogenesis, in the sewer reactors. Wastewater samples were then taken at 0, 20, 40, and 60 minutes after feeding for the analysis of dissolved inorganic sulfur and methane. Detailed procedures of the biofilms activity test can be found in Jiang et al. (2010).

2.2.4 Batch tests for the degradation of creatinine in sewer reactors

Batch tests in triplicate were conducted to investigate the degradation of creatinine under different sewer conditions. For each replicate of the batch test, wastewater newly taken from the same pumping station was warmed to 20^{o}C and its pH was adjusted to 7.5 using either 1M NaOH or 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 chemicals in wastewater (Chen et al. 2012a, Plosz et al. 2013, van Nuijs et al. 2012). The temperature- and pH-adjusted wastewater was then pumped into the RM and GS reactors using a peristaltic pump (Masterflex 7520-47). Care is taken to ensure that the liquid in each reactor was replaced with newly prepared wastewater. The CR reactor was manually filled with newly prepared wastewater from the top.

Working solution of creatinine (100 µg/mL) prepared in deionised water was spiked into each of the three reactors to obtain an initial creatinine concentration in the range of 1 µg/mL, which is similar to the range of creatinine in wastewater samples reported previously (Burgard et al. 2013, Chiaia et al. 2008).

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 conditions in the GS reactor and aerobic conditions in the CR (dissolved oxygen around 0.5 mg/L). Wastewater samples were then taken at

time 0, 0.25, 0.5, 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 regenerated cellulose syringe filters (Phenomenex, Australia). Six µl of 2M HCl was added to each vial to adjust the samples to approximately pH 2 to reduce microbial activity. The samples were then frozen at -20°C until analysis.

2.2.5 Wastewater sampling on Census Day

Twenty-four hour composite samples were collected from the inlets of 10 WWTPs on or around the 2011 Population Census Day in Australia (9th August 2011). Different sampling modes were used including continuous flow proportional, high-frequency volume or time proportional, depending on the equipment available at each treatment plant (Table S- 1). These WWTPs are from the Australian Capital Territory (1 WWTP), South Australia (2 WWTPs), Tasmania (1 WWTP) and Queensland (7 WWTPs). After collection, samples were preserved by acidifying to pH 2 with 2M HCl and shipped on ice (chilled or frozen) to the laboratory. Samples were then stored at -20°C until analysis. For analysis, thawed samples were filtered into 2-mL vials using 0.45 µm regenerated cellulose syringe filters, spiked with internal standard (creatinine-d3), and directly injected into LC-MS/MS system.

The samples collected around the Census Day were assumed to reflect the consumption/exposure/excretion of the census population in each sewer catchment. The catchment maps provided by WWTPs were sent to the Australian Bureau of Statistics (ABS) for population counts (Persons Enumerated or population residing in the catchment on Census Day). It is noted that census participation in Australia is compulsory and it is estimated that the error of the 2011 Census is approximately 1.7% of the population. For the purpose of this study the ABS Census values were not adjusted.

2.3 Results and Discussion

2.3.1 Biological activities in sewer reactors.

The results of the biological activities tests confirmed that RM, GS and CR reactors have produced the expected sewer conditions (Thai et al. 2014a). The RM reactor was under anaerobic condition, supported by the production of sulfide and methane. The

activity of sulfate-reducing bacteria and methanogenic archaea in the RM reactor was measured to be 4.3 ± 0.3 mg S/L-h and 18.9 ± 3.2 mg COD/L-h respectively, similar to previously reported values in both laboratory-scale and real sewers (Guisasola et al. 2008, Jiang et al. 2011a, Jiang et al. 2011b), confirming that the laboratory setup can mimic actual conditions of sewer pipes. Despite continuous aeration, the dissolved oxygen in the GS reactor was measured to be below 0.5 mg/L, indicating there is aerobic activity consuming oxygen. Similar to many typical gravity sewers, it is likely that anaerobic conditions also developed at the bottom of the reactor where oxygen could not reach. Measured anaerobic activity in the GS reactor is low in terms of sulfate reduction (0.17 ± 0.05 mg S/L-h) and methane generation (1.5 ± 0.15 mg COD/L-h). This is primarily due to the aerobic conditions, and also partly due to the continuous dissipation of gases through the air-water interface. No significant gas production activity was detected in the CR reactor as it lacked the sewer biofilms compartment. Detailed discussion can be found in Thai et al. (2013) where it demonstrated that the biological activities observed in the laboratory-scale reactors coincide well with those in real sewer systems.

2.3.2 Degradation of creatinine in the sewer reactors.

Since creatinine is very soluble in water (log Kow = -1.76) and has negligible vapour pressure we assumed that volatilisation and adsorption of creatinine were also negligible during the 12-h test period. Therefore, all losses of creatinine during the experiment in sewer reactors were attributed to the degradation of creatinine in wastewater.

Figure 2 shows the degradation profiles of creatinine under different sewer conditions. The graph represents the relative concentrations of creatinine during the tests with the initial concentrations regarded as 100%.

The results reveal that creatinine is relatively stable in wastewater alone (i.e. without biofilms) where the concentration of creatinine at 12 hours is not significantly different from the initial concentration (Figure 2). This result suggests that the microbial activity in the liquid-phase of the sewer system (wastewater) does not have considerable impact on the degradation of creatinine. It is in agreement with data from other studies where creatinine was observed to be either stable or degraded relatively slowly in the wastewater matrix. (Bisceglia et al. 2012, Brewer et al. 2012, Burgard et al. 2013)

Meanwhile, after 12 hours, degradation accounted for 27% of creatinine loss in the GS reactor while creatinine degraded almost completely in the RM reactor. The concentrations of creatinine were nearly depleted after 9 hours of batch test in the RM reactor. In the RM reactor, degradation can be considered complete after 9 hours. It should be noted that there seems to be a lag phase at the early stage in the RM reactor which may be due to the homogenization.

The data from the CR reactor re-affirmed the stability of creatinine reported previously in wastewater (Brewer et al. 2012, Burgard et al. 2013) and deionized water (Bisceglia et al. 2012) (f) which is reasonable because creatinine has been found to be stable in urine samples, a form of concentrated wastewater with minor microbial activities (Garde et al. 2003, Liu et al. 2012) (Table 4). Meanwhile, the loss of creatinine in the reactors with biofilms are significant ($p<0.01$), i.e. 27% and 99% for GS and RM reactors, respectively (Table 4). It is reasonable to assume that the microbial activity of the biofilms in the sewer reactors accelerates the degradation rate of creatinine compared to wastewater alone as it is the case for some illicit drugs (Thai et al., 2013). Only Bisceglia reported in his dissertation a similarly high degradation rate of creatinine in wastewater alone (Bisceglia et al. 2010). It is possible that the wastewater used in the experiment of Bisceglia (2010) has stronger microbial activity than those used in other studies (Brewer et al. 2012, Burgard et al. 2013).

While the significant loss of creatinine in biological sewer reactors was related to the existence of active sewer biofilms, the difference of degradation rates in RM and GS reactors was potentially because of the different type of biofilms and thus different biological processes. Some microbes were reported to use creatinine as alternative nitrogen sources to survive nitrogen limitation situations (Bendt et al. 2004) although this is not the case for the wastewater which had an ammonium level of around 50 mg N/L. Creatinine was also suggested to be utilized as a source of both carbon and nitrogen at the same time by various microorganisms (Shimizu et al. 1986, Yamada et al. 1985). Since anaerobic microorganisms prevail in the RM reactor while both aerobic/anaerobic microbes were present in the GS reactor it seems that the abundance of anaerobic microbes was related to the degradation rate observed.

The data obtained from the batch tests were analysed to identify the degradation kinetics. While the data of creatinine in CR reactor indicated no significant degradation was observed during the experiment, the degradation of creatinine in the GS and RM

reactors fits well with first order kinetics (Table 5). The degradation rate of creatinine in RM reactor is much faster than that in the GS reactor suggesting that anaerobic biofilms might be more active in degrading creatinine than aerobic biofilms as discussed in the previous section. Although this study did not determine the metabolic product(s) of creatinine degradation, it demonstrated that creatinine is susceptible to degradation under actual sewer conditions due to the prevalence of microbial activities and relatively long hydraulic retention time.

2.3.3 Measured load of creatinine and the population

The daily loads of creatinine were calculated (creatinine concentration × daily flow) for 10 WWTPs and compared with the corresponding population served by the WWTP to see if there is any correlation. No correlation was found between those two data sets (Figure 3 and Table S- 1). This is likely due to the different levels of degradation due to different sewer configurations and different lengths of residence time. The measured concentrations of creatinine at the WWTP are 1-3 orders of magnitude lower than the estimates from human excretion, which supported the results observed in laboratory scale sewers.

It is noted that the concentration of creatinine measured in most wastewater samples in this study are much lower (up to 2 orders of magnitude) compared to data reported previously in the US (Brewer et al. 2012, Chiaia et al. 2008). One possible explanation is that the hilly geography of Australian towns requires sewer systems with more rising main pipes than those in the US and thus results in much more degradation of creatinine in Australian sewer systems. The two WWTPs in Australia with the highest per capita loads of creatinine measured in this study (WWTP 6 and WWTP 10 in Table S- 1) also receive industrial waste which includes wastewater from slaughterhouses which potentially contains large quantities of creatinine in either waste blood or urine.

We applied the method using creatinine analysis in wastewater developed by Brewer et al. (2012) to estimate the creatinine excretion by the population. By using this method, our calculation from data of Chiaia et al. (2008) showed that the measured loads of creatinine in WWTPs in the US were 50% less than the expected load based on per capita excretion data. Brewer et al. (2012), using daily creatinine load to estimate the population, has underestimated the population by at least 50% (and as low as 15% of the maximum population). Those results also suggested that the loads

of creatinine from WWTPs cannot be used to directly estimate the number of people in the catchment without knowing the extent of creatinine degradation within the sewer system.

2.3.4 Implication for the applicability of creatinine in sewage epidemiology

The findings of the sewer reactor experiments indicate that the activity of microorganisms in the sewer system on creatinine is stronger than that in the wastewater alone due to the presence of biofilms on the sewer walls. This activity has significantly enhanced the degradation rate of creatinine in the sewer, especially under anaerobic condition (rising mains). This confirmed the results reported previously on the activity of sewer biofilms on the degradation of illicit drug biomarker (Thai et al., 2013) and sulfide production (Gutierrez et al. 2008, Mohanakrishnan et al. 2009).

The degradation of creatinine in the sewer system may limit its potential to be used as a biomarker for the estimation of population size or to be used as a normalisation chemical similar to its role in clinical chemistry (urinalysis) to compare any wastewater parameters among different WWTPs (or sewer catchments). Although both the reactor data and the data from different WWTPs indicated the degradability of creatinine in the sewer system, a controlled field study to confirm this finding is necessary.

It is also beneficial to investigate in detail the degradation pathway of creatinine in sewer to better understand the initiator of the process and see if one can assess the extent of creatinine degradation by using its transformed products/metabolites.

Meanwhile, we recognise the potential of creatinine to be used as a biomarker for monitoring population changes in a specific sewer catchment. The reasoning is that each sewer system would potentially function with reasonable reproducibility (i.e. consistent parameters that affect degradation) for creatinine degradation (average retention time, ratio of rising main/gravity sewer etc.) so that the level of degradation could be assumed similar among days. But this will not be practical, in our opinion, when the degradation of creatinine in the system render the load of creatinine negligible (i.e. only a small fraction of excreted creatinine can be measured at the WWTP).

As this study indicates the different effects of different sewers (GS and RM) on creatinine, more knowledge about the sewer system, such as the ratio of GS and RM sewer in addition to the average residence time, is required to understand/evaluate the degradation of creatinine in a sample collected in a specific sewer system.

The demonstrated capability of the sewer reactor to mimic real sewers in terms of types of biofilm, microbial populations and biological activity will promote them as essential tool to investigate the in-sewer fate of chemicals of interest including further studies about the transformation/degradation of creatinine in GS and RM for the purpose of applying them in the sewer epidemiology approach. However, some factors such as A/V ratio, flowing condition and hydraulic retention time should be carefully considered during the design of the lab-scale sewer reactors to best represent the actual sewer system.

2.4 Conclusions

This study evaluated the stability of creatinine under different sewer conditions using sewer reactors. It also compared the actual daily loads of creatinine in 10 WWTPs in Australia with the corresponding census populations. The main conclusions are:

1. Creatinine is stable in wastewater only but the simulated sewer conditions (RM and GS) enhanced its degradation, probably due to the presence of sewer biofilms.
2. Creatinine degradation under RM and GS conditions follows first order kinetics with degradation rate significantly higher under RM conditions than under GS conditions.
3. No correlation was found between daily loads of creatinine and populations in 10 WWTPs in Australia thus hindering the applicability of using creatinine as normalising biomarker in sewer epidemiology.
4. Further studies about degradation pathway of creatinine in the sewer system as well as a field trial are necessary to fully evaluate the potential of creatinine in sewer epidemiology.

2. Acknowledgements

Entox is a joint venture of The University of Queensland (UQ) and Queensland Health Forensic Science Services (QHFSS). This research is partly funded by the UQ Start-up Grant for Phong Thai. Phong Thai is funded by a UQ Postdoctoral Research Fellowship. Jochen Mueller is funded by an ARC Future Fellowship (FF

120100546). The establishment and operation of the sewer reactors at the Advanced Water Management Centre was supported by the Australian Research Council funded project LP0882016 “Corrosion and Odour Management in Sewers’.

2. Figures

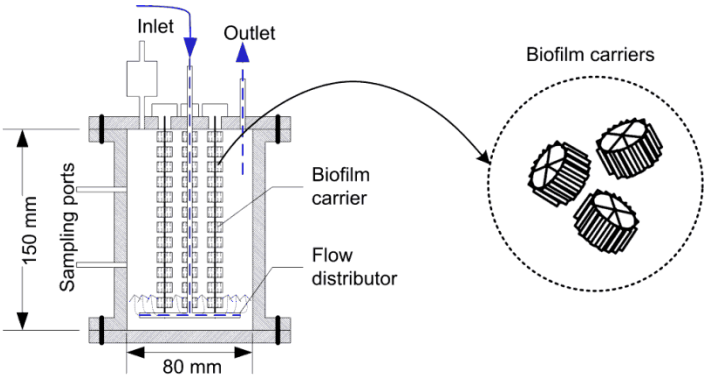


Figure 2 Sewer reactors with carriers to grow biofilms using wastewater.

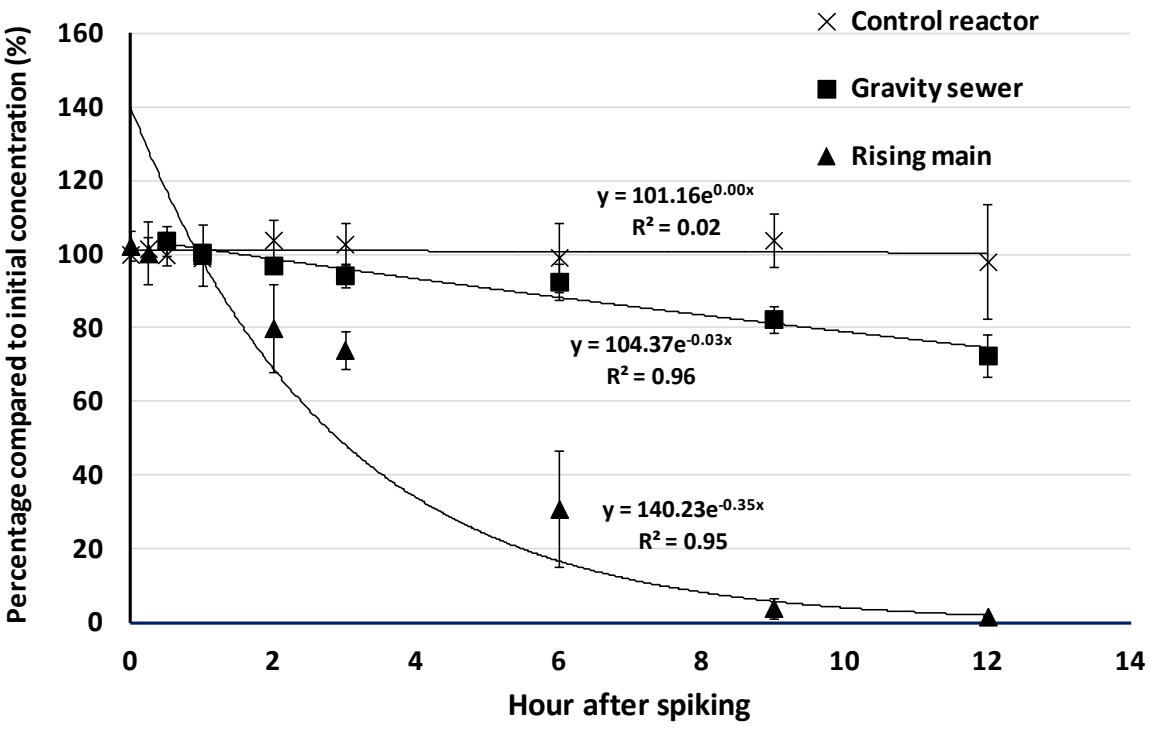


Figure 3 Degradation profiles of creatinine under different sewer conditions. Error bars represent the standard deviation of 3 replicates.

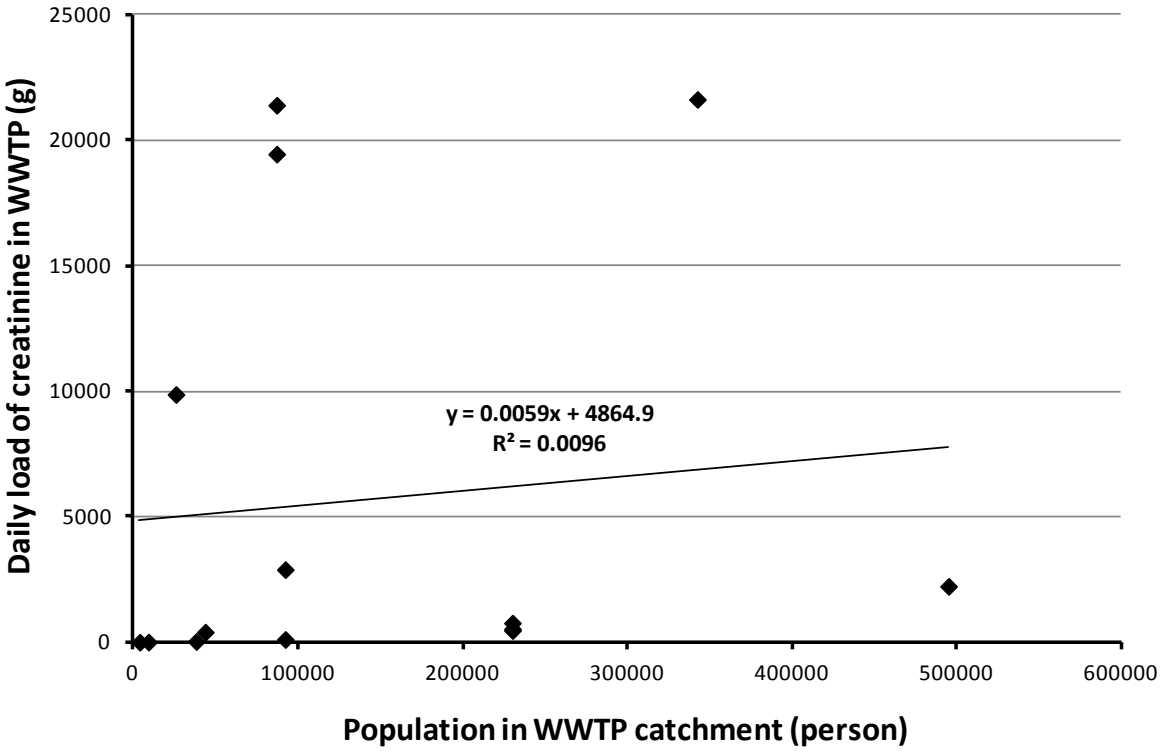


Figure 4 Daily load of creatinine vs population in 10 different WWTPs in Australia.

2. Tables

Table 4 Degradation of creatinine in wastewater.

	Chiaia *et al.* (2008)	Bisceglia *et al*. (2010)	Bisceglia *et al*. (2012)	Brewer *et al.* (2012)	Burgard *et al.* (2013)	This study pH = 7.5; 20 °C; 12 h.		
Experimental conditions	Wastewater collected from different WWTPs.	Wastewater 31°C; 23°C; 9°C; 24 h	Deionized water and wastewater pH = 12 55°C; 3 h	Wastewater collected from one WWTP. 4°C; 24 h	Wastewater20°C; 72 h	Waste-water	Gravity sewer (aerobic)	Rising main (an-aerobic)
Concentration range (mg/L)	0.22-1.5	0.06-0.09	0.31-0.55	~1.2	1.9-10.7	~1.0		
Degradation	n/a	Complete loss at 31°C; 23°C; 40% loss at 9°C	relatively stable	13% loss	Stable in first 48h. 17% loss in last 24h	Stable	27% loss after 12h	99% loss after 12h

Table 5 Linear regression of creatinine degradation in different sewer reactors.

	First order kinetics	
	Half-life (h)	R^2
Control (wastewater only)	n.a [a]	0.02 [a]
Gravity sewer (GS reactor)	24.8	0.96
Rising main (RM reactor)	2.0	0.95

[a] not significantly deviated from zero

Chapter 3: Assessment of pharmaceuticals and personal care products as population markers and the development of a population model

Chapter 2 investigations suggest creatinine is too unstable within the sewer environment and no correlation was observed between the daily mass load of creatinine in wastewater influent and the population size. Therefore we decided to assess pharmaceuticals and personal care products (PPCPs) as population markers and to avoid the limitations of using a sole population marker, develop a model which includes multiple population markers. Chapter 3 focuses on the assessment of a suite of PPCPs as population size markers and the development of a model using the best population size markers identified from this assessment.
The following publication is incorporated as Chapter 3:

O'Brien, J.W., Thai, P.K., Eaglesham, G., Ort, C., Scheidegger, A., Carter, S., Lai, F.Y., Mueller, J.F., 2014. A model to estimate the population contributing to the wastewater using samples collected on census day. Environ. Sci. Technol. 48, 517-525.

Peer–reviewed and published by Environmental Science and Technology
Accepted author manuscript

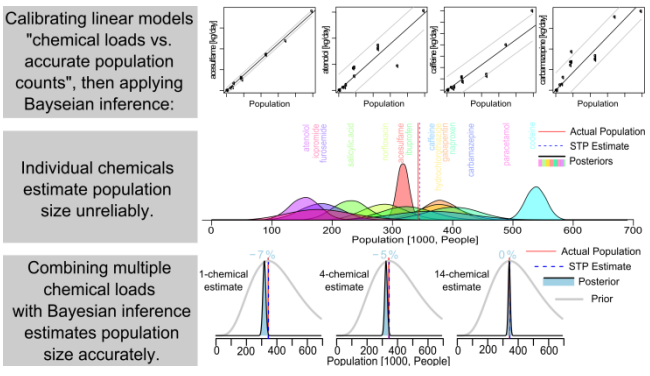
Title: A model to estimate the population contributing to the wastewater using samples collected on census day
Journal: Environmental Science and Technology
Authors: Jake W. O'Brien*[a], Phong K. Thai[a], Geoff Eaglesham[a], Christoph Ort[b,c], Andreas Scheidegger[c], Steve Carter[d], Foon Yin Lai[a], Jochen F. Mueller[a]

Affiliation: [a]The University of Queensland, The National Research Centre for Environmental Toxicology (Entox), 39 Kessels Road, Coopers Plains, QLD 4108, Australia
[b]The University of Queensland, Advanced Water Management Centre (AWMC), St. Lucia, QLD 4072, Australia
[c]Eawag, Swiss Federal Institute of Aquatic Science and Technology, CH 8600 Dübendorf, Switzerland
[d]Queensland Health Forensic and Scientific Services, 39 Kessels Road, Coopers Plains, QLD 4108, Australia
Corresponding Author: Present Address: Entox, 39 Kessels Road, Coopers Plains, QLD 4108, Australia. Tel: +61 (7) 3274 9120; Fax: +61 (7) 3274 9003; Email: j.obrien2@uq.edu.au

Abstract:

An important uncertainty when estimating per capita consumption of e.g. illicit drugs by means of wastewater analysis (often referred to as sewage epidemiology) relates to the size and variability of the *de facto* population in the catchment of interest. In the absence of a day-specific direct population count any indirect surrogate model to estimate population size lacks a standard to assess associated uncertainties. Therefore, the objective of this study was to collect wastewater samples at a unique opportunity, i.e. on a Census Day, as a basis for a model to estimate the number of people contributing to a given wastewater sample. Mass loads for a wide range of pharmaceuticals and personal care products were quantified in influents of ten sewage treatment plants (STP) serving populations ranging from approximately 3,500 to 500,000 people. Separate linear models for population size were estimated with the mass loads of the different chemical as the explanatory variable: fourteen chemicals showed good, linear relationships, with highest correlations for acesulfame and gabapentin. Bayesian inference was then used to estimate *de facto* population using the population size provided by STP staff as prior knowledge and updated that with the measured chemical mass loads. Cross validation showed that large populations can be estimated fairly accurately with a few chemical mass loads quantified from 24-h composite samples. In contrast, the prior knowledge for small population sizes cannot be improved substantially despite the information of multiple chemical mass loads. In the future, observations other than chemical mass loads may improve this deficit, since Bayesian inference allows including any kind of information relating to population size.

3. Abstract Art:



Key Words: wastewater, population, estimation, model, Bayesian Inference, pharmaceuticals

3.1 Introduction:

The first documented attempt for estimating the *de facto* population that is serviced by a sewage treatment plant (STP) was developed in the 1970s through a model developed for Ocean City in Maryland. This model was to measure tourist influx and adjust the number of rostered medical staff appropriately(Goldschmidt and Dahl 1976). The underlying assumption of the model was that population increase is correlated and thus can be estimated from water consumption. However, daily per capita water consumption may be variable for several reasons including seasonal variations, industrial wastewater input and ex/infiltration of water in the sewer(Daughton 2012a, Editorial 2009, Russo 2009).

The *de facto* population count, all people present in a given area, is an important parameter where chemical loads obtained through wastewater analysis (WWA) are used to estimate consumption or exposure of a given population which is sometimes referred to as ‘sewer epidemiology’. In particular, there is emphasis on using this technique for objective assessment of illicit drug use in the general population through WWA(Daughton 2012b). Wastewater analysis data are commonly normalised on a per capita basis, i.e. the amount of chemical that was consumed per 1000 people, per day in a particular catchment. It was recognised that a *de jure* population (usual residents) may not be adequate for this normalisation because: i) administrative regions for *de jure* populations may not coincide with geographic catchments of STPs, ii) *de jure* population estimates may be outdated since they are not carried out very frequently, and iii) transient changes are not accounted for(Daughton 2012a). Therefore, it was suggested to use a *de facto* population, i.e. the number of people that effectively contributed to the wastewater sample during sample collection since it appropriately accounts for population fluxes into or out of the catchment, i.e. regular commuters, visitors, tourists etc. People can travel daily in and out of catchments for a range of reasons—to work, to study, to go on holiday and various other activities—with visits varying both in timing and duration. The importance of these population movements has been recognised and discussed by

Charles-Edwards and Bell(Charles-Edwards and Bell 2012). Lai et al. highlighted that variations in the number of tourists may be substantial in specific locations, such as vacation destinations(Lai et al. 2013a).

Historically, and for practical reasons, wastewater samples are collected over periods of 24 hours and conceptually it would be desirable to also have reliable *de facto* population values on a daily basis. The calculation for estimating daily consumption of a chemical per person via WWA was first published by Zuccato et al.(2008) where i is the chemical of interest, C_i is the concentration of the chemical in the sample, F is the total daily volume of water during the sampling period, R_i the ratio of the molar mass of the parent chemical to its metabolite, E_i the average excretion of the chemical and P is the number of people who contributed to the sample.

$$Daily\ per\ capita\ drug\ consumption_i = \frac{C_i \cdot F \cdot \frac{R_i}{E_i}}{P}$$

Recently, a number of experimental approaches have been applied to address the uncertainty of P. For example, Van Nuijs et al.(2011c) normalised their yearlong data from the largest STP in Belgium through measuring biological oxygen demand, chemical oxygen demand, nitrogen and phosphorous. However, these markers are not human-specific as multiple sources can contribute to these loads within sewers including food waste and industrial wastewater(Castiglioni et al. 2013, Daughton 2012a). Instead, to estimate the *de facto* population, Lai et al.(2011) proposed an approach where human-specific markers were measured including both parent compounds and/or excreted metabolites of prescription pharmaceuticals (atenolol, gabapentin, hydrochlorothiazide, methadone and venlafaxine). The measured mass load of each pharmaceutical was then compared with the expected mass load according to records in the Australian Pharmaceutical Benefits Scheme database. The authors however acknowledged limitations associated with this approach such as accuracy of national annual consumption data and the stability of these chemicals in sewer systems. Therefore, they suggested another potential human marker, acesulfame (an artificial sweetener), because this chemical is not metabolised and is fully excreted after consumption. Additionally acesulfame is relatively persistent in wastewater(Brorström-Lundén et al. 2008, Buerge et al. 2009), and thus may be a useful population marker.

Daughton(2012a) alternatively proposed the concept of using endogenous markers for population estimation and put forward the potentials and limitations of creatinine and coprostanol. Brewer et al.(2012) used creatinine loads to assess relative trends, i.e. diurnal and between-day variations over multiple days at a single STP serving approximately 50,000 people. Burgard et al.(Burgard et al. 2013) also used creatinine to normalize their data for population change but this was for a much smaller population of >500 people and was sampled much closer to the source. There are, however, limitations with creatinine to be used as a single population size marker such as unknown stability in the sewer under varying conditions and there are potentially other sources of creatinine input such as abattoir waste(Martin-Tereso et al. 2010). Additionally, a recent survey in the United States identified that 34.1% of children and adolescents who use dietary supplements take creatine to build body mass(Evans et al. 2012) which may lead to increased excretion of its primary metabolite creatinine and bias results(Wyss and Kaddurah-Daouk 2000).

One key problem associated with the development and validation of population estimation models concerns the lack of accurate data of populations (i.e. the true number of people who contribute to a given wastewater sample) which can ultimately be used to validate such models. Thorough population censuses provide an opportunity to obtain such data with small error and thus should be utilised. Renaud(Renaud 2007) investigated coverage for five countries (Australia, Canada, United Kingdom, United States of America and Switzerland) and found undercoverage more prevalent and ranged from 1 to 3%. Overcount was only observed for the 2000 United States of America census with an estimated 0.5% overcoverage.

The aim of this study was to i) obtain accurate population data for 10 STP catchments in Australia on Census Day (a day-specific geo-referenced population count), ii) measure mass loads of chemicals and assess their individual capability for estimating population size and iii) use Bayesian inference to incorporate chemical markers which are representative of population size into a population estimation model and validate this model on a leave-one-out basis (cross validation).

3.2 Methods:

3.2.1 Measurement of daily chemical mass loads

3.2.1.1 Wastewater sampling

Twenty-four hour composite samples were collected from the raw inlets – before the primary clarifier – of ten STPs between the 9th and 11th of August 2011. The STPs cover urban, semi-rural and rural catchments located throughout Australia including the Australian Capital Territory (one STP), South Australia (two STPs), Tasmania (one STP) and Queensland (six STPs) with populations ranging from 3,500 to 640,000 (estimated number of people provided by STP operators). A summary of each site including sampling mode, daily wastewater volume and population can be found in Table 6. After collection samples were preserved by acidifying to pH 2 with 2M hydrochloric acid and shipped on ice (chilled or frozen) to the laboratory. The samples were stored at -20°C prior to analysis.

Table 6 Site summary of the sewage treatment plants and samples collected in this study. Enumerated Population on Census Day is the number of people who spent the majority of Census Day in that catchment area. Usual population is the number of people who reside in the STP catchment area. Best estimate of the model is the estimated number of people for that site using the Bayesian inference model calibrated in this manuscript.

STP Size	STP ID	Date Sampled (D/M/Y)	State	Sampling Mode	ΔT or ΔV	Daily Flow (Megalitres)	Nominal Population (STP operator estimate)	*de facto* (ABS census)	*de jure* Usual Population	Best estimate of our model (S14)	80% Inter-quantile of posterior	Ratio of 80% Interquantile Prior to 80% Interquantile Posterior for S14	% Difference of STP estimate and our best estimate	% Difference of ABS census and our best estimate
Small	STP 1	10/08/2011	South Australia	Volume Proportional	10 m^3	1.6	3,500	3,682	3,692	3,542	4,169	1.06	1.2	-3.8
	STP 2	10/08/2011	South Australia	Volume Proportional	20 m^3	3.8	10,000	8,995	9,272	9,398	9,818	1.26	-6.0	4.5
	STP 3	11/08/2011	Tasmania	Continuous Non-Flow Proportional	NA	12.6	28,000	25,698	25,992	29,912	18,768	1.87	6.8	16
	STP 4	09/08/2011	Queensland	Time Proportional	20 min	4.1	26,600	38,005	38,000	24,143	16,671	1.96	-9.2	-37
	STP 5	10/08/2011	Queensland	Time Proportional	60 min	10.2	40,000	43,513	43,648	50,442	21,083	2.42	26	16
Large	STP 6	10/08/2011	Queensland	Volume Proportional	100 m^3	21.4	100,000	86,882	86, 592	96,386	22,538	5.52	-3.6	11
	STP 6	11/08/2011	Queensland	Volume Proportional	100 m^3	21.2	100,000	86,882	86, 592	103,615	22,372	5.75	3.6	19
	STP 7	10/08/2011	Queensland	Time Proportional	20 min	15.6	96,000	92,104	92,486	85,590	19,648	6.2	-11	-7.1
	STP 7	11/08/2011	Queensland	Volume Proportional	20 min	15.2	96,000	92,104	92,486	87,132	19,623	6.27	-9.2	-5.4
	STP 8	09/08/2011	Queensland	Continuous Flow Proportional	NA	57.1	330,000	230,117	211,340	235,904	19,896	19.6	-29	2.5
	STP 8	10/08/2011	Queensland	Continuous Flow Proportional	NA	56.4	330,000	230,117	211,340	220,000	19.238	19.7	-33	-4.4
	STP 8	11/08/2011	Queensland	Continuous Flow Proportional	NA	58.0	330,000	230,117	211,340	243,855	19,263	19.8	-26	6.0
	STP 9	09/08/2011	Australian Capital Territory	Time Proportional	60 min	80.3	345,000	342,459	338,888	340,843	17,954	26.6	-1.2	-0.5
	STP 10	11/08/2011	Queensland	Time Proportional	20 min	127.4	643,000	495,027	474,520	454,490	15,130	49.6	-29	-8.2

ΔT = time between samples, ΔV = volume between samples, *de facto* (ABS census) = enumerated people on Census Day, *de jure* Usual Population = persons usually residing at the address

3.2.1.2 Analytical standards

Analytical grade formic acid was purchased from Sigma Aldrich (Castle Hill, Australia). Analytical grade hydrochloric acid 32% was purchased from Univar (Ingleburn, Australia). Water was purified through a MilliQ system (Millipore, 0.22 µm filtered, 18.2 mΩ cm^{-1}). Analytical grade pharmaceutical and personal care product (PPCP) solutions were prepared using only high purity standards (see SI for further information) and diluted into MilliQ water for a calibration set. Liquid chromatography grade acetonitrile was purchased from Merck (Darmstadt, Germany). Mobile phases were filtered using Sartorius Stedim 0.45 µm RC filters (Goettingen, Germany).

3.2.1.3 Chemical analysis

Wastewater contains many PPCP's which are usually measured by solid phase extraction (SPE) and concentration. We focussed on PPCP chemicals which could be directly measured in filtered (0.45 µm, 47 mm nylon filter membranes (Phenomenex, Lane Cove, Australia)) wastewater without the need for extraction and concentration using either negative or positive ionisation modes on LC-MS/MS because for near real-time population estimation it is important that the time, cost, sample preparation and analysis types required are minimised.

An in-house analytical method from Queensland Health Forensic and Scientific Services was optimised and applied for screening 96 PPCPs in wastewater. This method is an adaption of U.S. EPA Method 1694(U.S. Environmental Protection Agency 2007). Chemical analysis was performed using an AB/Sciex API 5500 QTrap mass spectrometer (AB/Sciex, Concord, Ontario, Canada) with an electrospray ionization (ESI) interface coupled to a Shimadzu Nexera HPLC system (Shimadzu Corp., Kyoto, Japan). Separation was achieved on a Luna C-18 (2) column (3 µm, 100 Å, LC Column 150 mm x 3 mm, Phenomenex) using a mobile phase gradient of 1 to 95 % acetonitrile with 0.1% formic acid. Instrumental parameters can be found in the Supporting Information.

For quantification both filtered samples and calibration standards (5 – 100 µg L^{-1}) were spiked with deuterated standards ranging from 20 to 200 µg L^{-1} depending on chemical sensitivity prior to direct injection analysis. Reagent blanks were prepared with MilliQ water acidified to pH2 with HCl. For quality control and assurance, all

samples including the reagent blanks were spiked with the deuterated standards and were run in triplicate. The results were averaged. Positive reagent blank results were averaged and subtracted from the measured concentration within the samples. The analytical method was reproducible with the LOD for most chemicals of 1 µg L^{-1} within wastewater and a linear quantitation range between 5 and 100 µg L^{-1}.

3.2.1.4 Population count on Census Day (ABS census)

The population census is costly and as such only occurs every five years in Australia. Sampling was therefore organised to correspond with the Census Day. Ten STPs participated in this study and samples were collected either on or around the 2011 ABS Census Day, the 11th August 2011 (Table 6). Each STP provided a map with boundaries of the physical catchment they serve. The Australian Bureau of Statistics (ABS) determined the population connected to each STP by intersecting geo-referenced census data available at the administrative level “Statistical Area 1” with the catchment maps. There are approximately 54,000 “Statistical Area 1” regions covering Australia with approximately 400 people per region. The data returned by the ABS was for both *Enumerated Population* (population count based on where people spent most of their time on Census Day, hence *de facto* population) and *Usual Population* (population count based on residential addresses, hence *de jure* population). A site summary is provided in Table 6 (ABS, Customised report, 2012). While census participation in Australia is compulsory, a number of quality assurance procedures are in place to ensure the accuracy of the data such as a post enumeration survey conducted a month after census where a sample survey is conducted and compared with the census data which indicated that the 2011 census undercounted the population by approximately 1.7%(Australian Bureau of Statistics (ABS) 2011). For the purpose of this study we used the *Enumerated Population* provided by the ABS (subsequently referred to ABS census) as *de facto* population.

3.2.2 Relationships of chemical mass loads with population size

As the aim of this study was to develop and calibrate a model for the *de facto* population using mass loads of a broad range of chemicals, two criteria were chosen to identify these chemicals. Criteria 1: the chemical must be measurable via direct

injection on LC-MSMS in all of the samples we collected. Criteria 2: the mass load of the chemical must show a correlation with population size. Therefore, daily mass loads were calculated for the chemicals which were measured in all of our samples and plotted against the census population. This unique reference data set covers a wide range of STPs allowing the calibration of a separate model for each chemical. The models are based on the reasonable assumption that the mass load entering the treatment plant per day of a chemical is proportional to the contributing population. This implies that the measured mass load m_i of chemical i should be modelled as:

$$m_i = \beta_i P + \epsilon_i$$

where P is the corresponding population, ϵ_i the error term, and β_i the estimated parameter for chemical i. The error term accounts for differences in consumption between locations and daily variations. It is assumed that ϵ_i is independent normally distributed with standard deviation σ_i that is independent of the population.
Basically, there are three options for the error term that will be discussed subsequently: ϵ_1) a relative error term, i.e. the standard deviation is proportional to P, ϵ_2) a constant error term (independent of P), and ϵ_3) an error term whose standard deviation decreases for large P.
Measurement errors are often modelled with a relative error term (ϵ_1). Conceptually, one might expect the error term: ϵ_3 the variability of an aggregate consumption of a large population, to be small, whereas a consumption pattern from a few individuals in a small population may lead to large deviations. However, in view of the available data (see Figure 4) there is neither evidence for ϵ_1 nor ϵ_3 and only the constant error term ϵ_2 seems to be substantiated.
With this error term and the estimated parameter β_i the predictive probability density $p_i(m|P)$ for mass load m_i for a given population is defined. In paragraph 2.4 all single chemical models are combined to estimate the population.

3.2.3 Population estimation

For the population estimation the probability distribution of the population given n measured mass loads, $p_{Pop}(P|m_1, \dots, m_n)$, is of interest. This distribution is calculated according to Bayes theorem:

$$p_{Pop}(P|m_1,\dots,m_n) = \frac{p_1(m_1|P)\ \dots p_n(m_n|P)p_{Pop}(P)}{\int p_1(m_1|P)\ \dots p_n(m_n|P)p_{Pop}(P)\mathrm{dP}}$$
$$\propto\ p_1(m_1|P)\ \dots p_n(m_n|P)p_{Pop}(P)$$

where $p_i(m_i|P)$ are the predictive probability densities of the chemical models (see 2.3) and $p_{Pop}(P)$ represents the prior knowledge about the population. For numerical computation the proportional relationship is sufficient, so that the integral in the denominator need not be evaluated. The resulting posterior distribution, $p_{Pop}(P|m_1,\dots,m_n)$, can be summarized by its mean and the uncertainty can be expressed by the standard deviation or quantiles for confidence intervals.
In the current study, the prior knowledge about the population $p(P)$ is parameterized as gamma distribution. The mode was set to the population estimates provided by STP operators and the standard deviation was assumed to be 50% of this value.

3.2.4 Assessing the influence of multiple chemicals

To assess how the estimate changes with inclusion of multiple chemicals, five estimations for the same catchment were made with different numbers of measured chemical loads. The first uses only the highest correlating substance for mass load versus population (acesulfame) and is labelled substance model 1 (S1). Each progressive model doubles the number of substances used for its estimate in the order of highest correlating substances for mass load versus population first. As there are 14 substances identified as suitable markers for population size, we therefore have 5 models with each named after the number of substances they contain (S1, S2, S4, S8, S14). A summary of the chemicals which are included in each model can be found in Table 7.

3.2.5 Cross-validation

The predictive capability of the model is validated with a leave-one-out cross-validation. Thereby, the parameters of the linear regression models (Section 2.3) are estimated without one location. For this location the population is then estimated based on measured mass loads and compared with the ABS census. This is repeated for all locations. The average of all errors provides an estimation of the predictive error.

3.2.6 Implementation

All calculations were programmed using the freely available statistics software package R(R Core Team 2012). The summary statistics of the posterior distribution are based on 10,000 samples obtained by importance sampling. A link to the code is provided in the Supporting Information (SI).

3.3 Results and Discussion:

3.3.1 Selection of chemicals

Of the 96 chemicals screened, only fourteen were quantifiable without extraction or concentration in most samples. Ten of these have known Australian per capita consumption as they are subsidized by the Australian Government through the Pharmaceutical Benefit Scheme (PBS). These are atenolol, carbamazepine, codeine, furosemide, gabapentin, hydrochlorothiazide, ibuprofen, naproxen, norfloxacin and paracetamol. Two are commonly found in food and beverages (acesulfame and caffeine), one is an imaging contrast medium (iopromide) and the other (salicylic acid) is a metabolite of acetylsalicylic acid which is used as the active ingredient in aspirin and other medications primarily for pain relief and as an anti-inflammatory. Linear regressions for mass loads versus population as counted on Census Day were computed for each individual chemical (summary of annual consumptions and linear regressions in Table 7). With the exception of iopromide (R^2=0.38, with one obvious outlier removed from the dataset, otherwise R^2=0.62, see SI) all selected markers showed strong correlations (R^2>0.8) between population and mass load. Therefore, they can be used as markers to estimate population size. Including or excluding iopromide in the estimation of population size does not affect results substantially since the weight of iopromide compared to the other substances is low. This is due to the low R^2 (see Table 7 and Figure S- 3), which is caused by the wide variation of iopromide mass loads, as a consequence of its highly variable spatio-temporal application pattern.

Table 7 Summary of chemical mass load versus population size, annual Australian consumption and inclusion in the Bayesian inference models

Chemical	Internal Standard	β_i = Slope of chemical mass load (L_i)[kg/day] versus population (*P*)[People] (95% Conf. Int.)	R^2	Total Australian Consumption (kg/year)	Included in Model				
					S1	S2	S4	S8	S14
Acesulfame	acesulfame-d_4	7.5e-6 (7.3e-6 – 7.7e-6)	0.995	Data not available	✓	✓	✓	✓	✓
Atenolol	atenolol-d_7	3.0e-7 (2.4e-7 – 3.5e-7)	0.823	6,906[a]					✓
Caffeine	caffeine-d_3	1.1e-5 (9.1e-6 – 1.3e-5)	0.869	Data not available					✓
Carbamazepine	carbamazepine-d_{10}	8.6e-8 (7.2e-8 – 1.0e-7)	0.849	13,436[a]					✓
Codeine	acetyl sulfamethoxazole-d_5	4.1e-7 (3.6e-7 – 4.6e-7)	0.908	4,132[a]				✓	✓
Furosemide	2,4-dichlorophenylacetic acid	1.2e-7 (9.6e-8 – 1.4e-7)	0.839	5,911[a]					✓
Gabapentin	acetyl sulfamethoxazole-d_5	9.8e-7 (9.0e-7 – 1.1e-6)	0.968	6,718[a]		✓	✓	✓	✓
Hydrochlorothiazide	hydrochlorothiazide-13C,d_2	2.1e-7 (1.9e-7 – 2.3e-7)	0.944	3,748[a]			✓	✓	✓
Ibuprofen	2,4-dichlorophenylacetic acid	9.2e-7 (8.1e-7 – 1.0e-6)	0.919	10,859[a]				✓	✓
Iopromide	2,4-dichlorophenylacetic acid	6.8e-7 (3.6e-7 – 1.0e-6)	0.377	Data not available					✓
Naproxen	acetyl sulfamethoxazole-d_5	4.2e-7 (3.7e-7 – 4.7e-7)	0.912	15,103[a]				✓	✓
Norfloxacin	norfloxacin-d_5	8.5e-8 (7.5e-8 – 9.5e-8)	0.929	1,678[a]			✓	✓	✓
Paracetamol	acetyl sulfamethoxazole-d_5	3.2e-5 (2.6e-5 – 3.9e-5)	0.811	609,543[a]					✓
salicylic acid	2,4-dichlorophenylacetic acid	5.2e-6 (4.6e-6 – 5.9e-6)	0.922	Data not available				✓	✓
a Data from DUSC Drug Utilisation Database									

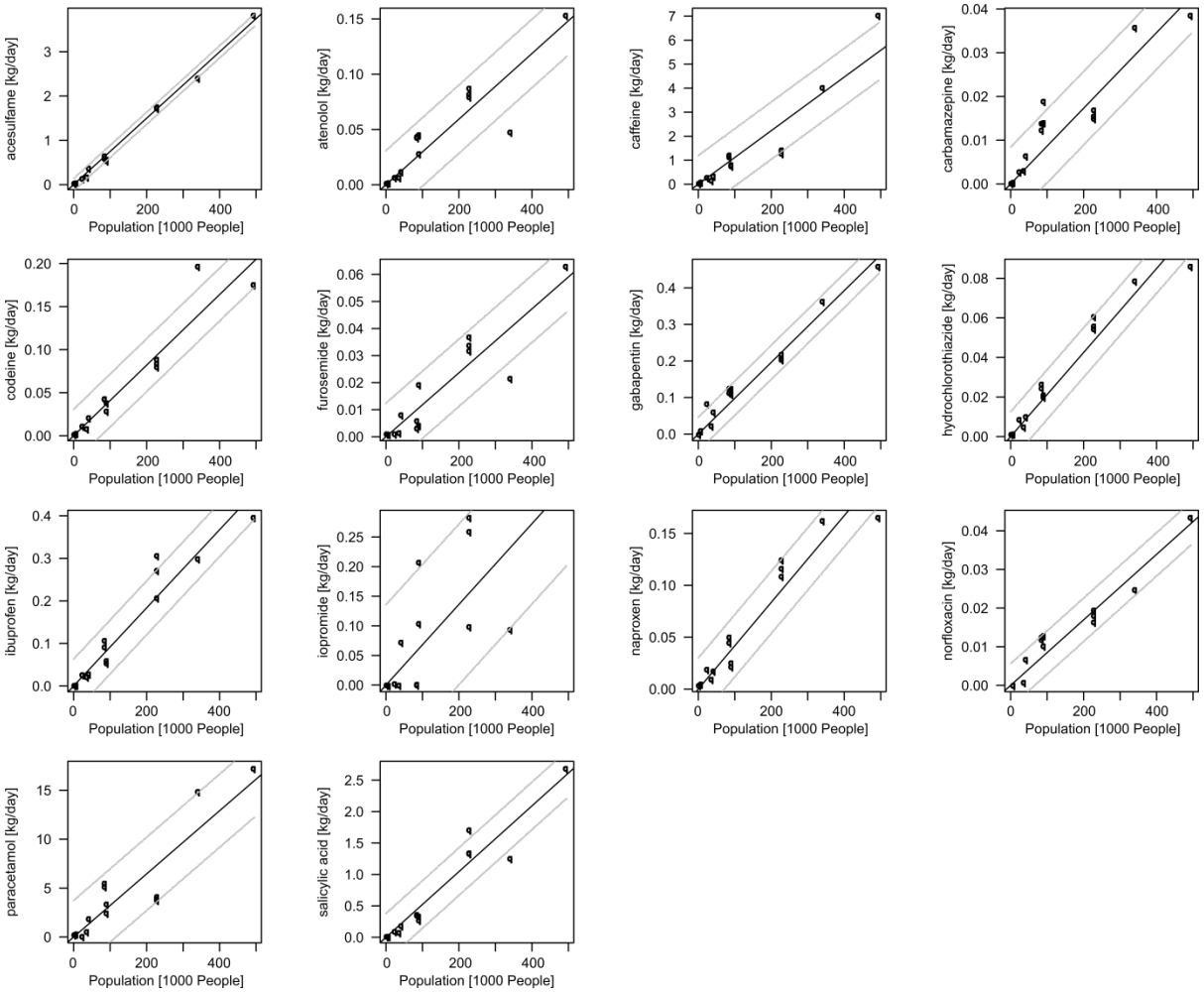


Figure 5 Population versus daily mass load of selected chemicals. The black line is the line of best fit. The grey lines indicate the 90% prediction interval.

3.3.2 Cross validation

To validate our approach we employed a leave-one-out cross validation. Bayesian updating lead to posterior distributions that are narrower than the priors (only 1.06-2.42x for small STPs, but 4.98-39.5x for big STPs, see Table 6). Since the chemicals for S1 and S2 are the ones with the biggest weight in the estimation process, adding more chemicals typically leads to no major change of the best estimate. However, the width of the posterior can in most cases be further reduced by adding more chemicals, not substantially for small populations though. This explains why STP estimates for small catchments cannot be improved. In contrast, for most large STPs substantial over/underestimation for STP estimates could be improved (refer to last two columns of Table 6).

Figure 6 illustrates two examples reflecting different population types, a large, urban and a small, rural population. These two cases are used to outline the possible, wide range of outcomes. The best estimate using all 14 chemicals for all sites including the 80% interquantile of the posterior distributions are summarized in Table 6.

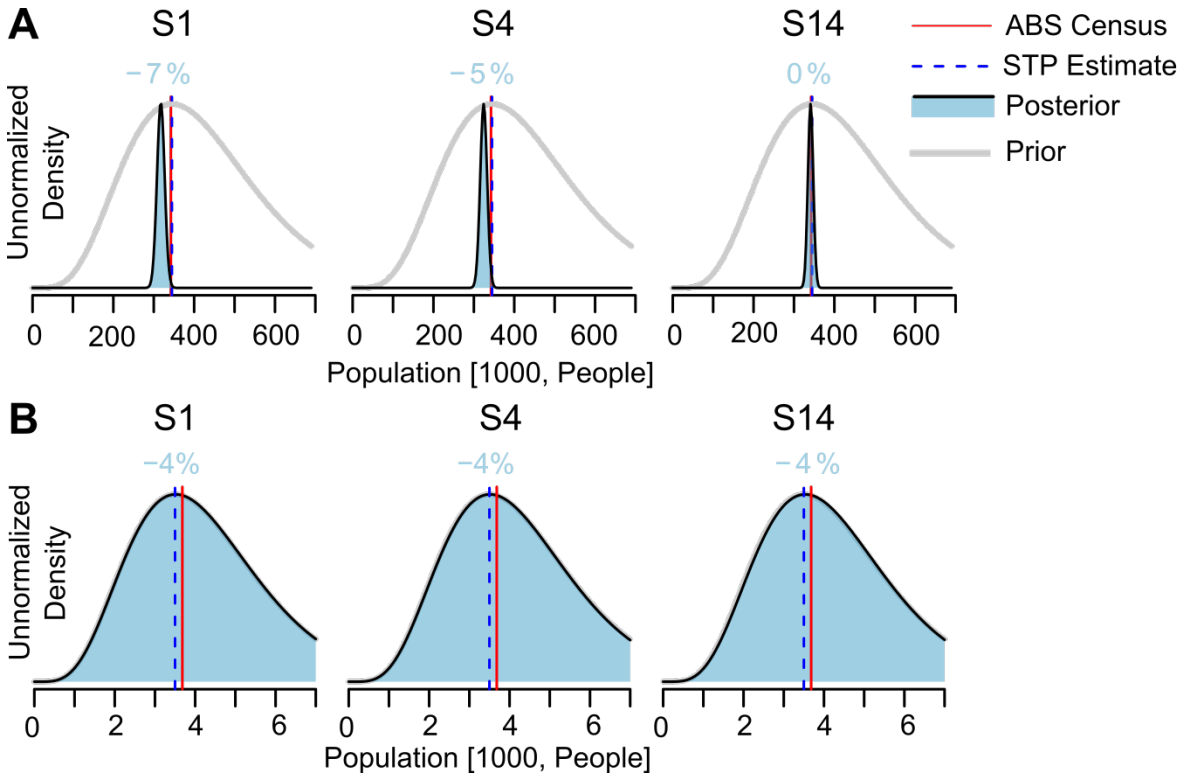


Figure 6 Bayesian inference population estimation models for A: Large, urban catchment B: Small, rural catchment. The blue, dashed line shows the population estimate provided by STP operators. The red line shows the day-specific, geo-referenced census population count (ABS, Customised report, 2012). The grey line is the assumed prior distribution and the black line (blue fill) indicates the posterior of the estimation based on mass loads of chemical markers (density). The %-value is the difference between the model's best estimate (maximum density) and the census population count. A: STP 9 B: STP 1 (see Table 6).

In the particular case of STP 9 which serves a large, urban catchment, the population estimate made by the STP operators was close to ABS census (Figure 5A). Using acesulfame as a single parameter (S1) to estimate population leads to an underestimate of 7% between the model's best estimate (maximum density of posterior distribution) and ABS census. Using four chemicals (S4) also underestimates the population size by 5% while using all 14 chemicals (S14) results in 0% difference between the model estimate and the ABS census count. Note that the 80% interquantile (difference between the 10% and 90% quantiles) decreases

from 23,795 (S1) to 16,216 (S14) demonstrating that the use of more chemicals leads to narrower posterior distributions in this case. This implies more confidence in the best estimate based on 14 chemicals compared to one chemical only. For STP 9 all three values, STP estimate, ABS census and best estimate based on measured chemical loads, are similar. However, the Bayesian inference does not merely confirm the STP estimate and ABS census, it also provides a realistic estimate for accuracy (width of posterior). Furthermore, substantial differences from STP estimates can be revealed with our model: e.g. the STP estimate for STP 9 was 330,000 people and our best estimate for three days was between 220,000 and 243,855, which is a quarter to one third less, but very close to ABS census (3% difference only).

In contrast, for STP 1, the smallest STP in this study, quantifying chemical loads in wastewater does not improve the population estimation: the posterior is almost congruent with the prior distribution. This is a direct consequence of the constant error term for the mass load models described in section 2.3. For a small catchment the predicted mass loads are small and so the corresponding relative errors are large. Therefore, the observed mass loads have a small weight and only little influence on the posterior. This is clearly visualized in Figure 6 for which S1 was carried out for each substance individually for both STP 9 and STP 1. When estimating the population based on a single compound only, the estimation is only unbiased if the consumption of the chemical in the catchment under investigation corresponds to the average consumption. This is best illustrated with acesulfame and gabapentin which showed the best correlation between population sizes and measured mass loads. If acesulfame is used as a single parameter (S1) the estimation for STP 9 is close to ABS census, however, if codeine is used as a single parameter, the population would be overestimated where as if atenolol was used as a single parameter the population would be underestimated as the consumption of these chemicals is different to that of the average consumption based on the other investigated STP catchments. This demonstrates that even in large STP catchments the aggregate consumption of an individual substance does not necessarily tend towards a nation-wide average. In small catchments this variation is expected to be even higher.

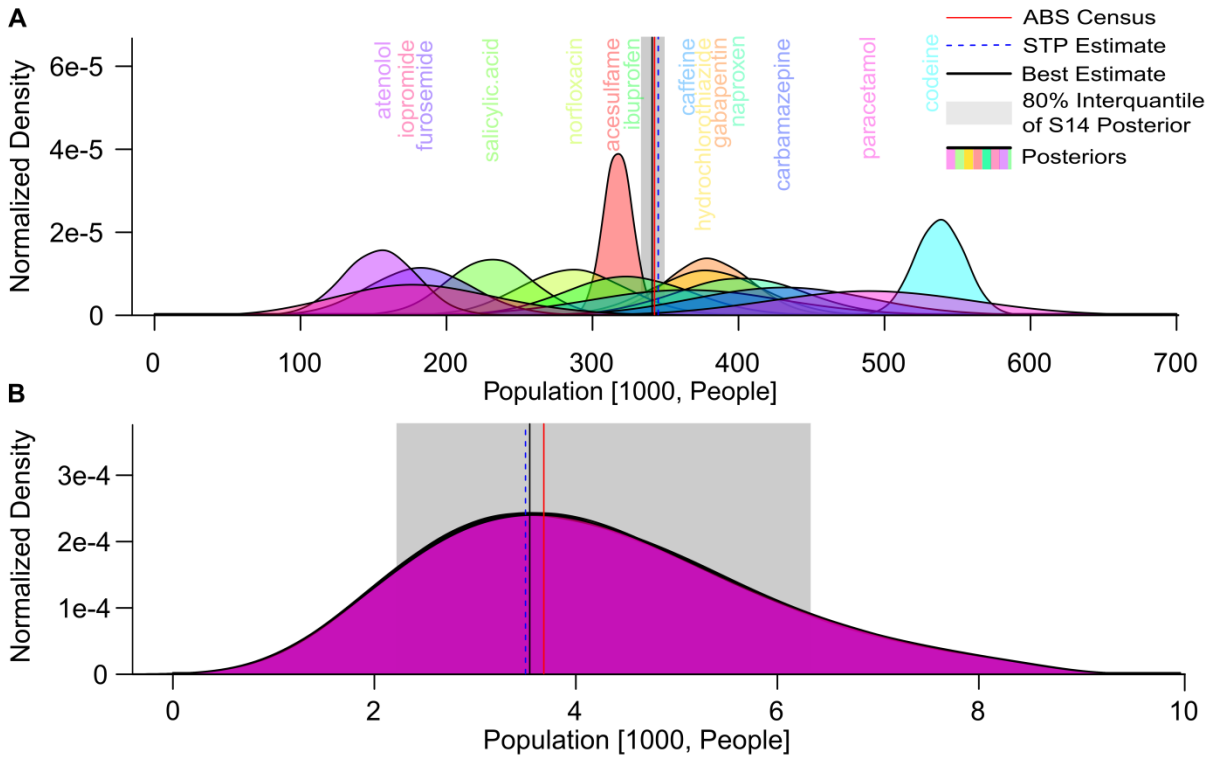


Figure 7 Individual substance distributions for estimating population size. A: Large, urban catchment (STP 9, see Table 6) B: Small, rural catchment (STP 1, see Table 6). The blue, dashed line shows the estimated population as provided by STP operators. The red line shows the day-specific, geo-referenced census population count (ABS, Customised report, 2012). The grey shading is the 80% Interquantile of the best estimate based on S14. The black lines (with coloured fill) indicate the posterior of the estimate based on the mass loads of each chemical marker (density). The width of the individual distributions reflects the goodness of fit of the individual models for each substance (Figure 4 and Table 7).

3.3.3 Estimating the population size in other catchments

It is expected that applying the Bayesian inference model with this calibration data to other large STPs in Australia would provide an accurate estimation of their population size as the calibration data collected represents over 6% of the Australian population and includes both urban and rural catchments. For applying the model to STPs in another country, unless there is confidence that the Australian calibration data is reflective of that country's consumption of the chemical markers, then the population estimation model may require recalibration with site specific chemical loads and accurate population counts. Therefore we propose three options of how to use the model where our calibration is understood to not be applicable to the study

site. As the most accurate population count is preferred, the first option requires calibrating the Bayesian inference model with chemical mass loads using the same approach as this study. This requires wastewater sampling on a day when specific population counts can be obtained for the geographical wastewater catchment areas and should include a range of STPs including small and large populations and covering a wide range of demographics. However, specific population counts may not be available and thus we propose the second option where another form of population count such as number of tax payers in the geographical wastewater catchment area is used. An easier but less accurate method is to use STP operator estimated population counts of the population sites for the calibration of the Bayesian inference model. All three scenarios are outlined in Table 8. In all three options, including a range of STPs of different population sizes within the same geographical area under investigation adds more confidence to the calibration data.

Table 8 Options for calibrating a Bayesian inference model for estimating population size

	Linear regression model calibration	
Population estimation method	Population count used to estimate linear models for each chemical/marker	Chemical mass loads
Option 0	Use Australian data (this paper/data set)	Only measure chemicals in STP for which estimation should be done
Option 1	Day specific census data for several STPs	Needed from several STPs
Option 2	Geographic Information System or alternative population count	1..n Site specific
Option 3	STP estimate	1..n Site specific

3.3.4 Options to recalibrate or apply the model elsewhere

Census counts are conducted infrequently or may not be evaluated and available to the same extent as in our study for the calibration and validation of a model. Conceptually, due to the expected correlation of pharmaceutical loads with population size, one could also make an estimation based on sales data. However, sales data is typically only available on a country wide basis and, hence, we cannot make reliable catchment-specific estimates due to unknown spatio-temporal variability. Even with the strong assumption of spatio-temporal homogeneity the following uncertainties remain: completeness of sales data (i.e. over the counter and internet), average compliance of us of medication, mean excretion rates and potential transformation in sewers. Uncertainty about all these parameters will affect estimations based on sales data to an unknown extent. Applying a Monte Carlo simulation may provide a distribution of results rather than only a point estimate, however, if no information on spatio-temporal variability is available, the confidence intervals may still not be realistic. In contrast, with our approach, all these uncertainties are considered

implicitly: i) systematic differences (e.g. incomplete sales data, non-compliance of use of medication) will lead to properly adjusted slopes in the linear regression models. ii) Random differences (e.g. day-to-day variation, demographics) will lead to bigger standard errors of estimated slopes, which in turn leads to wider confidence intervals of estimates obtained with Bayesian updating.

Monitoring annual sales amounts of the selected pharmaceuticals may reveal changes in consumption patterns, which would require a re-calibration of the model. If per capita consumption is similar (either in Australia or another country, compare Table 7) the data from this study can be used (Option 0, Table 8). Whenever there is a Census Day, samples should be collected to calibrate and validate a model as in this study (Option 1, will be repeated in Australia in 2016). Since population size is likely to vary any day thereafter, we can use pharmaceutical loads in wastewater to estimate a day-specific population that contributed to any given wastewater sample. Option 2 is an alternative to obtain an estimate of the population connected to a STP: e.g. through cell phone mapping[23] or the analyses of geo-referenced census data (available in some countries, however, mostly only for *de jure* population). Option 3 must be applied cautiously, as STP estimates may often be based on nutrient loads that have other sources (e.g. industry) and, therefore, overestimate the number of people (since the loads are population *equivalents*).

3. Acknowledgements

We thank the operators of the STPs for providing wastewater samples for this study and providing us with information about their catchment. We would also like to thank Dr Kristell Le Corre from the University of Queensland's Advanced Water Management Centre and Vanna Mabbott and Chris Raymond from The Drug Utilisation Sub-Committee of the Pharmaceutical Benefits Advisory Committee, Department of Health and Ageing, Commonwealth of Australia for providing Australian drug consumption data. Jake O'Brien receives an APA PhD scholarship. Phong Thai is funded by a UQ Postdoctoral Research Fellowship. Jochen Mueller is funded by an ARC Future Fellowship (FF 120100546). Entox is a joint venture of the University of Queensland and Queensland Health.

Supporting Information Available

Additional information including total daily wastewater flow versus population, chemical mass loads for each site, and chemical analysis instrumental parameters can be found in the Supporting Information which is freely available online at http://pubs.acs.org.

Chapter 4: Impact of in-sewer degradation of pharmaceutical and personal care product (PPCP) population markers on a population model

Using the model developed as part of Chapter 3 to estimate the population contributing to wastewater samples, a study was performed to assess the feasibility of determining *de facto* population size and the effect on estimating the population normalised drug consumption (PNDC) for a large community over an extended period. By analysing 311 daily composite wastewater influent samples for the chemicals outlined in Chapter 3 and determining the *de facto* population size using the model, it was found that for the given community, the *de facto* population was on average 32% higher than the *de jure* population and as a result any PNDC using only the *de jure* population would lead to a 22% systematic overestimation (Lai et al. 2015). Using the model, the uncertainty of the PNDC could be reduced by a factor of two hence demonstrating the importance of using such a model for future WBE studies.

One aspect of the model however which was not addressed was the suitability of the selected chemicals as population markers for other catchments and whether or not in-sewer degradation of these chemicals contributes to the uncertainty of the population model. As little is known about the in-sewer stability of these chemicals, Chapter 4 assesses the stability of the fourteen chemical markers under sewer conditions (both pressurised and gravity) using laboratory scale sewers. Using the in-sewer degradation data and the data from our population modelling paper we then assessed whether or not degradation was a major source of uncertainty for the model established in Chapter 3(O'Brien et al. 2014).

The following submitted publication is incorporated as Chapter 4:

O'Brien, J.W., Banks, A.P., Thai, P.K., Eaglesham, G., Ort, C., Scheidegger, A., Carter, S., Lai, F.Y., Mueller, J.F., 2014. A model to estimate the population contributing to the wastewater using samples collected on census day. Environ. Sci. Technol. (submitted 2nd June 2016)

In Review with Environmental Science and Technology
Submitted author manuscript

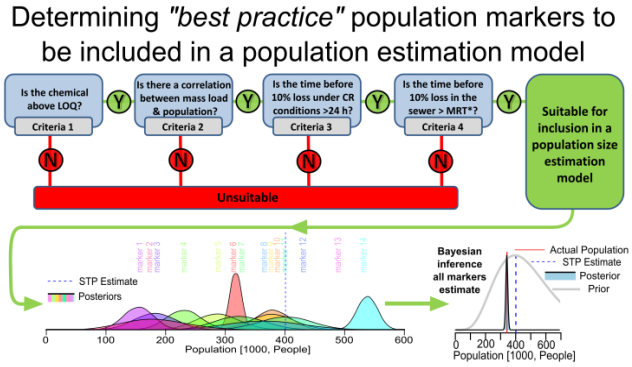
Title: Impact of in-sewer degradation of pharmaceutical and personal care products (PPCPs) population markers on a population model

Authors:
Jake W. O'Brien*[a]
Andrew Banks[a]
Jochen F. Mueller[a]
Guangming Jiang[b]
Andrew J. Novic[a]
Christoph Ort[c]
Geoff Eaglesham[a]
Zhiguo Yuan[b]
Phong K. Thai[a,d]

[a]The University of Queensland, The National Research Centre for Environmental Toxicology (Entox), 39 Kessels Road, Coopers Plains, QLD 4108, Australia
[b]The University of Queensland, Advanced Water Management Centre (AWMC), St. Lucia, QLD 4072, Australia
[c]Eawag, Swiss Federal Institute of Aquatic Science and Technology, CH 8600 Dübendorf, Switzerland

[d]International Laboratory for Air Quality and Health, Queensland University of Technology, Brisbane, QLD 4001, Australia
*j.obrien2@uq.edu.au
* Tel. +61 (0)7 3274 9009, Fax +61 (0)7 3274 9003

Abstract Art



4. Abstract

Wastewater-based epidemiology (WBE) has become a useful approach that has been adopted for monitoring of drug use at the population level and has been adopted to understanding consumption of and exposure to other chemicals. A key uncertainty of WBE is the size of the population which contributed to a given wastewater sample. Our previous study successfully developed and validated a Bayesian inference model to estimate the population size based on 14 chemical markers which are: (1) measurable via direct injection on LC-MSMS and (2) have mass loads which correlated well with population size (R^2 > 0.8). However, the potential uncertainty of the model prediction due to in-sewer degradation of these markers has not been evaluated. In this study we tested the stability of these 14 population markers under both gravity and rising main sewer conditions and assessed whether in-sewer degradation of individual population markers impacts the population model. We found that five markers (acesulfame, atenolol, carbamazepine, gabapentin and ibuprofen), which formed the core of our model, were stable in the sewers over the 12 h study period while the others were not (ranging from very little degradation to rapid degradation regardless of sewer conditions). Excluding the unstable population markers from the population model did not improve the precision of the population estimates using the previously developed model. Therefore, we now propose that the core markers to be included in our population model for other sites should meet two additional criteria: (3) having negligible degradation in wastewater to ensure the stability of chemicals

during wastewater collection; and (4) less than 10% in-sewer degradation could occur during the mean residence time of the WWTP sewer network.

4.1 Introduction:

Wastewater-based epidemiology (WBE) has become a useful approach for monitoring of drug use at the population level (Baker et al. 2014, Banta-Green et al. 2009, Burgard et al. 2014, Castiglioni et al. 2013, Lai et al. 2015, Lai et al. 2011, Ort et al. 2014b, van Nuijs et al. 2011b). Challenges remain to improve the accuracy of the approach and a key uncertainty associated with WBE relates to the population that has contributed to a given wastewater sample (Castiglioni et al. 2013) which is essential for the normalisation of the estimates. Normalised data is important to ensure that WBE is comparable across cities and even countries. A number of proxies have previously been proposed to address the population uncertainty. These include nutrients such as N and P, biological oxygen demand (BOD) and chemical oxygen demand (COD) (Been et al. 2014, van Nuijs et al. 2011c), creatinine (Brewer et al. 2012), caffeine and nicotine (Senta et al. 2015), as well as selected pharmaceuticals and personal care products (PPCPs) and an artificial sweetener (Lai et al. 2011). But none of the proposed population markers were calibrated against accurate population counts and thus were not validated yet. In our previous paper, a Bayesian inference model was developed to estimate *de facto* population size using 14 anthropogenic markers (Table 9) (O'Brien et al. 2014) using samples from 10 wastewater treatment plants (WWTPs) collected during the 2011 Australian Census. We subsequently applied this model successfully to support a study on daily drug use monitoring in a catchment in South East Queensland, Australia (Lai et al. 2015). However, we recognised that some chemicals may degrade in the sewer (Thai et al. 2014a). Therefore, it is important to investigate whether degradation during sewer passage contributes to the uncertainty of the population estimation model.

In-sewer degradation is a result of both hydrochemical and biotransformation processes in the sewer and as such is dependent on the chemistry (e.g. metals, micropollutants, nutrients, pH) of the wastewater, the bioactivity in the sewer and residence time of the wastewater in the sewer(Jelic et al. 2014), (McCall et al. 2015). In real sewers the chemistry, bioactivity and residence times are dependent on the flow and composition of water entering from the catchment at the time(McCall et al. 2015). Additionally, sewers are complex systems comprising of a multitude of pipes ranging from small, pressurised pipes called rising mains (RM) to large partially filled gravity fed pipes called gravity sewers

(GS). As such the surface area which biofilm can grow on is dependent on both the size of the pipe and how full the pipe remains. The ratio of area of biofilm versus volume of wastewater is thus termed the A/V ratio. Sewer networks comprise of sections ranging from low A/V to high A/V with differing residence times in each. A major limitation of in-sewer chemical degradation monitoring is that the observed degradation rates need to be statistically higher than the uncertainty of the chemical analysis and thus high A/V and residence times may be required. Using laboratory-scale sewer reactors overcomes this limitation as both the A/V and residence time can be controlled (Jiang et al. 2013, Thai et al. 2014a).

The aim of this study is to 1) assess the stability of the 14 population markers proposed in O'Brien et al. (2014) under sewer conditions; 2) assess whether potential degradation of these population markers in the sewer impacts the population model. From this we propose a protocol to assess whether a chemical can be used as a population marker.

4.2 Materials and Methods:

4.2.2 Chemicals and reagents

Analytical grade acetic acid was purchased from Sigma Aldrich (Castle Hill, Australia). Analytical grade hydrochloric acid 32% was purchased from Univar (Ingleburn, Australia). Water was purified through a MilliQ system (Millipore, 0.22 µm filtered, 18.2 mΩ cm^{-1}). High purity PPCP native and labelled analytical standards were purchased from various suppliers as outlined in the SI. Calibration standards were prepared in MilliQ water. Liquid chromatography grade methanol was purchased from Merck (Darmstadt, Germany). Mobile phases were filtered using Sartorius Stedim 0.45 µm RC filters (Goettingen, Germany).

4.2.3 Laboratory-scale sewer reactors

Laboratory-scale sewer reactors, with high A/V (70.9 m^2/m^3) which is similar to A/V ratios of small pipes, were used to investigate the biotransformation and degradation of the population markers. These reactors have been described elsewhere (Jiang et al. 2010, Thai et al. 2014a, Thai et al. 2014b). Briefly, the system comprised of laboratory-scale RM, GS and a control reactor (CR). Earlier studies using these reactors have found them suitable for studies regarding in-sewer processes, the control of sewer biofilm activities

and the in-sewer degradation of chemicals (Gutierrez et al. 2008, Jiang et al. 2011a, Jiang et al. 2010, Jiang et al. 2013, Mohanakrishnan et al. 2009, Thai et al. 2014a, Thai et al. 2014b). The sewer reactors are fed with domestic wastewater collected on a weekly basis from a local pumping station (Indooroopilly, Brisbane) which is typically at pH 7.5 with sulphide concentrations <3 mg S/L, sulfate between 10 and 25 mg S/L, total COD between 450 and 600 mg/L, soluble COD between 260 and 450 mg/L which contains volatile fatty acids between 50 to 120 mg COD/L (Thai et al. 2014b). The collected wastewater is stored at 4 °C and warmed to ~20 °C prior to feeding into the reactors. Feeding events occur at 6 hour intervals to mimic the typical hydraulic retention time of a real sewer (Hvitved-Jacobsen et al. 2013) and last approximately 2 minutes during which time one reactor volume (0.75 L) of wastewater is delivered to the reactor. Magnetic stirrers set to 250 rpm (Heidolph MR3000) homogenise the wastewater in the reactors. Further description of the reactors can be found in Thai et al. (2014a).

4.2.4 Batch tests for the degradation of PPCPs in sewer reactors

To investigate the degradation of the PPCPs under different sewer conditions, batch tests were conducted in triplicate. To meet OECD guideline No. 314, wastewater from the sampling pump station was warmed to 20 °C however no pH adjustment was necessary (OECD 2008). These conditions are comparable with other studies on the stability of chemicals under sewer conditions (Chen et al. 2012a, Plosz et al. 2013, Thai et al. 2014b, van Nuijs et al. 2012). Preliminary analysis of the wastewater was conducted to determine the spiking amount. Only furosemide, iopromide and norfloxacin required spiking for degradation to be measured accurately to give a spiked concentration of 5-10 µg/L above concentrations already present in the wastewater but within the range usually found in previous wastewater studies. For this purpose, a working solution of PPCP standards from 5 - 10 µg/mL was prepared in 50:50 methanol/deionised water and spiked in to the wastewater. Other chemicals can be tested with the residues already available in the wastewater matrix. The wastewater was then pumped using a peristaltic pump (Masterflex 7520-47) into the RM and GS reactors ensuring that the wastewater in each reactor was entirely replaced with fresh wastewater. Mixing occurred continuously for the duration of the batch tests using magnetic stirrers set to 250 rpm (Heidolph MR3000). This not only improved homogeneity of the wastewater but also enhanced surface aeration (dissolved oxygen of ~0.5 mg/L) producing both aerobic and anaerobic conditions in the GS reactor. The CR was basically the same as the GS except that the walls are regularly cleaned

hence preventing the growth of biofilms, however, mixing still occurred which enhanced the aerobic conditions for the CR (DO = 0.5 mg/L). Wastewater samples were taken at 0, 0.25, 0.5, 1, 2, 3, 6, 9 and 12 hours after the feeding event. Samples were immediately filtered using 0.20 µm PTFE syringe filters (Phenomenex, Australia) into 2 mL vials. Fifteen µL of 2 M HCl was added to each sample to acidify the sample to ~pH 2 and then samples were frozen at -20 °C prior to analysis.

4.2.5 Chemical Analysis

A slightly modified version of the chemical analysis outlined in O'Brien et al (2014) was performed using a Sciex QTrap 6500 (Sciex, Concord, Ontario, Canada) with electrospray ionization (ESI) interfaces coupled to Shimadzu Nexera HPLC systems (Shimadzu Corp., Kyoto, Japan). Separation was achieved on a Kinetix Biphenyl column (2.6 µm, 100 Å, LC Column 50 mm x 2.1 mm, Phenomenex) using a mobile phase gradient of 1 to 95 % methanol with 0.1% acetic acid. The QTrap 6500 was operated in scheduled multiple reaction monitoring (sMRM) mode with optimised parameters (Table S- 4). Data acquisition was performed using the Sciex software package Analyst Software 1.6 and quantification was performed using MultiQuant 3.0. Quantification was done using relative response factors of mass labelled internal standards.
For dissolved sulphide, samples were analysed within 24 h of sampling using an ion chromatograph with an UV and conductivity detector (Dionex ICS-2000). For methane analysis, BD vacutainer tubes were allowed to reach gas/liquid equilibrium overnight. Methane in the gas phase was measured by gas chromatography (Shimadzu GC-9A) equipped with a flame ionization detector. Concentrations of methane in sewage were calculated using mass balance and Henry's law (Guisasola et al. 2008).

4.4 Results and Discussion:

4.4.1 Biological activities in sewer reactors

The biological activity of the RM and GS reactors used in this experiment has previously been found to represent the biological activity and associated degradation of chemicals within actual sewers (Thai et al. 2014a). The control reactor showed no significant methane or sulphide gas production as it doesn't contain the sewer biofilms compartment as seen before (Thai et al. 2014b). The RM reactor produced sulphide and methane indicating that it was under anaerobic conditions. Activities of sulphate-reducing bacteria

and methanogenic archaea in the RM reactor were measured at 7.21 ± 0.74 mg S/L-h and 29.73 ± 0.63 mg COD/L-h respectively which is similar to previously reported values for both real and laboratory-scale sewers (Guisasola et al. 2008, Jiang et al. 2011a, Jiang et al. 2011b, Thai et al. 2014b). Dissolved oxygen in the GS reactor was measured below 0.33 mg/L despite continuous aeration which indicates aerobic activity consuming oxygen. It is also expected that anaerobic conditions may be present in the bottom of the reactor where oxygen could not reach. This is supported by the low reduction of sulphate (4.21 ± 0.77 mg S/L-h) and the low production of methane (14.8 ± 2.31 mg COD/L-h).

4.4.2 Degradation of PPCPs in the sewer reactors

Figure 7 shows the concentration profile of chemicals of interest in the system over time normalised to the concentration at the start of the experiment expressed in percent (i.e. C_t/C_0 x 100). To understand the degradation kinetics of the PPCP population markers, both linear regression (zero order) and pseudo first order regression were fitted to the data obtained from the batch tests (Table 9). For the pseudo first order, the regression intercept was set through 100% at time 0. The kinetic model chosen for the degradation of each compound was based on the fit to the model, i.e. the model with the higher R^2 value. For compounds where degradation observed was within the uncertainty of measurements, we concluded that no degradation had occurred over the studied period (12 hours) and thus these compounds are stable.

To our knowledge only Jelic et al. (2014) have investigated the fate of selected PPCPs under sewer conditions. That study however was conducted only in a pressurised sewer which is similar to that of the RM in our study. It is important to note though that the sewer reactors used for our experiment had fixed area to volume (A/V) ratios of 70.9 m^2/m^3 which is similar to A/V ratios of small pipes but higher than that of average sewers which typically use larger diameter pipes. Therefore the chemicals investigated in this study may have had more contact with biofilms and hence more degradation than would be expected in a typical sewer. This may explain why some discrepancy was observed between our results and that of Jelic et al. (2014) who had an A/V ratio of 8 m^2/m^3. As no other studies have looked at the degradation within a GS, we conducted a comparison between our results with available data on the removal efficiency of WWTPs for those PPCPs (Table 9). Such a comparison helps support our findings although we acknowledge that fate and transport processes during wastewater treatment are different from those during sewer passage (Blair et al. 2015, Liu and Wong 2013) (S1 SI).

It was observed that there are two groups of chemicals, those which had no measurable degradation (acesulfame, atenolol, carbamazepine, gabapentin and ibuprofen) and those which degraded over the studied period with half-life estimates ranging from 0.6 to 10 h in the GS and 0.6 to 11h in the RM. Detailed discussion for the degradation of each chemical can be found in the SI.

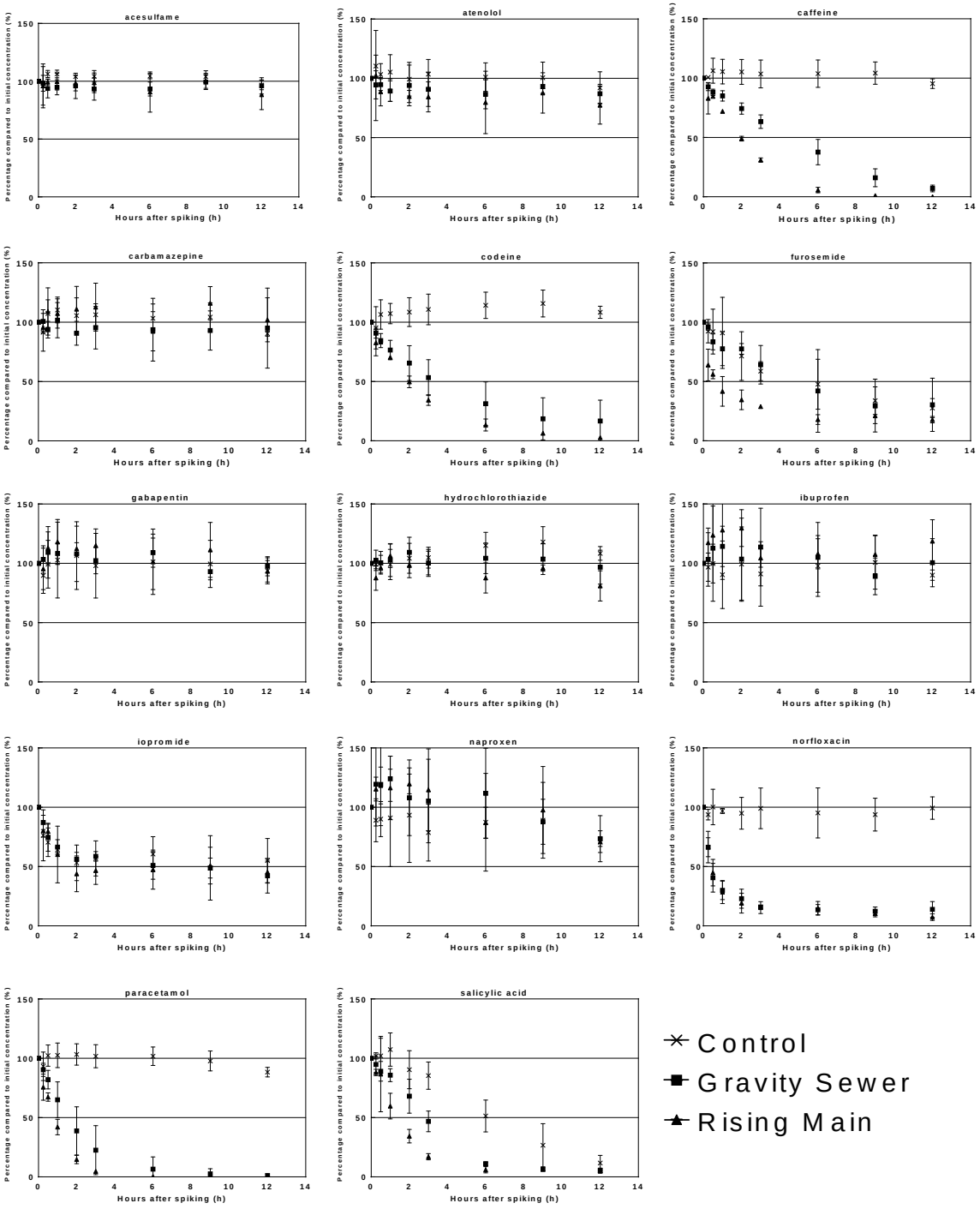


Figure 8 Degradation profiles of 14 population markers under different sewer conditions. Error bars represent the standard deviation of 3 replicate samples. The x axis is the time after the feeding event. The y axis is the concentration relative to the starting concentration (time zero) expressed as percent. Concentrations under control conditions are indicated by ×. Concentrations under gravity sewer (GS) conditions are indicated by ■. Concentrations under rising main (RM) conditions are indicated by ▲.

Table 9 Selection of kinetics models for degradation of the 14 population markers under sewer conditions. The model which fitted with the higher R^2 value was selected except where linear regressions indicated no significant degradation (i.e. less than the uncertainty of the measurement). The selected model for each compound under the particular sewer condition is indicated by bolded text. Time before 10% loss under each sewer condition was calculated using the selected model for each compound.

Chemical	Correlation between mass load and population size(O'Brien et al. 2014)		Linear regression (Zero order)						First-order Kinetics						Time before 10% loss (h)			WWTP Removal Efficiency %
			Control		Gravity Sewer		Rising Main		Control		Gravity Sewer		Rising Main		Control	Gravity Sewer	Rising Main	
	R^2	Ranking [a]	Slope (%/h)	R^2	Slope (%/h)	R^2	Slope (%/h)	R^2	Half-life (h)	R^2	Half-life (h)	R^2	Half-life (h)	R^2				
acesulfame	0.995	1	n.s.		n.s.		n.s.		>500	0.005	>500	0.000	100	0.075	>24	>24	>24	-(Buerge et al. 2009)
atenolol	0.823	12	n.s.		n.s.		n.s.		94	0.070	96	0.062	43	0.108	>24	>24	>24	<0-85.1%(Jelic et al. 2014)·(Luo et al. 2014)
caffeine	0.869	9	n.s.		-8.08 ± 0.37	0.952	-8.27 ± 0.8	0.809	200	0.024	4.0	0.967	1.8	0.981	>24	0.61	0.28	>49.9-99.6%(Blair et al. 2015)·(Luo et al. 2014)
carbamazepine	0.849	10	n.s.		n.s.		n.s.		150	0.024	170	0.011	>500	0.000	>24	>24	>24	<0-62.3%(Jelic et al. 2014)·(Blair et al. 2015)·(Ryu et al. 2014)·(Rodayan et al. 2014)·(Luo et al. 2014)
codeine	0.908	8	1.02 ± 0.49	0.117	-7.13 ± 0.65	0.784	-7.77 ± 0.73	0.820	>500	0.000	3.8	0.867	2.1	0.983	>24	0.57	0.32	13%(Rodayan et al. 2014)
furosemide	0.839	11	-6.08 ± 0.77	0.715	-5.9 ± 0.81	0.680	-4.57 ± 0.91	0.501	5.7	0.764	5.8	0.731	1.9	0.708	0.87	0.88	0.29	40 - 80%(Kasprzyk-Hordern et al. 2010)
gabapentin	0.968	2	n.s.		n.s.		n.s.		460	0.001	90	0.062	110	0.029	>24	>24	>24	0 - 84%(Kasprzyk-Hordern et al. 2010), 6.4%(Gurke et al. 2015)
hydrochloro-thiazide	0.944	3	1.39 ± 0.42	0.252	n.s.		-1.11 ± 0.48	0.178	>500	0.000	300	0.009	59	0.175	>24	>24	9.01	0(Radjenovic et al. 2009)·(Schroder et al. 2010) - 85%(Radjenovic et al. 2007)
ibuprofen	0.919	6	n.s.		n.s.		n.s.		320	0.002	71	0.029	270	0.004	>24	>24	>24	72 - 100%(Kasprzyk-Hordern et al. 2010)·(Luo et al. 2014)
iopromide	0.377	14	-2.26 ± 0.86	0.215	-3.55 ± 0.63	0.557	-2.99 ± 0.9	0.307	17	0.234	10	0.609	11	0.354	2.55	1.52	1.63	>80%(Kormos et al. 2011)
naproxen	0.912	7	n.s.		-3.15 ± 1.1	0.200	-3.26 ± 0.94	0.323	48	0.038	23	0.193	22	0.314	>24	3.17	3.07	43.3-98.6%(Ryu et al. 2014)·(Luo et al. 2014)
norfloxacin	0.929	4	n.s.		-4.46 ± 1.1	0.396	-4.89 ± 1.11	0.439	>500	0.001	0.6	0.819	0.6	0.864	>24	0.09	0.09	87 - 100%(Blair et al. 2015)·(Ternes 1998)·(Vieno et al. 2006)
paracetamol	0.811	13	n.s.		-8.31 ± 0.96	0.693	-6.74 ± 1.2	0.560	120	0.070	1.5	0.949	0.8	0.986	>24	0.22	0.12	98.7-100%(Ratola et al. 2012)·(Luo et al. 2014)
salicylic acid	0.922	5	-8.26 ± 0.55	0.899	-8.79 ± 0.66	0.850	-7.49 ± 1.14	0.635	5.2	0.869	2.6	0.953	1.3	0.929	1.21	0.40	0.20	89.6-100%(Ternes 1998)·(Lee et al. 2005)·(Luo et al. 2014)

a = population biomarker rankings are from O'Brien et al. (2014)(2014) with 1 being the best marker, 14 the worst
n.s. = not significantly different from zero; bolded values = kinetic model chosen

4.4.3 Implications of in-sewer degradation for PPCPs on a population model and recommendations for future population models

In terms of ranking potential population markers in the population model proposed in our earlier work (O'Brien et al. 2014), acesulfame was selected as the best marker due to a strong correlation between mass load and population size (Table 9) (R^2 = 0.995) followed by gabapentin (R^2 = 0.968), hydrochlorothiazide (R^2 = 0.944) and norfloxacin (R^2 = 0.929). We had assumed that these linear regressions would also take into account uncertainties such as degradation/transformation in the sewer, variability from the different sampling methods, and any differences in consumption and excretion in the different regions. Combined with the sewer degradation data obtained from this study, which is arguably from what would be considered a worst case scenario in terms of high levels of degradation processes, it can be suggested that uncertainty from degradation is not an issue for acesulfame, atenolol, carbamazepine, gabapentin and ibuprofen. Therefore, it is likely that the variability observed in the linear regressions for the mass loads of these population markers versus population size presented in O'Brien et al. (2014) reflects the variance associated with other factors such as differences in consumption and excretion as well as uncertainty associated with sampling and analysis.

To understand if there is a link between degradation and the uncertainty associated with the correlations between mass loads and population, we correlated the R^2 values for the correlation between mass load and population size from the previous paper (O'Brien et al. 2014) and the time taken for 10% loss under each of the sewer conditions in this study and found that there was no relationship (Spearman's rank correlation, $p < 0.05$) (Table S- 6). For example, norfloxacin was considered unstable under sewer conditions but had better correlation (R^2 = 0.912) between population size and mass load than carbamazepine (R^2 = 0.849) which was stable under all sewer conditions. One plausible explanation for why degradation did not affect the correlations between mass loads and population is that the sewer reactors had higher A/V ratios than that of average sewer systems. In our study, the A/V ratio was 70.9 m^2/m^3 which is about 2-10 times higher than that of average sewers which typically comprise of larger diameter pipes. In a typical sewer system, the residence time in small pipes is much shorter as the small pump stations usually have higher running frequency (Jelic et al. 2014). Therefore, the degradation observed in our

study is likely representative of a worst case scenario while in actual catchments (such as for the catchments in the model calibration data), the extent of degradation is much lower and probably did not affect the overall uncertainty of mass load measurements. For degradation to affect a correlation there must be a crossover between residence time and degradation (e.g. the observed degradation in some catchments would be higher than others because of longer residence times). We speculate that other factors may be responsible for the higher uncertainty but this requires further investigation.

To visualise the influence of stability on the uncertainty of our population estimate, we compared the coefficient of variation (CV) of the population estimate for each catchment based on all chemicals (s14 in the paper) and the stable chemicals identified in this study (Figure 8). For all WWTP catchment population estimates made using the model, the CV was lower (higher precision) by using all 14 compounds in the model than just the stable chemicals. Individual substance distributions for the three largest WWTPs are shown in SI Figure S- 5.

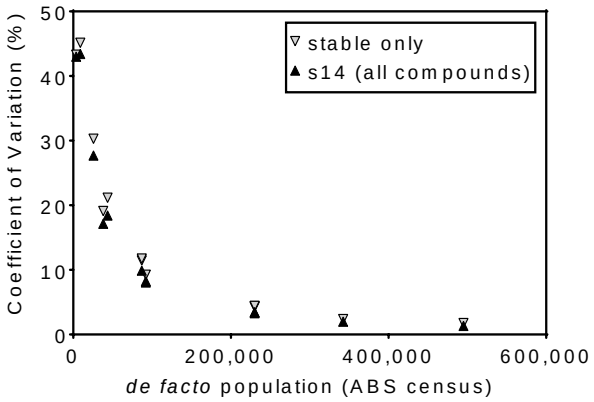


Figure 9 Coefficient of variation expressed as percentage for posterior estimates for population size for each of the WWTPs from O'Brien et al. (2014) using all chemicals (s14, indicated by ▲) and using only the chemicals identified as stable in the sewer by this study (indicated by ▼).

For future calibration of a population model, if the only chemicals identified are unstable in the sewer and/or where the stable chemicals have no relationship between mass load and population size, we propose to still use less stable markers but also include a metabolite/metabolites or transformation products which are produced from the degradation of the compound within the sewer. This will then

provide insight into degradation within the sewer and to also counterbalance the impact of the degradation of the parent chemical on the model.

4.4.4 Importance of the quality of calibration data used in a population model

The samples from our previous study used to calibrate the model covered a broad range of catchments from across Australia including small catchments, large catchments, different climate and geographic zones (from cold temperate to subtropical), inland and coastal catchments and catchments with potential cultural and socioeconomic differences. These factors may all influence the consumption, excretion and degradation of the population markers investigated and thus influence the calibration data. Despite these factors, the correlation between mass load and population size was still above $R^2 = 0.8$ for 13 of the 14 population markers.
In the model, an error term was included that accounts for differences in consumption between locations and daily variations of population size. It was implied that daily variations would include factors that would contribute to either the addition (increase in mass load) or removal (decrease in mass load) of a chemical in wastewater.
Degradation is therefore one variable in daily variations and its effect on the population model is the extent that the degradation of a chemical would affect the variance in the correlation between mass load and the population size. However, if the combined impact of A/V ratios and mean residence times are all similar among catchments, then the chemical would degrade to a similar extent and we would get a systematic underestimation of total consumption. In such a case, there is still a good correlation between mass load and population size (assuming relatively homogenous consumption and excretion) and thus the population estimate would be unaffected by the degradation. The quality of the population estimates is therefore dependent on the quality (representativeness) of the calibration data and as such, calibration data for a different area or country should include catchments of both long and short residence times as well as a combination of GS and RM sewers. By including these in the calibration data, variability due to degradation is covered implicitly.

4.4.5 Applicability to other sewer catchments

Degradation within sewers is dependent on the residence time in the sewer. Using data from the most comprehensive WBE study which covered 25 WWTPs in 2012 and 47 WWTPs in 2013 (Ort et al. 2014b), we correlated mean residence time of each WWTP who provided a mean residence time (n = 50) against population size (Spearman's rank correlation, $\rho < 0.05$) and found there is no relationship between mean residence time and population size (Figure S- 4). Therefore we consider that residence time is independent of population size and as such degradation can also be considered independent of population size.

In our previous study (O'Brien et al. 2014), chemicals for population estimation were chosen using two criteria: (1) the chemical must be measurable via direct injection on LC-MSMS in all of the collected samples; (2) the mass load of the chemical must show a correlation with population size ($R^2 > 0.8$). With the use of Bayesian inference to combine the chemicals which met these criteria into a population model it was apparent that they were capable of estimating the population size with high accuracy and that uncertainty reduced with the inclusion of more markers. While these two criteria were observed as suitable criteria for our specific catchments, to extend the applicability of the population model to any other catchments based on the knowledge about chemical degradation obtained in this study we now propose two additional criteria for "Best Practice" population markers: (3) degradation should be negligible under CR conditions to ensure the stability of chemicals during wastewater collection; and (4) the mean residence time of the WWTP sewer network (RM and/or GS) should be shorter than the time for 10% degradation to occur for the compound under sewer conditions. Degradation of less than 10% is considered acceptable for our population markers as it is the acceptable "Best Practice" tolerance for degradation of sewage drug biomarkers (Castiglioni et al. 2013). If no mean residence time is provided by WWTPs, we recommend using the 95th percentile of the mean residence time of all WWTPs (non-normally distributed) from the Ort et al. (2014b) study which was 10 hours. A protocol for selecting "Best Practice" suitable population markers based on these criteria is shown in Figure 9. Based on these criteria, for our calibration data and catchments, acesulfame, atenolol, carbamazepine, gabapentin and ibuprofen would all be considered "Best Practice" population markers particularly considering they had negligible degradation

under “high in-sewer degradation processes” and hence should be considered as potential population markers for all sites regardless of knowledge of mean residence time. Their stability was also greater than the longest mean residence time of the sewer catchments in the Ort et al. (2014b) study (15 hours). Hydrochlorothiazide can also be considered as a potential “Best Practice” population marker providing the sewer catchments do not have rising main sewers with mean residence times greater than 9 hours (Table 9). Furosemide, iopromide and salicylic acid were identified as highly degradable as all compounds degraded in the CR and thus should not be used as “Best Practice” marker (failing criteria 3). The use of naproxen, caffeine, codeine, norfloxacin and paracetamol as “Best Practice” markers would require further investigation as all had greater than 10% loss in <3 hours under both gravity and rising main sewer conditions (Table 9).

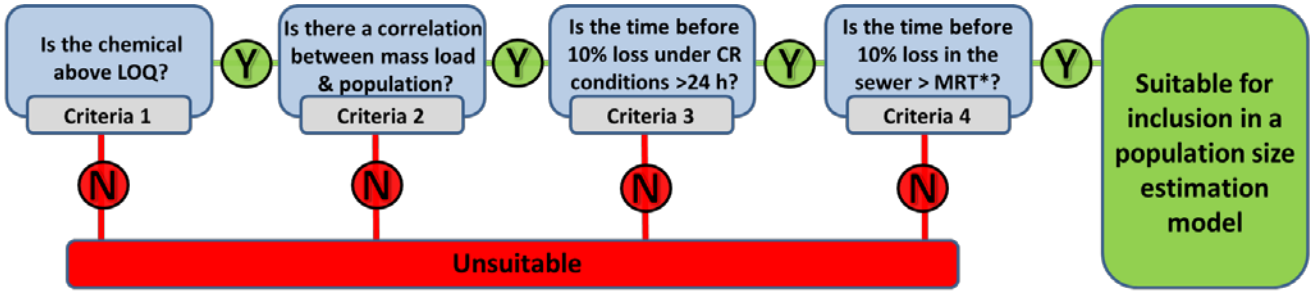


Figure 10 Selection criteria of “Best Practice” population markers based on measurable levels (i.e. above LOQ), degradation in wastewater and within the sewer and correlation between mass load and population. Y = Yes. N = No. LOQ = limit of quantification. MRT = mean residence time. * = if mean residence time is unknown, assume 10 h as it is the 95th percentile of the mean residence times from the largest wastewater-based epidemiology study(Ort et al. 2014b).

4. Acknowledgements

The authors would like to thank Andreas Scheidegger from Eawag for his invaluable assistance in the initial model development and for the advice he provided for this study. Entox is a joint venture of the University of Queensland (UQ) and Queensland Health. Entox is a member of the Queensland Alliance for Environmental Health Science (QAEHS). This project was conducted as part of an ARC Discovery project (DP150100645). Jake O’Brien and Andrew Novic are both funded by APA PhD Scholarships. Phong K. Thai is funded by the QUT Vice Chancellor Research Fellowship. Jochen Mueller is funded through the ARC Future Fellowship

(FT120100546). Guangming Jiang is funded by a Queensland State Government Early Career Accelerate Fellowship.

Chapter 5: Wastewater analysis of Census Day samples to investigate per capita input of organophosphorus flame retardants and plasticizers into wastewater

Having identified suitable markers of population size from wastewater in Australia and having developed a population estimation model which incorporates all of these markers, it was decided to evaluate the application of wastewater-based epidemiology for other compounds of interest. The target group selected were organophosphate flame retardants. Organophosphate esters have a broad range of uses such as plasticisers and flame retardants but there has been an increase in usage since the ban of some brominated flame retardants. The increase in use is of particular concern as some organophosphorous flame retardants (PFRs) have shown toxicity including carcinogenicity and neurotoxicity. There is very little data on their use in Australia and thus the aim of this chapter was to investigate the use of PFRs by the Australian population, using WBE analysis.

The following publication is incorporated as Chapter 5:

O'Brien, J.W., Thai, P.K., Brandsma, S.H., Leonards, P.E., Ort, C., Mueller, J.F., 2015. Wastewater analysis of Census day samples to investigate per capita input of organophosphorus flame retardants and plasticizers into wastewater. Chemosphere 138, 328-334.

Peer–reviewed and published by Chemosphere
Accepted author manuscript

Title:

Wastewater analysis of census day samples to investigate per capita input of organophosphorus flame retardants and plasticizers into wastewater

Authors:

Jake W. O'Brien[*a], Phong K. Thai[a,b], Sicco H. Brandsma[c], Pim E.G. Leonards[c], Christoph Ort[d,e], Jochen F. Mueller[a]

Affiliation:

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

[b]Queensland University of Technology, International Laboratory for Air Quality & Health, 2 George Street, Brisbane, QLD 4001, Australia[c]

Institute for Environmental Studies, VU University, De Boelelaan 1087, 1081 HV Amsterdam, The Netherlands

[d]Eawag, Swiss Federal Institute of Aquatic Science and Technology, CH 8600 Dübendorf, Switzerland

[e]The University of Queensland, Advanced Water Management Centre (AWMC), St. Lucia, QLD 4072, Australia

*Corresponding author: Tel.: +61 3274 9009 Fax: +61 (0)7 3274 9003 Email: j.obrien2@uq.edu.au

Email Addresses for each author:

Jake W. O'Brien: j.obrien2@uq.edu.au

Phong K. Thai: p.thai@uq.edu.au

Sicco Brandsma: sicco.brandsma@vu.nl

Pim E.G. Leonards: pim.leonards@vu.nl

Christoph Ort: Christoph.Ort@eawag.ch

Jochen F. Mueller: j.mueller@uq.edu.au

Highlights:

- Organophosphorus esters (PFRs) were measured in Australian wastewater influent
- Similar PFR concentrations were found in Australian wastewater as in US and Europe

- Per capita PFR loads into STPs were calculated using Census Day influent samples
- We estimate annual use of PFRs in Australia and release to the aquatic environment

5. Abstract:

The use of organophosphate esters (PFRs) as flame retardants and plasticizers has increased due to the ban of some brominated flame retardants. There is however some concern regarding the toxicity, particularly carcinogenicity and neurotoxicity, of some of the PFRs. In this study we applied wastewater analysis to assess use of PFRs by the Australian population. Influent samples were collected from eleven wastewater treatment plants (STPs) in Australia on Census Day and analysed for PFRs using gas chromatography coupled with mass spectrometry (GC-MS). Per capita mass loads of PFRs were calculated using the accurate census head counts. The results indicate that tris(2-butoxyethyl) phosphate (TBOEP) has the highest per capita input into wastewater followed by tris(2-chloroisomethylethyl) (TCIPP), tris(isobutyl) phosphate (TIBP), tris(2-chloroethyl) phosphate (TCEP) and tris(1,3-dichloroisopropyl) phosphate (TDCIPP). Similar PFR profiles were observed across the Australian STPs and a comparison with European and U.S. STPs indicated similar PFR concentrations. We estimate that approximately 2.1 mg.person^{-1}.day^{-1} of PFRs are input into Australian wastewater which equates to 16 tonnes per annum.

Keywords:
Organophosphorus esters, Flame retardants, Plasticizers, Census, Wastewater analysis, Australia

5.1 Introduction

The use of organophosphate esters as flame retardants (referred to here as PFRs but sometimes referred to as OPEs or OPFRs) has increased due to the restrictions implemented on the use of some brominated flame retardants (Brommer et al. 2012, Reemtsma et al. 2008, Stapleton et al. 2009, United States Environmental Protection Agency 2005). In 2006, 20% of flame retardants in Europe were PFRs (van der Veen

and de Boer 2012). In addition to their use as flame retardants, some PFRs are also used as plasticizers, antifoaming agents and as additives for lubricants and hydraulic fluids (Marklund et al. 2005b). Tris(1,3-dichloroisopropyl) phosphate (TDCIPP) and tris(phenyl) phosphate (TPHP) are primarily used as flame retardants in polyurethane foams (Marklund et al. 2003, Reemtsma et al. 2008, Stapleton et al. 2009) whereas other organophosphates such as tris(butyl) phosphate (TNBP) and tris(2-butoxyethyl) phosphate (TBOEP) are primarily used as antifoaming agents and plasticizers (Marklund et al. 2003, 2005b). In addition, TNBP and TPHP may be used as antiwear agents and/or extreme pressure additives in hydraulic fluids, lubricants and transmission and motor oils (Agency for Toxic Substances and Disease Registry 1997). The global use of PFRs in 2011 was estimated at 292 000 tonnes (Townsend Solutions 2012) which is up from 186 000 tonnes in 2001 (Marklund et al. 2005b).

There is concern regarding the toxicity of PFRs. Tris(2-chloroethyl) phosphate (TCEP) and TDCIPP are proven carcinogens. Tris(2-chloroisopropylethyl) (TCIPP) and TBOEP are suspected to be carcinogenic ((WHO) 1998, 2000). Tris(methylphenyl) phosphate (ortho-TMPP), TPHP and TNBP are all considered neurotoxins ((WHO) 1990, 1991a, b). Other concerns include neurodevelopment issues from exposure to TDCIPP, TCEP and TCIPP (Dishaw et al. 2011) and an association between decreased semen quality in men from exposure to TDCIPP and TPHP (Meeker and Stapleton 2010). TCEP, TCIPP and TDCIPP are all Priority Existing Chemicals as they are industrial chemicals with reasonable grounds that manufacturing, handling, storing, using or disposing of the chemical gives rise, or could give rise, to a risk of adverse health and/or environmental effects (NICNAS 2014).

PFRs may enter the environment via processes such as abrasion, volatilisation and leaching (Marklund et al. 2003, van den Eede et al. 2011, Wensing et al. 2005). Studies have indicated that indoor air, particularly within public buildings, may have higher PFR concentrations than outdoor air. PFR concentrations in air have been measured as high as 950 $ng.m^{-3}$ (Marklund et al. 2005a). Additionally, high concentrations of PFRs have been measured in indoor dust with levels as high as 5.5 $g.kg^{-1}$. These high levels are mainly attributed to TBOEP which is found in floor polishes and waxes for linoleum floors and can account for up to 97% of the total

PFR concentrations. (Marklund et al. 2003). PFRs in dust may be a concern to humans as dust appears to be a relevant exposure pathway for certain flame retardants such as decabromodiphenyl ether assuming adults ingest on average 20 $mg.d^{-1}$ of dust and toddlers 50 $mg.d^{-1}$ (Brommer et al. 2012, Jones-Otazo et al. 2005).

In Australia, there is no record of manufacturing PFRs and the only import data available is for TCEP and TCIPP as they are considered Priority Existing Chemicals (NICNAS 2001). This data shows that in 2001 the total import of TCEP and TCIPP was 410 tonnes and it was expected that 20 to 40 tonnes of TDCIPP would be imported. As there is no import data available for the other OPEs, a method is required to assess which PFRs are present in Australia and which may pose a health risk.

Waste streams, including wastewater, are an important sink for many chemicals and wastewater analysis is increasingly being used as a tool for assessing changes in the consumption, exposure to and use of chemicals (Daughton 2012b). PFRs have previously been measured in municipal wastewater in Europe and United States; with an emphasis on their removal during wastewater treatment (Ratola et al. 2012). To date, only two studies have measured the load of PFRs entering wastewater treatment plants (STPs) (Marklund et al. 2005b, Schreder and La Guardia 2014) and one study measured the load of PFRs in wastewater effluent (Phillips and Chalmers 2009). Additionally, a European wide study of 89 STPs found PFRs in all samples, however, they report only concentrations and not loads (Loos et al. 2012). To date, there are no published data on PFRs in Australian wastewater.

The aims of this study are to i) identify key PFRs in wastewater influent ii) assess daily mass loads of PFRs and their associated per capita release into the wastewater stream, iii) evaluate differences between the per capita input of PFRs into wastewater of the different treatment plant catchments. This may form a basis for future assessment of changes in the release and associated use of these chemicals in Australia.

5.2 Materials and Methods:

5.2.1 Standards and materials

Solvents were pro-analysis quality or HPLC grade. Acetone, methanol, hexane were obtained from Promochem, (Wesel, Germany) and toluene from Fisher Scientific (Loughborough, UK). Standards of tris(2-chloroethyl)phosphate (TCEP), tris(1,3-dichloroisopropyl)phosphate (TDCIPP), tris(phenyl) phosphate (TPHP), tris(butyl) phosphate (TNBP), 2-ethylhexyl diphenyl phosphate (EHDPP) and tris(2-butoxyethyl)phosphate (TBOEP) were supplied by Sigma-Aldrich Chemie B.V. (Zwijndrecht, the Netherlands). Tris(isobutyl) phosphate (TIBP) was supplied by Merck (Darmstadt, Germany) and tris(2-chloroisopropyl)phosphate (TCIPP) from Ehrenstorfer (Augsburg, Germany). The internal standards TPHP-$_{d15}$ and TNBP-$_{d27}$ were supplied by respectively, Sigma-Aldrich Chemie B.V. (Zwijndrecht, the Netherlands) and Cambridge Isotope Laboratories, Inc. (Andover, MA, USA). M6TBOEP, TCEP-$_{d12}$ and TDCIPP-$_{d15}$ were supplied by Wellington Laboratories Inc. (Guelph, ON, Canada). Formic acid 98% was obtained from Merck (Darmstadt, Germany). Oasis® MCX cartridges (150 mg/6 cc) were purchased from Waters, (Milford, MA, USA).

5.2.2 Sample collection:

Twenty-four hour composite samples were collected from the raw inlets – before the primary clarifier – of eleven STPs during the week of the 2011 Australian Census (i.e. between the 9th and 11th of August 2011). Thorough population censuses such as the Australian census provide unique opportunities to obtain accurate population data such as head count with small error. In Australia, participating in census is compulsory. There are also quality assurance procedures in place to ensure the accuracy of the data such as a post enumeration survey conducted a month after census which indicated that the 2011 census was undercounted by only approximately 1.7%(Australian Bureau of Statistics (ABS) 2011). Sampling wastewater influent during census allows for wastewater influent chemical loads to be normalised per capita with accuracy (O'Brien et al. 2014). The STPs sampled cover a variety of demographics and are representative of urban, semi-rural and rural catchments. For three of the sites, two days of samples were collected and for

one site three days of samples were collected. Ten catchments provided maps of the catchment boundaries in order for the Australian Bureau of statistics to determine accurate population estimates which ranged from approximately 3,500 in the smallest catchment to approximately 500,000 in the largest. For the site where accurate population could not be provided due to the lack of a catchment map, the population was calculated using mass loads of 14 chemicals in samples from that STP and the calibration data from a population estimation model (O'Brien et al. 2014). A brief description of the sampling methods and catchment for each STP is provided in Table 10. This includes minimum and maximum temperatures as well as daily rainfall however as rainfall during the sampling period was minimal (median 0 mm, maximum = 3.8 mm) we consider stormwater runoff into the sewer negligible. The collected samples were preserved by acidifying to pH 2 with 2M hydrochloric acid and shipped on ice (chilled or frozen) to the laboratory. The samples were stored and transported frozen at -20°C to the University of Amsterdam (VU) where they remained frozen prior to extraction and analysis.

Table 10 Site summary of the sewage treatment plants and samples collected for this study

STP ID	Date Sampled	State	Sampling Mode	ΔT or ΔV (~T)	Daily Flow (ML)	Temperature (min - max)	Rainfall (mm)	Population Size
STP 1	10/08/2011	SA	V	10 m^3 (9 min)	1.6	5.0 – 12.8 °C	3.8	3682
STP 2	10/08/2011	SA	V	20 m^3 (8 min)	3.8	5.3 – 10 °C	3.0	8995
STP 3	11/08/2011	TAS	C	C	12.6	6.9 – 9.6 °C	0.2	25 698
STP 4	09/08/2011	QLD	T	20 min	4.1	5.6 – 21.7 °C	0	38 005
STP 5	10/08/2011	QLD	T	60 min	10.2	5.5 – 23.7 °C	0	43 513
STP 6	10/08/2011	QLD	V	100 m^3 (7 min)	21.4	3.5 – 17.4 °C	0	86 882
STP 6	11/08/2011	QLD	V	100 m^3 (7 min)	21.2	7.4 – 17.9 °C	0	86 882
STP 7	10/08/2011	QLD	T	20 min	15.6	5.0 – 22.6 °C	0	92 104
STP 8	10/08/2011	QLD	T	15 min	35.0	5.6 – 23.1 °C	0	171 807*
STP 8	11/08/2011	QLD	T	15 min	35.0	8.2 – 20.4 °C	0	171 8 07*
STP 9	9/08/2011	QLD	CF	C	57.1	9.8 – 21.9 °C	0	211 340
STP 9	10/08/2011	QLD	CF	C	56.4	8.2 – 22.3 °C	0	211 340
STP 9	11/08/2011	QLD	CF	C	58.0	11.3 – 21 °C	0	211 340
STP 10	09/08/2011	ACT	T	60 min	80.3	-0.2 – 13.1 °C	0.4	342 459
STP 11	11/08/2011	QLD	T	20 min	127.4	7.8 – 19.7 °C	0	495 027

ACT = Australian Capital Territory. QLD = Queensland. SA = South Australia. TAS = Tasmania. C = continuous. F = flow proportional. T = time proportional. ΔT = time between samples. ΔV = volume between samples. ~T = average time between samples (Daily Flow/ ΔV). Population size is the enumerated *de facto* population in the catchment on Census Day (9th Aug 2011). * = population estimated using the Bayesian inference population estimation model of O'Brien et al. (2014).

5.2.3 Extraction:

The analytical method used in this study is based on the method of Brandsma et al. (2014) further modified by Martinez Moral et al. (2014) for liquid samples being extracted using a solid phase extraction method. In brief, wastewater samples were centrifuged at 1700 g for 10 minutes. 10 mL aliquots of the supernatants were fortified with 50 µL internal standards (TNBP-$_{d27}$ = 1813 ng/mL, M6TBOEP = 1600 ng/mL, TCEP-$_{d12}$ = 1600 ng/mL, TDCIPP-$_{d15}$ = 1600 ng/mL, TPHP-$_{d15}$ = 1812 ng/mL), vortexed and refrigerated overnight at 4°C. Oasis MCX cartridges (150 mg/6 cc) were conditioned with 5 mL methanol and 5 mL MilliQ water at a flow rate of 1 mL per minute. Samples were loaded drip wise and the tubes and cartridges were rinsed with 3 mL pH2 formic acid in MilliQ water. The cartridges were then dried under vacuum for 30 minutes before elution with 6 mL methanol and 4 mL toluene. Eluates were concentrated using a gentle stream of nitrogen and a water bath held at 30°C to a final volume of 100 µL and transferred to vials.

5.2.4 Analysis:

Analysis of PFRs was performed on a GC/MS (HP6890 GC Agilent Technologies, Amstelveen, The Netherlands) with separation on a BPX5 capillary column (25 m x 0.22 mm ID, 0.25 µm film thickness, SGE Analytical Science, Rotterdam, the Netherlands). The MS was run in the electron impact ionization (EI) mode. Full details are outlined in Brandsma et al. (2014). Selective ion monitoring (SIM) was performed, as given in Table 11.
Quantification was performed using external calibration lines and labelled internal standards. The labelled internal standard TPHP-$_{d15}$ was used for TPHP and EHDPP, TNBP-$_{d27}$ for TNBP and TIBP, TCEP-$_{d12}$ for TCEP and TCIPP, TDICPP-$_{d15}$ for TDCIPP and M6TBOEP for TBOEP. As the internal standards were spiked prior to extraction, the internal standards correct for both extraction losses and matrix effects.

Table 11 GC-EI-MS retention time and monitoring ions of the PFRs studied.

Compound	Acronym[a]	Retention time (min)	Quantifier	Qualifier	LOD (µg/L)
Tris(butyl) phosphate d27*	TNBP-$_{d27}$	8.80	167	231, 99	N/A
Tris(butyl) phosphate	TNBP	8.96	155	211, 99	3.0
Tris(isobutyl) phosphate	TIBP	7.75	155	211, 99	0.8
Tris(2-chloroethyl) phosphate d12*	TCEP-$_{d12}$	10.06	261	213	N/A
Tris(2-chloroethyl) phosphate	TCEP	10.16	249	251	0.2
Tris(2-chloroisopropyl) phosphate	TCIPP	10.37	277	279, 201	1.1
Tris(1,3-dichloroisopropyl)phosphate d15*	TDCIPP-$_{d15}$	14.88	394	197	N/A
Tris(1,3-dichloroisopropyl)phosphate	TDCIPP	15.02	381	379, 191	0.03
Tris(2-butoxy-[13C2]-ethyl) phosphate*	M6TBOEP	15.49	303	231, 157	N/A
Tris(2-butoxyethyl) phosphate	TBOEP	15.49	199	299	0.1
Tris(phenyl) phosphate d15 *	TPHP-$_{d15}$	15.64	341	239	N/A
Tris(phenyl) phosphate	TPHP	15.72	326	325, 215	0.2
2-Ethylhexyldiphenyl phosphate	EHDPP	15.78	251	250, 362	0.1
* = chemical used as an internal standard					

5.2.5 QA/QC

To investigate the extraction efficiency and the blank levels, i) MilliQ water was fortified with 50 µL internal standard solution (concentration ~1600 µg/L) and ii) MilliQ water was fortified with 50 µL internal standard and native PFRs (concentration ~1600 µg/L), then these were extracted, concentrated and analysed as outlined above. The observed blank levels are given in Table S- 9 and ranged from 0.01 to 0.21 µg/L for EHDPP, TPhP, TBOEP, TCEP, TCIPP, TDCIPP. Somewhat higher blank levels were observed for TIBP and TNBP with averages of 0.29 and 1.0 and µg/L, respectively. Average recovery of the PFRs in the spiked MilliQ water ranged from 76 – 114%. In addition, a wastewater sample consisting of wastewater from 5 of the STPs was homogenised, split into four aliquots, two of which were fortified only with PFR internal standards, the other two with both internal standards and native OPEs. These samples were extracted to check extraction efficiency and for matrix effects. The average recovery for the spiked wastewater was comparable with the spiked MilliQ water and ranged between 70 – 112% with the exception of TIBP (50%) and TNBP (53%). All the analysed wastewater samples were corrected for the blank values and labelled internal standards, however not for the recovery standards. The limit of detection (LOD) for the PFR method is defined as three times the concentration measured in the blank (Table 11).

5.3 Results and Discussion

5.3.1 Concentration of PFRs in wastewater

Table S- 7 gives the concentrations of the various PFRs detected in wastewater samples collected from around Australia. TBOEP, TCIPP and TDCIPP were detected in all samples. TCEP was detected in all but one sample. TIBP was detected in six samples while EHDPP was detected in five. TPHP was only detected in one sample. TNBP was not detected in any of the samples.
TBOEP had the highest concentrations which ranged from 0.4 to 6.6 µg/L with a median of 4.4 µg/L followed by TCIPP, (0.5 to 4.1 µg/L, median = 2.5 µg/L), TIBP (1.1 to 1.6 µg/L with a median of 1.4 µg/L), TCEP (0.2 to 0.6 µg/L with a median of 0.3 µg/L) and TDCIPP (0.05 to .3 µg/L with a median of 0.1 µg/L. In the most recent US EPA survey, production volumes for TBOEP, TNBP and TCEP were listed as

high production volume chemicals with individual production volumes of between 1 and 10 million pounds annually (Environmental Protection Agency 2010).

5.3.2 PFR load in wastewater of different Australian catchments

The daily load of the PFRs coming into each STP was calculated by multiplying the concentration measured in the sample by the total volume (estimated from flow) of wastewater entering each treatment plant for the sampled 24 hour period.
A linear regression was performed for daily mass loads of TBOEP, TCEP, ∑TCIPP and TDCIPP entering wastewater treatment plants against the population size as these were the most frequently detected compounds and were found in samples from all sites, except for TCEP which couldn't be detected at Site 2. The population data is considered accurate as the samples were collected either on or within two days of the 2011 Census. This data indicates that the mass load entering wastewater for these compounds correlates strongly with the population size with $r^2 \geq 0.87$ which may indicate that Australians, regardless of location, use similar products containing OPEs. As the samples are from a range of catchments and population sizes, it is therefore assumed they have different sewer networks comprising of gravity sewers, rising mains and pumping stations, the strong correlations for linear regressions between population size and mass load for these PFRs may be indicative that the PFRs are not significantly degraded in the sewer.

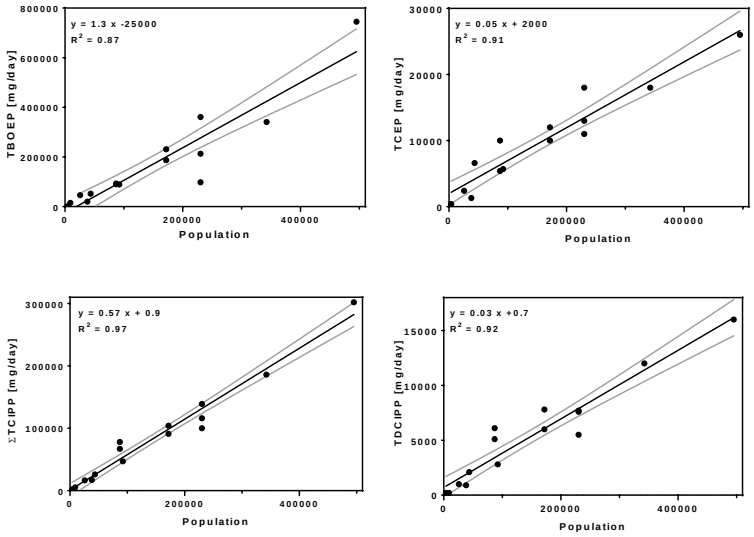


Figure 11 Population versus daily mass load of PFRs which were measured at the majority of sites. The black line is the line of best fit. The grey lines indicate the 90% confidence interval of the regression line.

In order to compare the level of PFR discharge into the sewer system among different STPs, we determined daily per capita PFR input into wastewater by dividing the mass load of each PFR by the population size. A comparison of the individual PFR to the sum of the detected PFR (referred to here as a PFR profile) is shown in Figure 12. This data indicates that the input of ΣOPE per capita input into wastewater is between approximately 0.85 and 2.6 mg/person/day with a mean of 2.1 mg/person/day. Data <LOD were excluded. Statistical analysis suggests that there are no significant outliers in this data set (Grubbs' test, significance level: 0.05 two sided).

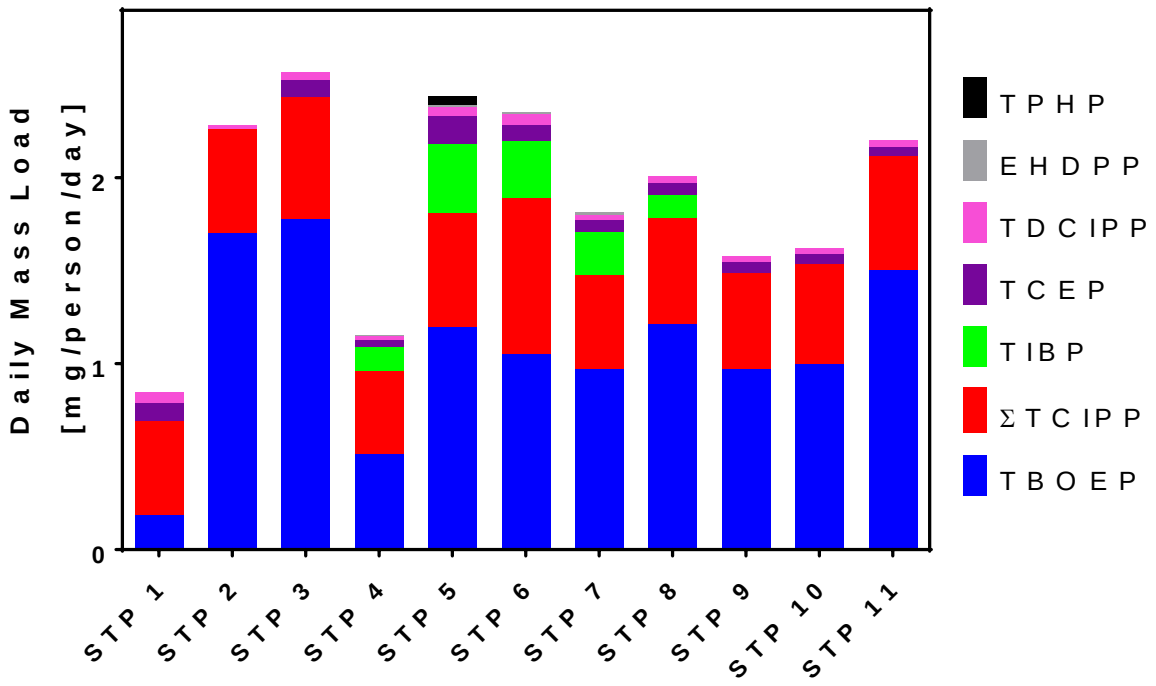


Figure 12 Daily per capita input of PFR mass loads into wastewater for eleven STP catchments in Australia. For STPs 6 and 7, the mean of two consecutive days sampling are reported. For STP 9, the mean of three consecutive days is reported (coefficient of variation for TDCIPP = 0.17, TCEP = 0.26, ∑TCIPP = 0.16, TBOEP = 0.58).

5.3.3 Comparison of PFRs in wastewater between Australia and other countries

For comparison between the data from this study and other countries we have to revert to the concentrations in the influent water samples acknowledging that water use and associated dilution may vary substantially between treatment plants and sampling periods. Overall we find similar (within an order of magnitude)

concentrations to those observed in other studies. For example, the concentration of TBOEP in Australian wastewater influent (median = 4.4 µg/L) is similar to those observed in Sweden (median = 9.1 µg/L) (Marklund et al. 2005b). For TCIPP the median concentration in Australia (2.5 µg/L) is similar to both Sweden and United States medians (2.0 and 1.2 µg/L respectively). TCEP concentrations in our study (median = 0.3 µg/L) were also similar to those reported in Swedish and US studies (median = 0.5 and 0.7 µg/L respectively). The concentration of TDCIPP in Australia (median = 0.1 µg/L) was similar to that of Sweden (median = 0.3 µg/L) but much lower than the United States influent (median = 1.9 µg/L). TIBP was not measured in either the Swedish or United States influent. Overall there is reasonably good agreement in the concentrations and associated profiles of PFRs entering wastewater among the few studies available (Figure 14).

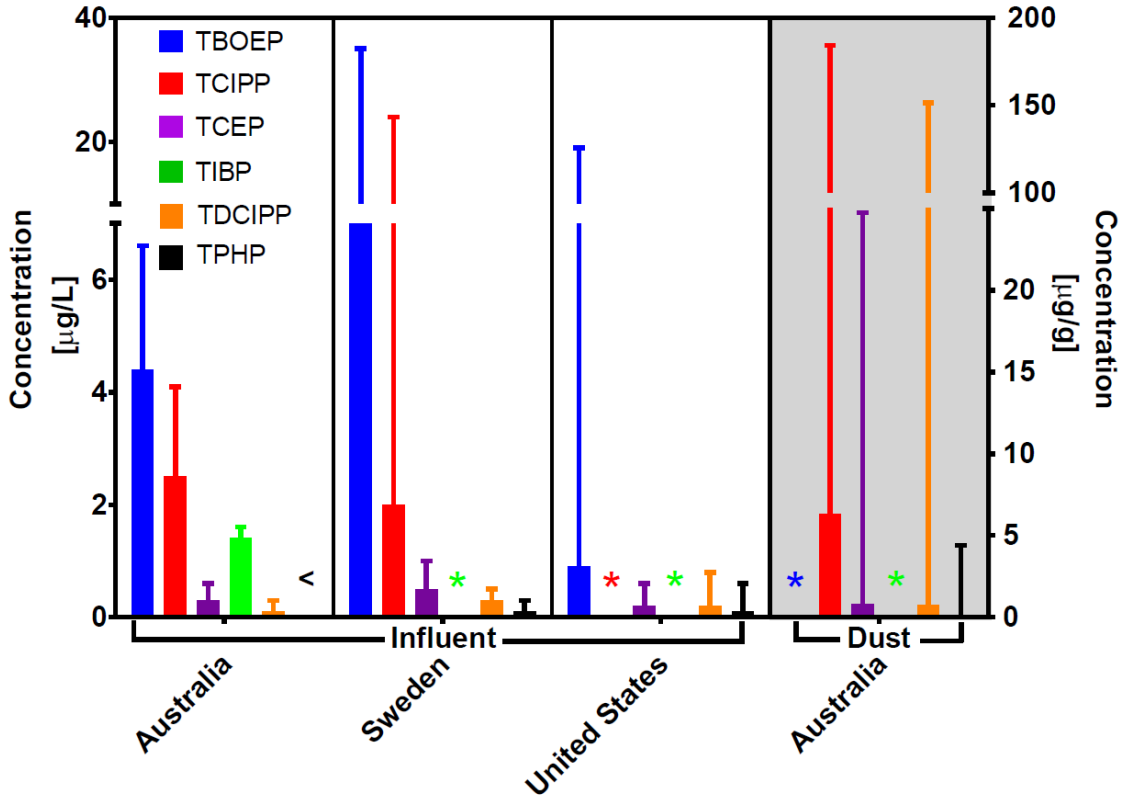


Figure 13 Country comparison of concentration of PFRs in wastewater influent as well as dust. Australian influent data (STPs=11). Sweden influent data (STPs=7) is from Marklund et al. (2005b). United States influent data (STPs=2) is from Schreder and La Guardia (2014). Australia dust data (n=238) is from Brommer (2014). Bars indicate the median. Lines indicate the maximum. < indicate that the median was below the detection limit. * indicate that the compound matching that colour was not analysed.

5.3.4 Annual Australian PFR discharge into STPs and the aquatic environment

On Australian Census Day 2011 when the samples were collected, the population was 21.5 million. Given this population size and that the daily load of PFRs in wastewater is approximately 2.1 mg/person/day, the yearly load of PFRs input into Australian wastewater is approximately 16 tonnes. As a third of this load is chlorinated PFRs, this may be an environmental issue as chlorinated PFRs are considered resistant to microbial degradation and there is no significant removal from wastewater (Meyer and Bester 2004). It is therefore estimated that the load of PFRs entering STPs in Australia is likely to be similar to that entering the aquatic environment. However, it is important to note that this is an estimate only and without further study on the removal efficiencies of the PFRs in the STPs assessed, we cannot be certain of the PFR loads entering the Australian aquatic environment. Additionally, the amount of PFR discharged into the Australian aquatic environment may be slightly under-estimated in this study because we did not include a small proportion of PFR mass that could adsorb onto the particulate phase of the wastewater. Recently both Marklund et al. (2005b) and Schreder and La Guardia (2014) suggested that between 1 – 5 % of the PFRs that are in use enter the wastewater stream annually. Based on the annual load estimated from the sampling during the census period (16 tonnes) and the above estimate that 1 – 5 % of the in-use PFRs we come up with a ball park estimate of 320 – 1600 tonnes of PFRs were in use at the time of this study (ie. 2011). NICNAS (2001) estimated that the import of TCEP and TCIPP in 2001 was about 410 tonnes which is similar to the total amount of 100 – 500 tonnes that is estimated from our study using the Marklund et al. (2005b) and Schreder and La Guardia (2014) approach. However continuous import must have resulted in a much higher total amount of these chemicals being in circulation hence we estimate that the release into wastewater is probably well below 1 % of the PFRs that are in use within Australia at the time. These data indicate that TDCIPP, which hadn’t been imported into Australia before 2001 (NICNAS 2001), has been imported into Australia and suggest that in 2011 somewhere between 6 and 30 tonne per were in use. It is noteworthy that TBOEP was found at higher loads than when compared for example with TCEP, TCIPP and TDCIPP which are priority existing chemicals – and thus our data may suggest that it could be considered for inclusion into the list of priority existing chemicals.

If we consider the annual load of PFRs entering STPs and the individual removal efficiencies of STPs for each PFR (Table 12), the combined annual load of PFRs discharged to the environment from all STPs in Australia is approximately 6.7 tonnes. ∑TCIPP is the largest contributor to this load (69%) whereas TBOEP, which had the highest input into wastewater, only contributes about 13% to the annual Australian STPs discharge load.

Table 12 Estimated annual loads of PFRs in Australian effluent based on the influent data and STP removal efficiencies. a removal estimate is from Olofsson (2012). b removal estimate is from Meyer and Bester (2004). TCEP, ∑TCIPP and TDCIPP are considered not significantly removed (Meyer and Bester 2004).

	EHDPP	TPHP	TBOEP	TCEP	∑TCIPP	TDCIPP	TIBP	TNBP	∑OPEs
STP Removal Efficiency	81%[a]	58%[b]	89%[b]	not removed[b]	not removed[b]	not removed[b]	84%[b]	54%[b]	
Effluent Load (tonne.year^{-1})	0.02	0.2	0.9	0.5	4.6	0.3	0.3	NA	6.7

5.3.5 Comparison with data from other sources

Schreder and La Guardia (2014) determined that laundry wastewater may be the primary source for PFRs entering STPs, possibly as a result of household dust accumulating on clothing and transferring to the laundry wastewater (Schreder and La Guardia 2014). Currently there is only one study which measured PFRs in Australian dust (Brommer 2014). This study focused on the OPEs: TNBP, TCEP, TCIPP, TPHP, EHDPP, TDCIPP and TMPP in five indoor microenvironments: living rooms, bedrooms, cars, couches and mattresses (Brommer 2014). In order to establish whether or not the profile of PFRs in the dust are reflective of what is in the wastewater influent, the median concentration from all dust microenvironments were averaged to give a single value for each PFR and graphed with the Australian wastewater influent (Figure 3, right part of figure using the secondary y axis). Although TBOEP had the highest concentration and mass load in Australian

wastewater influent, it was not measured in the dust samples. EHDP and TIBP were also not measured in the dust. However, the profile for the dust appears to agree with the wastewater influent in that the order of highest to lowest is TCIPP, then TCEP and then TDCIPP. Therefore, inclusion of TBOEP in future analysis of dust samples may provide further evidence for whether or not indoor dust is the dominant source of PFRs in wastewater in addition to giving a better estimate of the sum of PFRs in dust.

5.4 Conclusion:

To our knowledge this is the first study to investigate PFRs in Australian wastewater. The data indicate that TBOEP has the highest concentration followed by TCIPP, TDCIPP and TCEP. Using accurate census population, it is estimated that on average a load of 2.1 mg/person/day PFRs enter Australian wastewater. Additionally, good correlation was observed for the four main contributors (TBOEP, TCIPP, TDCIPP and TCEP) to the PFR load and the population size ($r^2 \geq 0.87$). The concentration of PFRs in Australian wastewater influent is similar to U.S. and European wastewater influents. This study also confirms that TDCIPP has been imported into Australia and that expected PFR usage and observed usage are similar. Approximately 6.7 tonnes of PFRs are estimated to be discharged to the Australian aquatic environment every year. This dataset may be useful for further evaluation of time and spatial trends in Australia and suggests that wastewater analysis may be a useful tool for assessing PFR usage in other countries.

5. Acknowledgements

We would like to thank the operators of the STPs for providing wastewater samples for this study. Additionally we would like to thank Sandra Brommer and Stuart Harrad for sharing their PFR in Australian dust data. This project was conducted as part of a secondment between the VU University Amsterdam and The University of Queensland as part of the INTERFLAME project, supported by the European Union Seventh Framework Programme FP7/2007-2013 under grant agreement 295138. We would like to thank the IVM for hosting and for the training provided by Jacco Koekoek and Martin van Velzen. Jake O'Brien receives an APA PhD scholarship. Phong Thai is funded by a UQ Postdoctoral Research Fellowship. Jochen Mueller is

funded by an ARC Future Fellowship (FF 120100546). Entox is a joint venture of The University of Queensland and Queensland Health.

Chapter 6: Conclusion: key findings, final discussion and future research

6.1 Conclusion

The field of WBE has received much attention particularly for its ability to objectively measure illicit drug consumption with even the European Monitoring Centre including wastewater analysis in its *Perspectives on Drug*s report (EMCDDA 2016). In Australia, a national WBE drug monitoring strategy has been developed as WBE is recognised as providing a comprehensive picture of illicit drug markets and an effective way to measure the response of law enforcement to the illicit drug market ((ACC) 2016).

While the applications of WBE are becoming broader and the number of target analytes are increasing, the core principle remains the same. The approach relies on the principle that any substance when ingested, is excreted as the active parent compound and/or their metabolites and that these ultimately enter the urban sewer networks. The concentrations measured in the wastewater itself is thus representative of consumption of a particular chemical by the population served by the sewer network. Therefore, by measuring concentrations of target compounds (C_i) in wastewater samples which are representative of whole days, multiplying by the daily flow (F), correcting for excretion factors ($\frac{R_i}{E_i}$) and dividing by the population (P) we calculate the amount of a chemical consumed on a per capita basis. This is outlined by the equation in Zuccato et al. (Zuccato et al. 2008) where consumption of a target chemical (i) is normalized to per 1000 people:

$$Daily\ chemical\ consumption_i \left(\frac{\frac{mg}{day}}{1000\ people} \right) = \frac{C_i + F + \frac{R_i}{E_i}}{P}$$

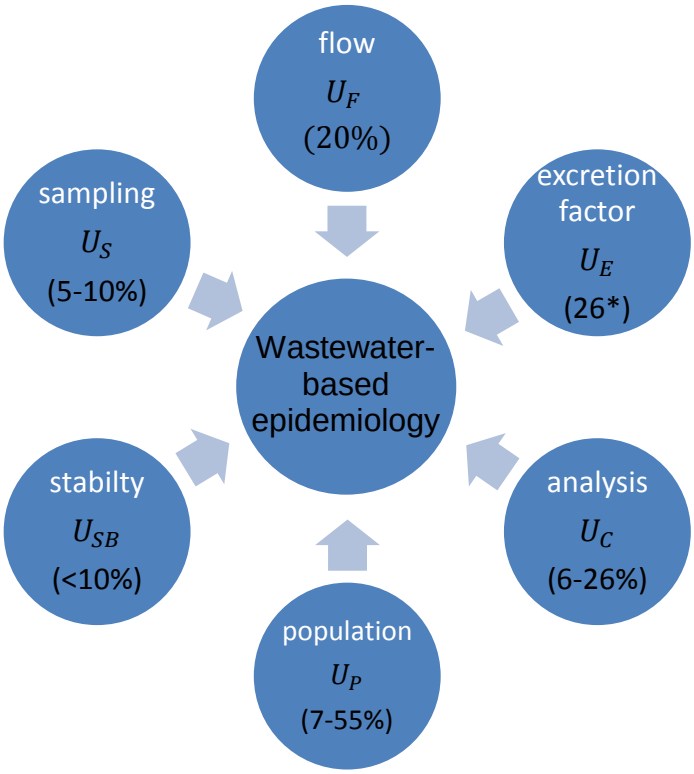


Figure 14 Wastewater-based epidemiology required elements and associated uncertainties. Uncertainty assessments based on Castiglioni et al. (2013) (Castiglioni et al. 2013) and Lai et al. (2011) (Lai et al. 2011). F = total daily flow. S = sampling. E = excretion factor. C = concentration of chemical. P = population. P = population. * = based on cocaine.

The accuracy of this estimate is dependent on accuracy of each of the parameters in the equation. Each of these parameters has its own uncertainties associated with it. An assessment of the uncertainties of WBE conducted by Castiglioni et al (2013) (Castiglioni et al. 2013) found that even using best practice methods, uncertainties still remained high. In an international interlaboratory study using real samples, it was found that the uncertainty of chemical analysis as expressed by relative standard deviation, RSD was 1 – 34% even when using analyte specific labelled internal standards and internal quality controls. The uncertainty of the sampling method can be reduced to between 5 and 10% using best practice sampling methods such as continuous flow-proportional sampling but is still dependent on the concentration variation of the target chemicals, population size, number of users, pharmacokinetics, operation of pump stations, in addition to the characteristics of the catchment such as exfiltration, special events and layout of the sewer catchment. In addition, there's uncertainty from the degradation of the target analytes both within the collected samples and within the sewer network which is estimated at

approximately 10%. The consumption estimate also relies on excretion data which in some cases may not exist or may be based on very small sample sizes. As an example, the uncertainty of the cocaine excretion profile is approximately 26%. The largest uncertainty however was identified for the estimation of population size with the uncertainty of population size estimated as between 7 and 55%.

As the uncertainty of population size was identified as the largest uncertainty, the overall aim of this thesis was to evaluate and propose an approach to reduce this uncertainty. The novelty of this PhD thesis lies in the unique wastewater sample set which were collected during a population census thus allowing for accurate head counts of the *de facto* population who contributed to each sample to be obtained. This sample set was then used to identify markers of *de facto* population size and to accurately calibrate per capita mass loads of potential population markers for inclusion in a population size model. Through identifying population size markers within wastewater samples and including them in a model, both the uncertainty of population size and flow were able to be reduced and thus overall improving upon the uncertainty of WBE.

6.2 Key Findings

In Chapter 2 a method was developed to accurately quantify creatinine concentration in wastewater influent using LC-MS/MS. Creatinine is a previously used marker of population size. Using laboratory-scale sewer reactors with conditions representative of both gravity sewers (GS) and rising main (RM) (pressure) sewers, we found that creatinine, while stable in collected samples, is unstable under sewer conditions and that this is likely due to the presence of sewer biofilms. The degradation followed first order kinetics and the degradation rate was significantly higher under RM conditions than when under GS conditions. Using samples collected from 10 different WWTP catchments during the 2011 Australian census for which accurate population data were obtained, an assessment between population size and creatinine daily mass load was made but no correlation was found. Therefore it can be concluded that creatinine is an unsuitable marker for estimating *de facto* population size.

In Chapter 3 a method was developed to accurately quantify 96 chemicals in wastewater influent using LC-MS/MS. Wastewater influent samples were collected from 10 different WWTP catchments during the 2011 Australian census for which accurate population data was obtained. These samples were analysed using the developed LC-MS/MS method. An assessment between population size and the daily mass load of the fourteen chemicals detected in all samples was made. Thirteen of these fourteen chemicals were found to have good correlation between daily mass load and population size ($R^2 > 0.8$). The good correlation between population and daily mass load suggested that these chemicals are suitable markers to estimate population size. Bayesian inference was then used to incorporate the correlation between mass load and population size for all fourteen chemicals into a single population size estimation model. The predictive capability of the model was validated using a leave-one-out approach for all sites which could then be compared to the census population sizes. It was found that Bayesian updating using all fourteen chemicals resulted in a significant improvement over the uncertainty of population size estimates provided by WWTP operators (width of the posterior for small WWTP catchments became 1.1 and 2.4 times narrower, but for the large WWTP catchments this was between 5.0 and 40 times narrower). It was also found that the width of the posterior was dependent on the number of chemicals included in the model with all fourteen providing the narrowest posterior and thus the most confident population estimate. Another finding from this study was that when estimating the population size using a single compound only, significant under/overestimation could be observed even for large WWTP catchments. This demonstrates that the aggregate consumption of an individual substance does not necessarily tend towards a nation-wide average.

In Chapter 4 we evaluated the impact of in-sewer degradation on the suitable population markers identified in Chapter 3**.** Additionally we assessed whether or not degradation of these chemicals impacts the population model. Using the same laboratory-scale sewer reactors used in Chapter 2, i.e. conditions representative of both RM and GS, it was determined that five of the markers were stable under all conditions over the 12 hour study period while the others were not. For the chemicals which were unstable, this ranged from very little degradation over the

study period and only under certain conditions to rapid degradation regardless of sewer conditions.

We applied the population model using only the chemicals which met the following four criteria: (1) measurable via direct injection on LC-MSMS; (2) have mass loads which correlate well with population size ($R^2 > 0.8$); (3) have negligible degradation in wastewater to ensure the stability of chemicals during wastewater collection; and (4) less than 10% in-sewer degradation could occur during the mean residence time in the WWTP sewer network. It was determined that the precision of the estimates did not improve and thus the combination of uncertainties associated with other factors such as variation in excretion and homogeneity of consumption may have greater influence on the uncertainty of the model. Regardless, to ensure that degradation does not influence the uncertainty of a population model, core chemicals to be used within the model should be chosen based on the 4 criteria outlined above.

By combining the knowledge gained from Chapters 2 to 4, a framework can be defined that can be applied to assess suitability of potential markers of population size. This is shown in Figure 16.

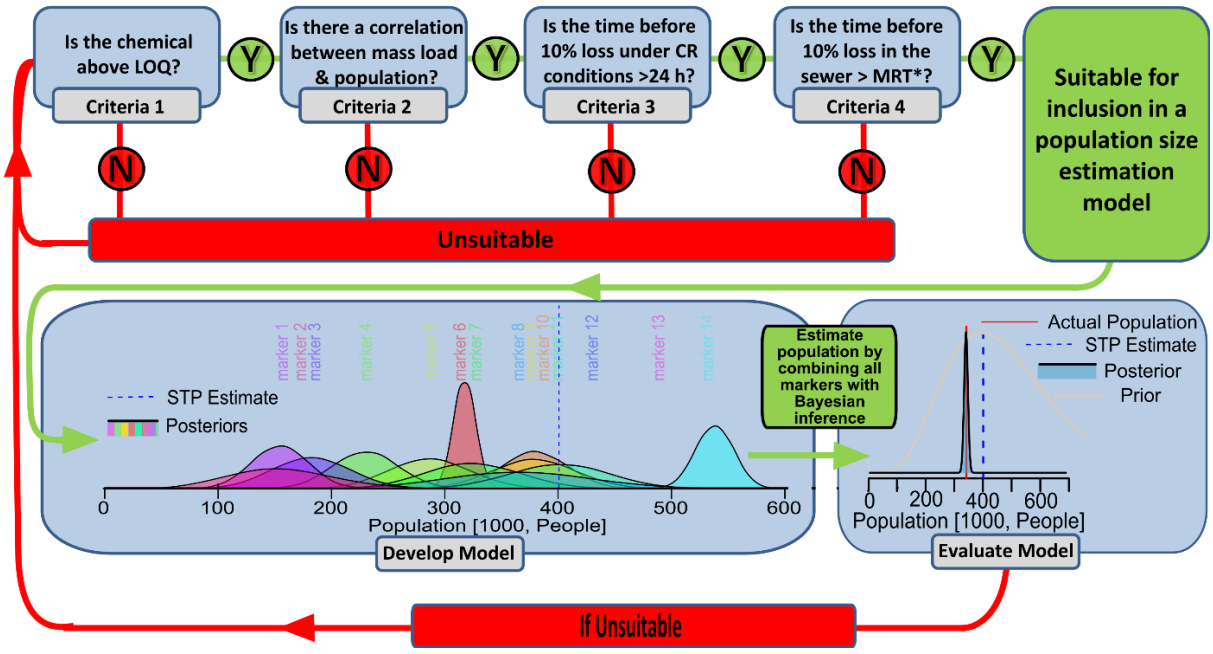


Figure 15 Framework to assess suitability of potential markers of population size.

In Chapter 5, to assess whether WBE can be expanded to chemicals other than those previously assessed (illicit drugs, alcohol and tobacco), a method was developed to analyse PFRs in wastewater influent to determine per capita loads of PFRs entering WWTPs in Australia. It was estimated that 2.1 mg person^{-1} day^{-1} of PFRs enter Australian wastewater and that there is a good correlation between the loads of the four main contributors (TBOEP, TCIPP, TDCIPP and TCEP) and population size ($r^2 \geq 0.87$). This study also confirmed that TDCIPP had been imported into Australia, a fact which was not previously known. Additionally, it was estimated that 6.7 tonnes of PFRs are discharged to the Australian aquatic environment every year.

6.3 Final discussion

6.3.1 Degradation of chemicals in the sewer

While the laboratory-scale sewer reactors used in Chapters 2 and 4 have been shown to provide conditions similar to real sewers, the area to volume (A/V) ratio was much higher than real sewers and thus observed degradation may not be representative. Additionally, we only monitored the degradation of the parent compounds and did not do an assessment of transformation products which may be useful in determining whether or not observed changes of mass loads in collected samples are a result of lower consumption or degradation in the sewer. Since conducting the sewer degradation experiments, new methods for analysing some metabolites/transformation products have been developed. One such method is for paraxanthine which is a major metabolite of caffeine. Thus for future in-sewer degradation paraxanthine could be a useful marker for indicating that caffeine is metabolised within the sewer. Additionally, since conducting the sewer degradation experiments (Chapters 2 and 4) a pilot sewer has been built onsite at a nearby WWTP which can be directly fed with fresh wastewater influent. This particular pilot sewer comprises of both rising main (RM) and gravity sewer (GS) pipes of similar dimensions to those within the sewer catchment. As the A/V ratio of the pilot sewer will be similar to those within actual sewer networks, it can be used to better understand in-sewer degradation/transformation. In addition, as the sewer can be

directly fed with fresh wastewater from the catchment, the biofilms are expected to be more reflective of those in the actual sewers within the catchment.

6.3.2 Expansion of wastewater-based epidemiology to assessing per capita input of organophosphorus flame retardants and plasticisers into wastewater

While this particular study was the first to determine per capita loads of PFRs entering WWTPs, confirmation of human exposure was not possible as only the parent compounds were monitored. Since conducting the analysis for this study, other studies have evaluated PFR concentrations, in particular metabolites, in urine which can thus be used to confirm exposure (Van den Eede et al. 2014). Through evaluation of the concentrations of these metabolites in urine and the already low levels of PFRs detected in wastewater, we determined that using the current analytical methods we would not be able to quantify the metabolites in wastewater and that higher sample volume and or better limits of detection would be required.

6.4 Future Research

6.4.1 How far can we go with the model?

Since publication, we have applied the population model developed in Chapter 3 to determine *de facto* population size for 311 daily wastewater influent samples from a large WWTP in South East Queensland (Lai et al. 2015). We found that the population uncertainty on average could be improved by a factor of 2. However, the relative day-to-day pattern of drug consumption was similar regardless of the type of normalization as daily illicit drug loads appeared to vary substantially more than the population. The time and effort put into calculating daily *de facto* population estimates using this method in this case then can probably not be justified.
This raises the questions of when and where should we apply the model? When collecting the wastewater samples for the calibration data of the population model, we also asked the WWTP operators to provide population estimates which is the normal practice for WBE studies. In some cases large discrepancies were identified between census counts and the WWTP operator estimates and thus the model may be useful for providing a better estimate of the actual population. This is particularly

useful where per capita consumption of chemicals is to be compared between regions.

6.4.2 Modelling of sub populations

The accuracy and uncertainty of the population model is impacted by the homogeneity of consumption of chemicals within the population being investigated and within the calibration data populations. Some chemicals currently included in the population model, such as atenolol and hydrochlorothiazide, are only consumed by people with disease. Atenolol is a beta blocker and hydrochlorothiazide a diuretic hence both are used primarily for the treatment of cardiovascular disease. In Australia, cardiovascular disease affects one in six people and is responsible for 30% of deaths. Moreover, the processes leading to cardiovascular disease increase with age. While consumption of these chemicals is thought to be relatively homogenous in the population overall, depending on demographic factors within a particular community (e.g. where the average age is higher), the model may not accurately estimate the population size for this community using these chemicals in the model. However, while this is a limitation of the model, the monitoring and modelling of these specific chemicals may reveal domestic migration patterns of people with cardiovascular disease.

Additionally, where other chemicals are identified which are representative of other subpopulations, then these also may be used to determine specific demographics of communities and/or their migration patterns. Such examples include chemicals only consumed and or excreted by females (gender specific hormones may be useful for this), chemicals only consumed/excreted by younger people and so on.

For this to become a reality, a model needs to be calibrated where accurate demographic data can be correlated with mass loads of chemicals. This may be possible during the likes of the Australian Census where in addition to accurate head counts, demographic data such as age, ethnicity, gender, internal migration, income, level of education and occupation are collected ((ABS) 2015).

6.4.3 Using the population model for other countries

To assess the applicability of the population model in other countries, we analysed the same suite of chemicals in wastewater samples from China and applied our calibration data to estimate the population for each site based on this (Appendix 5) (Gao et al. 2016). It was in general observed that consumption of these chemicals was higher in Australia which would lead to underestimation of population sizes if used to estimate populations for China. In this case, we chose to recalibrate the model using WWTP operator population estimates and the mass loads of chemicals calculated from these samples. Therefore, in future studies to identify markers of population size it would be beneficial to focus on detecting endogenous markers in order to develop a universal population size model.

6.4.5 When to recalibrate the population model?

Ideally the best time to calibrate is when representative samples can be collected from populations of known size such as during census. Additionally, the model can be recalibrated if new exogenous population markers are identified which can then be analysed in previously collected samples with associated accurate head count data. The best case scenario is that markers are identified that are human specific, excreted homogenously, are uninfluenced by demographic (i.e. no age, gender or ethnic differences) and are stable within wastewater. In this scenario, calibration data could be collected once from multiple catchments for which accurate head counts can be obtained and used universally without site specific calibration. As mentioned in Chapter 3, the model is not limited to using only relationships between chemical mass loads and population size but other parameters such as mobile phone usage data (Charles-Edwards 2016) can be incorporated.

6.4.6 What other uncertainties of WBE should be addressed?

As shown in this thesis, the uncertainty of population size for WBE can be reduced. Further work is still required however to identify universal markers which can be used to provide better per capita chemical consumption comparisons between cities and countries. Other uncertainties should also be reduced to improve WBE data. For

example, Ort et al. (2010) (Ort et al. 2010b) reduced the uncertainty of sampling through use of a continuous flow-proportional sampling method whereby sampling uncertainties of <10% can be achieved (Castiglioni et al. 2013). As the quality of WBE also relies on the quality of the measured flow data, using a population model as described in this thesis to determine population size, the uncertainty from systematic flow measurement errors can be cancelled out. The quality and uncertainty of chemical analysis can be improved through following quality guidelines such as use of matched internal standards and use of quality assurance/quality controls. The stability of target compounds in wastewater and within the sewer for many target compounds is either limited or not known. This thesis produced some stability data for certain target chemicals both in wastewater and within the sewer. Further research however is required as stability is chemical dependant. Through identifying both homogenously consumed and excreted chemicals which are stable under certain conditions and others which are not, it may be possible to better understand the degradation processes occurring in individual catchments and back-calculate mean residence times based on these profiles.

Another uncertainty which is also compound dependant is that of excretion and the excretion profile. Excretion profiles can depend on many factors such as route of administration, frequency of use, and weight, age and ethnicity of the consumer. In addition, the profile of a chemical may change when consumed alongside other substances. Such studies are limited, or may be hampered by ethical issues where toxic substances such as illicit drugs are involved, and typically such studies use small sample sizes. Therefore the uncertainty inherent in excretion profiles remains largely unknown for many compounds. One alternative may be using the wastewater itself to refine the excretion factors by combining the available pharmacokinetic data with wastewater analysis data. Such a study has recently been applied for refining the excretion factors for both codeine and methadone and showed promising results (Thai et al. 2016).

Bibliography

(ABS), A.B.o.S. (2015) Topics to be Included in the 2016 Census. Census of Population and Housing: Nature and Content, A., 2016 (ed), Canberra, Australia.

(ACC), A.C.C. (2013) Illicit drug data report 2011-12, Canberra, Australia.

(ACC), A.C.C. (2016) 4 Intelligence operations.

(MIIT), M.o.I.a.I.T. (2013) Chinese Medicine Yearbook 2013. China, M.o.I.a.i.T.o.t.P.s.R.o. (ed), Beijing.

(WHO), W.H.O. (1990) EHC-110. Tricresyl Phosphate, Geneva, Switzerland.

(WHO), W.H.O. (1991a) EHC-111. Triphenyl Phosphate, Geneva, Switzerland.

(WHO), W.H.O. (1991b) EHC-112. Tributyl Phosphate, Geneva, Switzerland.

(WHO), W.H.O. (1998) EHC-209. Tris(chloropropyl) Phosphate and Tris(2-chloroethyl) Phosphate, Geneva, Switzerland.

(WHO), W.H.O. (2000) EHC-218. Flame Retardants: Tris(2-butoxyethyl) Phosphate, Tris(2-Ethylhexyl) Phosphate and Tetrakis(Hydroxymethyl) Phosphonium Salts, Geneva, Switzerland.

Agency for Toxic Substances and Disease Registry (1997) Toxicological Profile for Hydraulic Fluids. Services, D.o.H.a.H. (ed), Atlanta, GA.

Ambre, J., Fischman, M. and Ruo, T.-i. (1984) Urinary Excretion of Ecgonine Methyl Ester, a Major Metabolite of Cocaine in Humans. Journal of Analytical Toxicology 8(1), 23-25.

Ambre, J., Ruo, T.I., Nelson, J. and Belknap, S. (1988) Urinary Excretion of Cocaine, Benzoylecgonine, and Ecgonine Methyl Ester in Humans. Journal of Analytical Toxicology 12(6), 301-306.

Andreottola G., B.L., Poggiali L., Zaffaroni C (1994) A methodology for the estimation of unit nutrient and organic loads from domestic and non-domestic sources. European Water Pollution Control 4(6), 7.

Australian Bureau of Statistics (ABS) (2011) Undercount Adjustment Factors, Canberra.

Australian Bureau of Statistics (ABS) (2012) 4364.0.55.001 - Australian Health Survey: First Results, 2011-12 Australian Bureau of Statistics, Canberra.

Avdeef, A., Barrett, D.A., Shaw, P.N., Knaggs, R.D. and Davis, S.S. (1996) pH-Metric logP .7. Octanol-, chloroform-, and propylene glycol dipelargonat-water partitioning of morphine-6-glucoronide and other related opiates. Journal of Medicinal Chemistry 39(22), 4377-4381.

Avdeef, A., Box, K.J., Comer, J.E.A., Hibbert, C. and Tam, K.Y. (1998) pH-Metric logP 10. Determination of Liposomal Membrane-Water Partition Coefficients of Ionizable Drugs. Pharmaceutical Research 15(2), 209-215.

Baker, D.R., Barron, L. and Kasprzyk-Hordern, B. (2014) Illicit and pharmaceutical drug consumption estimated via wastewater analysis. Part A: chemical analysis and drug use estimates. The Science of the total environment 487, 629-641.

Baker, D.R., Ocenaskova, V., Kvicalova, M. and Kasprzyk-Hordern, B. (2012) Drugs of abuse in wastewater and suspended particulate matter--further developments in sewage epidemiology. Environ Int 48, 28-38.

Banta-Green, C.J., Field, J.A., Chiaia, A.C., Sudakin, D.L., Power, L. and De Montigny, L. (2009) The spatial epidemiology of cocaine, methamphetamine and 3,4-methylenedioxymethamphetamine (MDMA) use: a demonstration using a population measure of community drug load derived from municipal wastewater. Addiction 104(11), 1874-1880.

Barone, J.J. and Roberts, H.R. (1996) Caffeine consumption. Food and Chemical Toxicology 34(1), 119-129.

Baselt, R.C. (2008) Disposition of Toxic Drugs and Chemicals in Man, eighth ed., Biomedical Publications, Foster City, CA.

Been, F., Bijlsma, L., Benaglia, L., Berset, J.-D., Botero-Coy, A.M., Castiglioni, S., Kraus, L., Zobel, F., Schaub, M.P., Bücheli, A., Hernández, F., Delémont, O., Esseiva, P. and Ort, C. (2016) Assessing geographical differences in illicit drug consumption—A comparison of results from epidemiological and wastewater data in Germany and Switzerland. Drug and Alcohol Dependence 161, 189-199.

Been, F., Rossi, L., Ort, C., Rudaz, S., Delemont, O. and Esseiva, P. (2014) Population normalization with ammonium in wastewater-based epidemiology: application to illicit drug monitoring. Environ Sci Technol 48(14), 8162-8169.

Beermann, B., Dalen, E., Lindstrom, B. and Rosen, A. (1975) On the fate of furosemide in man. Eur. J. Clin. Pharmacol. (9), 57-61.

Beermann, B. and Groschinsky-Grind, M. (1977) Pharmacokinetics of hydrochlorothiazide in man. Eur. J. Clin. Pharmacol. (12), 297-303.

Bendt, A.K., Beckers, G., Silberbach, M., Wittmann, A. and Burkovski, A. (2004) Utilization of creatinine as an alternative nitrogen source in Corynebacterium glutamicum. Arch Microbiol 181(6), 443-450.

Bisceglia, K.J., Roberts, A.L. and Lippa, K.A. (2012) A hydrolysis procedure for the analysis of total cocaine residues in wastewater. Analytical and Bioanalytical Chemistry 402(3), 1277-1287.

Bisceglia, K.J., Roberts, A.L., Schantz, M.M. and Lippa, K.A. (2010) Quantification of drugs of abuse in municipal wastewater via SPE and direct injection liquid

chromatography mass spectrometry. Analytical and Bioanalytical Chemistry 398(6), 2701-2712.

Blair, B., Nikolaus, A., Hedman, C., Klaper, R. and Grundl, T. (2015) Evaluating the degradation, sorption, and negative mass balances of pharmaceuticals and personal care products during wastewater treatment. Chemosphere 134, 395-401.

Blanchard, J., Sawers, S.J.A., Jonkman, J.H.G. and Tang-Liu, D.D.S. (1985) Comparison of the urinary metabolite profile of caffeine in y9oung and elderly males. Brit. J. Clin. Pharmacol. (19), 225-232.

Brandsma, S.H., de Boer, J., van Velzen, M.J. and Leonards, P.E. (2014) Organophosphorus flame retardants (PFRs) and plasticizers in house and car dust and the influence of electronic equipment. Chemosphere 116, 3-9.

Brewer, A.J., Ort, C., Banta-Green, C.J., Berset, J.-D. and Field, J.A. (2012) Normalized Diurnal and Between-Day Trends in Illicit and Legal Drug Loads that Account for Changes in Population. Environmental Science & Technology 46(15), 8305-8314.

Brommer, S. (2014) Human exposure to organophosphate flame retardants, University of Birmingham.

Brommer, S., Harrad, S., Van, d.E.N. and Covaci, A. (2012) Concentrations of organophosphate esters and brominated flame retardants in German indoor dust samples. Journal of environmental monitoring : JEM 14(9), 2482-2487.

Brorström-Lundén, E., Svenson, A., Viktor, T., Woldegiorgis, A., Remberger, M., Kaj, L., Dye, C., Bjerke, A. and Schlabach, M. (2008) Measurements of sucralose in the Swedish screening program 2007 - part I; sucralose in surface waters and STP samples, IVL Swedish Environmental Research Institute, Stockholm.

Bu, Q., Wang, B., Huang, J., Deng, S. and Yu, G. (2013) Pharmaceuticals and personal care products in the aquatic environment in China: A review. Journal of Hazardous Materials 262, 189-211.

Bu, Q., Wang, B., Huang, J., Liu, K., Deng, S., Wang, Y. and Yu, G. (2016) Estimating the use of antibiotics for humans across China. Chemosphere 144, 1384-1390.

Buerge, I.J., Buser, H.R., Kahle, M., Muller, M.D. and Poiger, T. (2009) Ubiquitous Occurrence of the Artificial Sweetener Acesulfame in the Aquatic Environment: An Ideal Chemical Marker of Domestic Wastewater in Groundwater. Environmental Science & Technology 43(12), 4381-4385.

Buerge, I.J., Kahle, M., Buser, H.-R., Müller, M.D. and Poiger, T. (2008) Nicotine derivatives in wastewater and surface waters: application as chemical markers for domestic wastewater. Environmental Science & Technology 42(17), 6354-6360.

Burgard, D.A., Banta-Green, C. and Field, J.A. (2014) Working upstream: how far can you go with sewage-based drug epidemiology? Environmental Science & Technology 48(3), 1362-1368.

Burgard, D.A., Fuller, R., Becker, B., Ferrell, R. and Dinglasan-Panlilio, M.J. (2013) Potential trends in Attention Deficit Hyperactivity Disorder (ADHD) drug use on a college campus: Wastewater analysis of amphetamine and ritalinic acid. Science of The Total Environment 450, 242-249.

Byrd, G.D., Chang, K.M., Greene, J.M. and deBethizy, J.D. (1992) Evidence for urinary excretion of glucuronide conjugates of nicotine, cotinine, and trans-3'-hydroxycotinine in smokers. Drug Metab Dispos 20(2), 192-197.

Castiglioni, S., Bagnati, R., Melis, M., Panawennage, D., Chiarelli, P., Fanelli, R. and Zuccato, E. (2011) Identification of cocaine and its metabolites in urban wastewater and comparison with the human excretion profile in urine. Water Res 45(16), 5141-5150.

Castiglioni, S., Bijlsma, L., Covaci, A., Emke, E., Hernandez, F., Reid, M., Ort, C., Thomas, K.V., van Nuijs, A.L.N., de Voogt, P. and Zuccato, E. (2013) Evaluation of Uncertainties Associated with the Determination of Community Drug Use through the Measurement of Sewage Drug Biomarkers. Environmental Science & Technology 47(3), 1452-1460.

Castiglioni, S., Senta, I., Borsotti, A., Davoli, E. and Zuccato, E. (2015) A novel approach for monitoring tobacco use in local communities by wastewater analysis. Tob Control 24(1), 38-42.

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

Charles-Edwards, E. (2016) Demography for Planning and Policy: Australian Case Studies. Wilson, T., Charles-Edwards, E. and Bell, M. (eds), pp. 31-49, Springer International Publishing, Cham.

Charles-Edwards, E. and Bell, M. (2012) Estimating the Service Population of a Large Metropolitan University Campus. Applied Spatial Analysis and Policy 6(3), 209-228.

Chen, C., Kostakis, C., Gerber, J.P., Tscharke, B.J., Irvine, R.J. and White, J.M. (2013) Towards finding a population biomarker for wastewater epidemiology studies. The Science of the total environment.

Chen, C., Kostakis, C., Irvine, R.J., Felgate, P.D. and White, J.M. (2012a) Evaluation of pre-analysis loss of dependent drugs in wastewater: stability and binding assessments. Drug Testing and Analysis 5(8), 6.

Chen, H., Li, X. and Zhu, S. (2012b) Occurrence and distribution of selected pharmaceuticals and personal care products in aquatic environments: a comparative

study of regions in China with different urbanization levels. Environmental Science and Pollution Research 19(6), 2381-2389.

Chiaia, A.C., Banta-Green, C. and Field, J. (2008) Eliminating Solid Phase Extraction with Large-Volume Injection LC/MS/MS: Analysis of Illicit and Legal Drugs and Human Urine Indicators in US Wastewaters. Environmental Science & Technology 42(23), 8841-8848.

Cone, E.J., Sampson-Cone, A.H., Darwin, W.D., Huestis, M.A. and Oyler, J.M. (2003) Urine Testing for Cocaine Abuse: Metabolic and Excretion Patterns following Different Routes of Administration and Methods for Detection of False-Negative Results. Journal of Analytical Toxicology 27(7), 386-401.

Cox, E.R., Motheral, B.R., Henderson, R.R. and Mager, D. (2003) Geographic Variation in the Prevalence of Stimulant Medication Use Among Children 5 to 14 Years Old: Results From a Commercially Insured US Sample. Pediatrics 111(2), 237-243.

Dal Pozzo, A., Donzelli, G., Rodriquez, L. and Tajana, A. (1989) “In vitro” model for the evaluation of drug distribution and plasma protein-binding relationships. International Journal of Pharmaceutics 50(2), 97-101.

Dalgaard, L. and Larsen, C. (1999) Metabolism and excretion of citalpram in man: identification of O-acyl- and N-glucuronides. Xenobiotica (29), 1033-1041.

Daughton, C.G. (2001) Pharmaceuticals and Care Products in the Environment, pp. 348-364, American Chemical Society.

Daughton, C.G. (2011) Reviews of Environmental Contamination and Toxicology, Vol 210. Whitacre, D.M. (ed), pp. 59-110, Springer, New York.

Daughton, C.G. (2012a) Real-time estimation of small-area populations with human biomarkers in sewage. Science of The Total Environment 414(0), 6-21.

Daughton, C.G. (2012b) Using biomarkers in sewage to monitor community-wide human health: Isoprostanes as conceptual prototype. Science of The Total Environment 424(0), 16-38.

Daughton, C.G. and Ruhoy, I.S. (2009) Environmental footprint of pharmaceuticals: The significance of factors beyond direct excretion to sewers. Environmental Toxicology and Chemistry 28(12), 2495-2521.

Davis, M., Labadarios, D. and Willians, R.S. (1976) Metabolism of paracetamol after therapeutic and hepatotoxic doese in man. J. Int.Med.Res. (4), 40-45.

de Leon, M.P., Roncucci, L., di Donato, P., Sacchetti, C., Pezcoller, C., Annoni, C., Bertani, C., Rebecchi, P., Balli, F., Galli, D. and Carulli, N. (1987) Fecal Neutral Steroids in Normal Conditions and in Patients with Polyps or Cancer of the Large Bowel. Cancer Research 47(1), 305-310.

Dishaw, L.V., Powers, C.M., Ryde, I.T., Roberts, S.C., Seidler, F.J., Slotkin, T.A. and Stapleton, H.M. (2011) Is the PentaBDE replacement, tris (1,3-dichloro-2-propyl) phosphate (TDCPP), a developmental neurotoxicant? Studies in PC12 cells. Toxicology and Applied Pharmacology 256(3), 281-289.

Du, P., Li, K., Li, J., Xu, Z., Fu, X., Yang, J., Zhang, H. and Li, X. (2015) Methamphetamine and ketamine use in major Chinese cities, a nationwide reconnaissance through sewage-based epidemiology. Water Research 84, 76-84.

Duan, Y.P., Meng, X.Z., Wen, Z.H. and Chen, L. (2013) Acidic pharmaceuticals in domestic wastewater and receiving water from hyper-urbanization city of China (Shanghai): Environmental release and ecological risk. Environmental Science and Pollution Research 20(1), 108-116.

Editorial (2009) Estimating population now an impossible task, Ocean City Today, Maryland: Ocean City.

EMCDDA, E.M.C.f.D.a.D.A. (2016) Wastewater analysis and drugs — a European multi-city study, p. 7, Lisbon.

Environmental Protection Agency (2010) Non-confidential 2006 IUR Records by Chemical, including Manufacturing, Processing and Use Information, US Environmental Protection Agency.

EPA, U.S. (2012) Estimation Program Interface (EPI) Suite Version 4.11.

Evans, M., Jr., Ndetan, H., Perko, M., Williams, R. and Walker, C. (2012) Dietary Supplement Use by Children and Adolescents in the United States to Enhance Sport Performance: Results of the National Health Interview Survey. The Journal of Primary Prevention 33(1), 3-12.

Gao, J., O'Brien, J., Du, P., Li, X., Ort, C., Mueller, J.F. and Thai, P.K. (2016) Measuring selected PPCPs in wastewater to estimate the population in different cities in China. The Science of the total environment 568, 164-170.

Gao, J., O'Brien, J., Lai, F.Y., van Nuijs, A.L., He, J., Mueller, J.F., Xu, J. and Thai, P.K. (2015) Could wastewater analysis be a useful tool for China? - A review. J Environ Sci (China) 27C, 70-79.

Garde, A.H., Hansen, Å.M. and Kristiansen, J. (2003) Evaluation, including effects of storage and repeated freezing and thawing, of a method for measurement of urinary creatinine. Scandinavian Journal of Clinical and Laboratory Investigation 63(7-8), 521-524.

Goldschmidt, P.G. and Dahl, A.W. (1976) Demoflush Estimating Population in Seasonal Resort Communities. Growth and Change 7(2), 44-48.

Government, A. Climate Data Online. Meterology, B.o. (ed).

Grabic, R., Fick, J., Lindberg, R.H., Fedorova, G. and Tysklind, M. (2012) Multi-residue method for trace level determination of pharmaceuticals in environmental

samples using liquid chromatography coupled to triple quadrupole mass spectrometry. Talanta 100, 183-195.

Guisasola, A., de Haas, D., Keller, J. and Yuan, Z. (2008) Methane formation in sewer systems. Water Res 42(6-7), 1421-1430.

Gurke, R., Rossler, M., Marx, C., Diamond, S., Schubert, S., Oertel, R. and Fauler, J. (2015) Occurrence and removal of frequently prescribed pharmaceuticals and corresponding metabolites in wastewater of a sewage treatment plant. The Science of the total environment 532, 762-770.

Gutierrez, O., Mohanakrishnan, J., Sharma, K.R., Meyer, R.L., Keller, J. and Yuan, Z. (2008) Evaluation of oxygen injection as a means of controlling sulfide production in a sewer system. Water Res 42(17), 4549-4561.

Hansch, C., Leo, A. and Hoekman, D. (1995) Exploring QSAR. Hydrophobic, Electronic, and Steric Constants. , American Chemical Society, Washington DC.

Harman, C., Reid, M. and Thomas, K.V. (2011) In Situ Calibration of a Passive Sampling Device for Selected Illicit Drugs and Their Metabolites in Wastewater, And Subsequent Year-Long Assessment of Community Drug Usage. Environmental Science & Technology 45(13), 5676-5682.

Hickey, S., McIlwraith, F. and Alati, R. (2011) Queensland trends in ecstasy and related drug markets 2014 Findings from the Ecstasy and Related Drugs Reporting System (EDRS) Australian Drug Trend Series No. 144.

Hodges, J.E.N., Holmes, C.M., Vamshi, R., Mao, D. and Price, O.R. (2012) Estimating chemical emissions from home and personal care products in China. Environmental Pollution 165, 199-207.

Hopper, J.W., Su, Z., Looby, A.R., Ryan, E.T., Penetar, D.M., Palmer, C.M. and Lukas, S.E. (2006) Incidence and patterns of polydrug use and craving for ecstasy in regular ecstasy users: An ecological momentary assessment study. Drug and Alcohol Dependence 85(3), 221-235.

Hukkanen, J., Jacob, P. and Benowitz, N.L. (2005) Metabolism and Disposition Kinetics of Nicotine. Pharmacological Reviews 57(1), 79-115.

Hvitved-Jacobsen, T., Vollertsen, J. and Nielsen, A.H. (2013) Sewer processes: microbial and chemical process engineering of sewer networks, CRC press.

Jelic, A., Rodriguez-Mozaz, S., Barcelo, D. and Gutierrez, O. (2014) Impact of in-sewer transformation on 43 pharmaceuticals in a pressurized sewer under anaerobic conditions. Water Res 68C, 98-108.

Jernal, M., Ouyang, Z., Chen, B.C. and Teitz, D. (1999) Quantitation of the acid and lactone forms of artorvastatin and its biotransformation products in human serum by high-performance liquid chromatography with electrospray tandem mass spectrometry. Rap. Comm. Mass Spect (13), 1003-1015.

Jiang, G., Gutierrez, O., Sharma, K.R., Keller, J. and Yuan, Z. (2011a) Optimization of intermittent, simultaneous dosage of nitrite and hydrochloric acid to control sulfide and methane productions in sewers. Water Res 45(18), 6163-6172.

Jiang, G., Gutierrez, O., Sharma, K.R. and Yuan, Z. (2010) Effects of nitrite concentration and exposure time on sulfide and methane production in sewer systems. Water Res 44(14), 4241-4251.

Jiang, G., Gutierrez, O. and Yuan, Z. (2011b) The strong biocidal effect of free nitrous acid on anaerobic sewer biofilms. Water Research 45(12), 3735-3743.

Jiang, G., Sharma, K.R., Guisasola, A., Keller, J. and Yuan, Z. (2009) Sulfur transformation in rising main sewers receiving nitrate dosage. Water Res 43(17), 4430-4440.

Jiang, G., Sharma, K.R. and Yuan, Z. (2013) Effects of nitrate dosing on methanogenic activity in a sulfide-producing sewer biofilm reactor. Water Res 47(5), 1783-1792.

Jones-Otazo, H.A., Clarke, J.P., Diamond, M.L., Archbold, J.A., Ferguson, G., Harner, T., Richardson, G.M., Ryan, J.J. and Wilford, B. (2005) Is House Dust the Missing Exposure Pathway for PBDEs? An Analysis of the Urban Fate and Human Exposure to PBDEs. Environmental Science & Technology 39(14), 5121-5130.

Jones, H.E., Hickman, M., Kasprzyk-Hordern, B., Welton, N.J., Baker, D.R. and Ades, A.E. (2014) Illicit and pharmaceutical drug consumption estimated via wastewater analysis. Part B: Placing back-calculations in a formal statistical framework. The Science of the total environment 487.

Kasprzyk-Hordern, B., Dinsdale, R.M. and Guwy, A.J. (2010) The removal of pharmaceuticals, personal care products, endocrine disruptors and illicit drugs during wastewater treatment and its impact on the quality of receiving waters (vol 43, pg 363, 2009). Water Research 44(6), 2076-2076.

Khan, U. and Nicell, J.A. (2011) Refined sewer epidemiology mass balances and their application to heroin, cocaine and ecstasy. Environment International 37(7), 1236-1252.

Khan, U. and Nicell, J.A. (2012) Sewer epidemiology mass balances for assessing the illicit use of methamphetamine, amphetamine and tetrahydrocannabinol. Science of The Total Environment 421, 144-162.

Khan, U., van Nuijs, A.L.N., Li, J., Maho, W., Du, P., Li, K., Hou, L., Zhang, J., Meng, X., Li, X. and Covaci, A. (2014) Application of a sewage-based approach to assess the use of ten illicit drugs in four Chinese megacities. Science of The Total Environment 487, 710-721.

Kormos, J.L., Schulz, M. and Ternes, T.A. (2011) Occurrence of Iodinated X-ray Contrast Media and Their Biotransformation Products in the Urban Water Cycle. Environmental Science & Technology 45(20), 8723-8732.

Lai, F.Y. (2013) Measuring population illicit drug use through wastewater analysis: temporal trends, spatial differences and specific events.

Lai, F.Y., Anuj, S., Bruno, R., Carter, S., Gartner, C., Hall, W., Kirkbride, K.P., Mueller, J.F., O'Brien, J.W., Prichard, J., Thai, P.K. and Ort, C. (2015) Systematic and Day-to-Day Effects of Chemical-Derived Population Estimates on Wastewater-Based Drug Epidemiology. Environ Sci Technol 49(2), 10.

Lai, F.Y., Bruno, R., Hall, W., Gartner, C., Ort, C., Kirkbride, P., Prichard, J., Thai, P.K., Carter, S. and Mueller, J.F. (2013a) Profiles of illicit drug use during annual key holiday and control periods in Australia: wastewater analysis in an urban, a semi-rural and a vacation area. Addiction 108(3), 556-565.

Lai, F.Y., Bruno, R., Leung, H.W., Thai, P.K., Ort, C., Carter, S., Thompson, K., Lam, P.K. and Mueller, J.F. (2013b) Estimating daily and diurnal variations of illicit drug use in Hong Kong: a pilot study of using wastewater analysis in an Asian metropolitan city. Forensic Sci Int 233(1-3), 126-132.

Lai, F.Y., O'Brien, J., Bruno, R., Hall, W., Prichard, J., Kirkbride, P., Gartner, C., Thai, P., Carter, S., Lloyd, B., Burns, L. and Mueller, J. (2016a) Spatial variations in the consumption of illicit stimulant drugs across Australia: A nationwide application of wastewater-based epidemiology. Science of The Total Environment.

Lai, F.Y., O'Brien, J., Thai, P.K., Hall, W.D. and Mueller, J. (2016b) Trends in methamphetamine residues in wastewater in metropolitan and regional cities in south-east Queensland, 2009-2015. Med J Aust 204(4), 151-152.

Lai, F.Y., O'Brien, J.W., Thai, P.K., Hall, W., Chan, G., Bruno, R., Ort, C., Prichard, J., Carter, S., Anuj, S., Kirkbride, K.P., Gartner, C., Humphries, M. and Mueller, J.F. (2016c) Cocaine, MDMA and methamphetamine residues in wastewater: Consumption trends (2009-2015) in South East Queensland, Australia. The Science of the total environment.

Lai, F.Y., Ort, C., Gartner, C., Carter, S., Prichard, J., Kirkbride, P., Bruno, R., Hall, W., Eaglesham, G. and Mueller, J.F. (2011) Refining the estimation of illicit drug consumptions from wastewater analysis: Co-analysis of prescription pharmaceuticals and uncertainty assessment. Water Research 45(15), 4437-4448.

Lai, F.Y., Thai, P.K., O'Brien, J., Gartner, C., Bruno, R., Kele, B., Ort, C., Prichard, J., Kirkbride, P., Hall, W., Carter, S. and Mueller, J.F. (2013c) Using quantitative wastewater analysis to measure daily usage of conventional and emerging illicit drugs at an annual music festival. Drug and Alcohol Review 32(6), 594-602.

Lee, H.B., Peart, T.E. and Svoboda, M.L. (2005) Determination of endocrine-disrupting phenols, acidic pharmaceuticals, and personal-care products in sewage by solid-phase extraction and gas chromatography-mass spectrometry. Journal of chromatography. A 1094(1-2), 122-129.

Lehmann, B.V., Wan, S.H., Riegelman, S. and Becker, C. (1973) Renal contribution to overall metabolism of drugs IV: biotransformation of salicylic acid to salicyluric acid in man. J. Pharmaceut. Sci. (62), 1483-1486.

Lemberger, L., Bergstrom, R.F. and al., R.L.W.e. (1985) Fluoxetine: clinical pharmacology and physiologic disposition J. Clin. Psychiat (46), 14-19.

Li, J., Hou, L., Du, P., Yang, J., Li, K., Xu, Z., Wang, C., Zhang, H. and Li, X. (2014) Estimation of amphetamine and methamphetamine uses in Beijing through sewage-based analysis. Science of The Total Environment 490, 724-732.

Lindberg, R.H., Wennberg, P., Johansson, M.I., Tysklind, M. and Andersson, B.A.V. (2005) Screening of human antibiotic substances and determination of weekly mass flows in five sewage treatment plants in Sweden. Environmental Science & Technology 39(10), 3421-3429.

Lintz, W., Erlacin, S., Frankus, E. and Uragg, H. (1981) Metabolismus von Tramadol bei Mensch und Tier. Arz. Forsch. (31), 1932-1943.

Liu, J.L. and Wong, M.H. (2013) Pharmaceuticals and personal care products (PPCPs): a review on environmental contamination in China. Environ Int 59, 208-224.

Liu, X., Ji, K. and Choi, K. (2012) Endocrine disruption potentials of organophosphate flame retardants and related mechanisms in H295R and MVLN cell lines and in zebrafish. Aquatic Toxicology 114–115(0), 173-181.

Loos, R., Negrão, R., Comero, S., Conduto, A., Ghiani, M., Lettieri, T., Locoro, G., Paracchini, B., Tavazzi, S. and Gawlik, B. (2012) EU wide monitoring survey on waste water treatment plant effluents. Institute for Environment and Sustainability, Luxembourg.

Luo, Y., Guo, W., Ngo, H.H., Nghiem, L.D., Hai, F.I., Zhang, J., Liang, S. and Wang, X.C. (2014) A review on the occurrence of micropollutants in the aquatic environment and their fate and removal during wastewater treatment. The Science of the total environment 473-474, 619-641.

Mao, W., Vu, H., Xie, Z., Chen, W. and Tang, S. (2015) Systematic Review on Irrational Use of Medicines in China and Vietnam. PloS one 10(3), e0117710.

Marklund, A., Andersson, B. and Haglund, P. (2003) Screening of organophosphorus compounds and their distribution in various indoor environments. Chemosphere 53(9), 1137-1146.

Marklund, A., Andersson, B. and Haglund, P. (2005a) Organophosphorus flame retardants and plasticizers in air from various indoor environments. Journal of Environmental Monitoring 7(8), 814-819.

Marklund, A., Andersson, B. and Haglund, P. (2005b) Organophosphorus flame retardants and plasticizers in Swedish sewage treatment plants. Environmental Science & Technology 39(19), 7423-7429.

Martin-Tereso, J., Derks, M., van Laar, H., Mulder, K., den Hartog, L.A. and Verstegen, M.W.A. (2010) Urinary calcium excretion in non-lactating dairy cows in

relation to intake of fat-coated rice bran. Journal of Animal Physiology and Animal Nutrition 94(1), 129-136.

McCall, A.-K., Bade, R., Kinyua, J., Lai, F.Y., Thai, P.K., Covaci, A., Bijlsma, L., van Nuijs, A.L.N. and Ort, C. (2015) Critical review on the stability of illicit drugs in sewers and wastewater samples. Water Research 88, 14.

McIlwraith, F., Belovic, T., Hickey, S. and Alati, R. (2012a) Queensland trends in ecstasy and related drug markets 2011: findings from the Ecstasy and Related Drugs Reporting System (EDRS).

McIlwraith, F., Hickey, S. and Alati, R. (2012b) Queensland drug trends 2011: findings from the Illicit Drug Reporting System (IDRS), NDARC.

Meeker, J.D. and Stapleton, H.M. (2010) House Dust Concentrations of Organophosphate Flame Retardants in Relation to Hormone Levels and Semen Quality Parameters. Environmental Health Perspectives 118(3), 318-323.

Metcalfe, C., Tindale, K., Li, H., Rodayan, A. and Yargeau, V. (2010) Illicit drugs in Canadian municipal wastewater and estimates of community drug use. Environmental Pollution 158(10), 3179-3185.

Meyer, J. and Bester, K. (2004) Organophosphate flame retardants and plasticisers in wastewater treatment plants. Journal of Environmental Monitoring 6(7), 599-605.

Mills, R.F.N., Adams, S.S. and al, E.E.C.e. (1973) The metabolism of ibuprofen. Xenobiotica (3), 589-598.

Mohanakrishnan, J., Gutierrez, O., Sharma, K.R., Guisasola, A., Werner, U., Meyer, R.L., Keller, J. and Yuan, Z. (2009) Impact of nitrate addition on biofilm properties and activities in rising main sewers. Water Res 43(17), 4225-4237.

Morselli, P.L. and Frigerio, A. (1975) Metabolism and pharmacokinetics of carbamazepine. Drug Metab. Rev. (4), 97-113.

Muth, E.A., Moyer, J.A. and al, J.T.H.e. (1991) Biochemical, neurophysiological, and behaviorial effects of WY-45,233, its enantiomers, and other identified metabolits of the antidepressant venlafaxine. Drug Dev. Res. (23), 191-199.

NICNAS (2001) Trisphosphates - Priority Existing Chemical Assessment Report No. 17, Sydney.

NICNAS (2014) Priority Existing Chemical (PEC) Assessments, Sydney.

O'Brien, J.W., Thai, P.K., Eaglesham, G., Ort, C., Scheidegger, A., Carter, S., Lai, F.Y. and Mueller, J.F. (2014) A model to estimate the population contributing to the wastewater using samples collected on census day. Environmental Science & Technology 48(1), 517-525.

OECD (2008) Test No. 314: Simulation Tests to Assess the Biodegradability of Chemicals Discharged in Wastewater, OECD Publishing.

Olofsson, U. (2012) Removal processes in sewage treatment plants-Sludge quality and treatment efficiency of structurally diverse organic compounds.

Ort, C., Eppler, J.M., Scheidegger, A., Rieckermann, J., Kinzig, M. and Sörgel, F. (2014a) Challenges of surveying wastewater drug loads of small populations and generalizable aspects on optimizing monitoring design. Addiction 109(3), 472-481.

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

Ort, C., Lawrence, M.G., Rieckermann, J. and Joss, A. (2010b) Sampling for Pharmaceuticals and Personal Care Products (PPCPs) and Illicit Drugs in Wastewater Systems: Are Your Conclusions Valid? A Critical Review. Environmental Science & Technology 44(16), 6024-6035.

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. and Thomas, K.V. (2014b) Spatial differences and temporal changes in illicit drug use in Europe quantified by wastewater analysis. Addiction 109(8), 1338-1352.

Peng, X., Yu, Y., Tang, C., Tan, J., Huang, Q. and Wang, Z. (2008) Occurrence of steroid estrogens, endocrine-disrupting phenols, and acid pharmaceutical residues in urban riverine water of the Pearl River Delta, South China. Science of The Total Environment 397(1–3), 158-166.

Phillips, P. and Chalmers, A. (2009) Wastewater effluent, combined sewer overflows, and other sources of organic compounds to Lake Champlain. Journal of the American Water Resources Association 45(1), 45-57.

Plosz, B.G., Reid, M.J., Borup, M., Langford, K.H. and Thomas, K.V. (2013) Biotransformation kinetics and sorption of cocaine and its metabolites and the factors influencing their estimation in wastewater. Water Res 47(7), 2129-2140.

Postigo, C., de Alda, M.J.L. and Barcelo, D. (2010) Drugs of abuse and their metabolites in the Ebro River basin: Occurrence in sewage and surface water, sewage treatment plants removal efficiency, and collective drug usage estimation. Environment International 36(1), 75-84.

Postigo, C., de Alda, M.L. and Barcelo, D. (2011) Evaluation of drugs of abuse use and trends in a prison through wastewater analysis. Environ Int 37(1), 49-55.

Prichard, J., Lai, F.Y., Kirkbride, P., Bruno, R., Ort, C., Carter, S., Hall, W., Gartner, C., Thai, P.K. and Mueller, J.F. (2012) Measuring drug use patterns n Queensland through wastewater analysis. Trends and Issues in Crime and Criminal Justice (442), 1.

R Core Team (2012) R: A language and environment for statistical computing., R Foundation for Statistical Computing, Vienna, Austria.

R.Wise (1984) Norfloxacin- a review of pharmacology and tissue penetration. J. Antimicrob. Chemother. (13 (Suppl. B)), 59-64.

Radjenovic, J., Petrovic, M. and Barcelo, D. (2007) Analysis of pharmaceuticals in wastewater and removal using a membrane bioreactor. Anal Bioanal Chem 387(4), 1365-1377.

Radjenovic, J., Petrovic, M. and Barcelo, D. (2009) Fate and distribution of pharmaceuticals in wastewater and sewage sludge of the conventional activated sludge (CAS) and advanced membrane bioreactor (MBR) treatment. Water Res 43(3), 831-841.

Ran, B., Qiu, Z., Qiu, Y., Lu, J., Xue, M. and Dong, Z. (2013) Using mobile phone location data in urban planning practice with big data environment. City era, Collaborative Planning—2013 Urban Planning Summit, China, Beijing.

Randall, C. and Baselt, R. (2004) Disposition of toxic drugs and chemicals in man, Biomedical Publications, sixth edition 2002. 19-Code de la santé publique. Livre sixième. Lutte contre le dopage. Dalloz. Art. L 3611-1 à Art. L 3534.

Ratola, N., Cincinelli, A., Alves, A. and Katsoyiannis, A. (2012) Occurrence of organic microcontaminants in the wastewater treatment process. A mini review. Journal of Hazardous Materials 239–240(0), 1-18.

Reemtsma, T., Quintana, J.B., Rodil, R., Garcı´a-López, M. and Rodrı´guez, I. (2008) Organophosphorus flame retardants and plasticizers in water and air I. Occurrence and fate. TrAC Trends in Analytical Chemistry 27(9), 727-737.

Reeves, P.R., McAinsh, J., McIntosh, D.A.D. and Winrow, M.J. (1978) Matabolism of atenolol in man. Xenobiotica (8), 313-320.

Reid, M.J., Langford, K.H., Morland, J. and Thomas, K.V. (2011) Analysis and interpretation of specific ethanol metabolites, ethyl sulfate, and ethyl glucuronide in sewage effluent for the quantitative measurement of regional alcohol consumption. Alcoholism, clinical and experimental research 35(9), 1593-1599.

Renaud, A. (2007) Estimation of the coverage of the 2000 census of population in Switzerland: Methods and results. Survey Methodology 33(2), 12.

Rodayan, A., Majewsky, M. and Yargeau, V. (2014) Impact of approach used to determine removal levels of drugs of abuse during wastewater treatment. The Science of the total environment 487, 731-739.

Russo, B. (2009) Questions surround ocean city demoflush estimates., Maryland Coast Dispatch, Inc., Berlin, MD.

Ryu, J., Oh, J., Snyder, S.A. and Yoon, Y. (2014) Determination of micropollutants in combined sewer overflows and their removal in a wastewater treatment plant (Seoul, South Korea). Environ Monit Assess 186(5), 3239-3251.

Sangster, J. (1993) LOGK_{OW} Databank Sangster Research Labs, Montreal, Canada.

Sangster, J. (1994) LOGKOW Databank (a databank of evaluated octanol-water partition coefficeints on microcomputer diskette). , Sangster Research Laboratories, Montreal, Quebec, Canada.

Schreder, E.D. and La Guardia, M.J. (2014) Flame Retardant Transfers from U.S. Households (Dust and Laundry Wastewater) to the Aquatic Environment. Environmental Science & Technology.

Schroder, H.F., Gebhardt, W. and Thevis, M. (2010) Anabolic, doping, and lifestyle drugs, and selected metabolites in wastewater--detection, quantification, and behaviour monitored by high-resolution MS and MS(n) before and after sewage treatment. Anal Bioanal Chem 398(3), 1207-1229.

Schwarz, H.J. (1979) Pharmacokinetics and metabolism of temazepam in man and several animal species. Brit. J. Clin. Pharmacol. (8), 23S-29S.

Segre, E.J. (1975) Naproxen metabolism in man. J. Clin. Pharmacol. (15), 316-323.

Seiler, R.L., Zaugg, S.D., Thomas, J.M. and Howcroft, D.L. (1999) Caffeine and pharmaceuticals as indicators of waste water contamination in wells. Ground Water 37(3), 405-410.

Senta, I., Gracia-Lor, E., Borsotti, A., Zuccato, E. and Castiglioni, S. (2015) Wastewater analysis to monitor use of caffeine and nicotine and evaluation of their metabolites as biomarkers for population size assessment. Water Res 74C, 23-33.

Shimizu, S., Kim, J.M., Shinmen, Y. and Yamada, H. (1986) Evaluation of two alternative metabolic pathways for creatinine degradation in microorganisms. Archives of Microbiology 145(4), 322-328.

Sim, W.-J., Lee, J.-W. and Oh, J.-E. (2010) Occurrence and fate of pharmaceuticals in wastewater treatment plants and rivers in Korea. Environmental Pollution 158(5), 1938-1947.

Sindicich, N.a.B., L. (2013) Australian Trends in Ecstasy and related Drug Markets 2011. Findings from the Ecstasy and Related Drugs Reporting System (EDRS). p. 182, National Drug and Alcohol Research Centre, University of New South Wales, Sydney.

Stapleton, H.M., Klosterhaus, S., Eagle, S., Fuh, J., Meeker, J.D., Blum, A. and Webster, T.F. (2009) Detection of Organophosphate Flame Retardants in Furniture Foam and U.S. House Dust. Environmental Science & Technology 43(19), 7490-7495.

Sui, Q., Huang, J., Deng, S., Yu, G. and Fan, Q. (2010) Occurrence and removal of pharmaceuticals, caffeine and DEET in wastewater treatment plants of Beijing, China. Water Research 44(2), 417-426.

Sui, Q., Wang, D., Zhao, W., Huang, J., Yu, G., Cao, X., Qiu, Z. and Lu, S. (2015) Pharmaceuticals and consumer products in four wastewater treatment plants in

urban and suburb areas of Shanghai. Environmental Science and Pollution Research 22(8), 6086-6094.

Sun, Q., Li, M., Ma, C., Chen, X., Xie, X. and Yu, C.-P. (2016) Seasonal and spatial variations of PPCP occurrence, removal and mass loading in three wastewater treatment plants located in different urbanization areas in Xiamen, China. Environmental Pollution 208, Part B, 371-381.

Takada, H. and Eganhouse, R.P. (1998) Molecular markers of anthropogenic waste, pp. 2883-2940, John Wiley & Sons: New York.

Ternes, T.A. (1998) Occurrence of drugs in German sewage treatment plants and rivers. Water Research 32(11), 3245-3260.

Thai, P.K., Jiang, G., Gernjak, W., Yuan, Z., Lai, F.Y. and Mueller, J.F. (2014a) Effects of sewer conditions on the degradation of selected illicit drug residues in wastewater. Water Res 48, 538-547.

Thai, P.K., Lai, F.Y., Bruno, R., van Dyken, E., Hall, W., O'Brien, J., Prichard, J. and Mueller, J.F. (2016) Refining the excretion factors of methadone and codeine for wastewater analysis - Combining data from pharmacokinetic and wastewater studies. Environ Int 94, 307-314.

Thai, P.K., O'Brien, J., Jiang, G., Gernjak, W., Yuan, Z., Eaglesham, G. and Mueller, J.F. (2014b) Degradability of creatinine under sewer conditions affects its potential to be used as biomarker in sewage epidemiology. Water Res 55, 272-279.

Thomas, K.V., Bijlsma, L., Castiglioni, S., Covaci, A., Emke, E., Grabic, R., Hernández, F., Karolak, S., Kasprzyk-Hordern, B., Lindberg, R.H., Lopez de Alda, M., Meierjohann, A., Ort, C., Pico, Y., Quintana, J.B., Reid, M., Rieckermann, J., Terzic, S., van Nuijs, A.L.N. and de Voogt, P. (2012) Comparing illicit drug use in 19 European cities through sewage analysis. Science of The Total Environment 432, 432-439.

Thomas, K.V. and Reid, M.J. (2011) What Else Can the Analysis of Sewage for Urinary Biomarkers Reveal About Communities? Environmental Science & Technology 45(18), 7611-7612.

Townsend Solutions (2012) 2012 Plastic Additives Global Market Study. Chapter 9 – Flame retardants, p. 657.

U.S. Environmental Protection Agency (2007) Method 1694: Pharmaceuticals and Personal Care Products in Water, Soil, Sediment, and Biosolids by HPLC/MS/MS. Office of Water, O.o.S.a.T., Engineering and Analysis Division (4303T) (ed), p. 77, Washington DC.

United States Environmental Protection Agency (2005) Environmental Profiles of Chemical Flame-Retardant Alternatives for Low-Density Polyurethane Foam.

van den Eede, N., Dirtu, A.C., Neels, H. and Covaci, A. (2011) Analytical developments and preliminary assessment of human exposure to organophosphate flame retardants from indoor dust. Environment International 37(2), 454-461.

Van den Eede, N., Heffernan, A.L., Aylward, L.L., Hobson, P., Neels, H., Mueller, J.F. and Covaci, A. (2014) Age as a determinant of phosphate flame retardant exposure of the Australian population and identification of novel urinary PFR metabolites. Environment International 74, 1-8.

van der Veen, I. and de Boer, J. (2012) Phosphorus flame retardants: properties, production, environmental occurrence, toxicity and analysis. Chemosphere 88(10), 1119-1153.

Van Havere, T., Vanderplasschen, W., Lammertyn, J., Broekaert, E. and Bellis, M. (2011) Drug use and nightlife: more than just dance music. Substance abuse treatment, prevention, and policy 6(1), 1.

van Nuijs, A.L., Gheorghe, A., Jorens, P.G., Maudens, K., Neels, H. and Covaci, A. (2014) Optimization, validation, and the application of liquid chromatography-tandem mass spectrometry for the analysis of new drugs of abuse in wastewater. Drug Test Anal 6(7-8), 861-867.

van Nuijs, A.L., Pecceu, B., Theunis, L., Dubois, N., Charlier, C., Jorens, P.G., Bervoets, L., Blust, R., Meulemans, H., Neels, H. and Covaci, A. (2009a) Can cocaine use be evaluated through analysis of wastewater? A nation-wide approach conducted in Belgium. Addiction 104(5), 734-741.

van Nuijs, A.L., Pecceu, B., Theunis, L., Dubois, N., Charlier, C., Jorens, P.G., Bervoets, L., Blust, R., Neels, H. and Covaci, A. (2009b) Cocaine and metabolites in waste and surface water across Belgium. Environ Pollut 157(1), 123-129.

van Nuijs, A.L.N., Abdellati, K., Bervoets, L., Blust, R., Jorens, P.G., Neels, H. and Covaci, A. (2012) The stability of illicit drugs and metabolites in wastewater, an important issue for sewage epidemiology? Journal of Hazardous Materials 239-240, 19-23.

van Nuijs, A.L.N., Castiglioni, S., Tarcomnicu, I., Postigo, C., de Alda, M.L., Neels, H., Zuccato, E., Barcelo, D. and Covaci, A. (2011a) Illicit drug consumption estimations derived from wastewater analysis: A critical review. Science of The Total Environment 409(19), 3564-3577.

van Nuijs, A.L.N., Mougel, J.F., Tarcomnicu, I., Bervoets, L., Blust, R., Jorens, P.G., Neels, H. and Covaci, A. (2011b) A one year investigation of the occurrence of illicit drugs in wastewater from Brussels, Belgium. Journal of Environmental Monitoring 13(4), 1008-1016.

van Nuijs, A.L.N., Mougel, J.F., Tarcomnicu, I., Bervoets, L., Blust, R., Jorens, P.G., Neels, H. and Covaci, A. (2011c) Sewage epidemiology - A real-time approach to estimate the consumption of illicit drugs in Brussels, Belgium. Environment International 37(3), 612-621.

van Nuijs, A.L.N., Pecceu, B., Theunis, L., Dubois, N., Charlier, C., Jorens, P.G., Bervoets, L., Blust, R., Neels, H. and Covaci, A. (2009c) Spatial and temporal variations in the occurrence of cocaine and benzoylecgonine in waste- and surface water from Belgium and removal during wastewater treatment. Water Research 43(5), 1341-1349.

Vieno, N.M., Tuhkanen, T. and Kronberg, L. (2006) Analysis of neutral and basic pharmaceuticals in sewage treatment plants and in recipient rivers using solid phase extraction and liquid chromatography–tandem mass spectrometry detection. Journal of Chromatography A 1134(1–2), 101-111.

Vollmer, K.O., Hodenberg, A.v. and Kolle, E.V. (1986) Pharmacokinetics and metabolism of gabapentin in rat dog and man. Arz. Forsch. (36), 830-839.

Wang, Z., Zhang, X.-H., Huang, Y. and Wang, H. (2015) Comprehensive evaluation of pharmaceuticals and personal care products (PPCPs) in typical highly urbanized regions across China. Environmental Pollution 204, 223-232.

Wangia, V. and Shireman, T.I. (2013) A review of geographic variation and Geographic Information Systems (GIS) applications in prescription drug use research. Research in Social and Administrative Pharmacy 9(6), 666-687.

Wensing, M., Uhde, E. and Salthammer, T. (2005) Plastics additives in the indoor environment--flame retardants and plasticizers. The Science of the total environment 339(1-3), 19-40.

Whelan, M.J., Hodges, J.E.N., Williams, R.J., Keller, V.D.J., Price, O.R. and Li, M. (2012) Estimating surface water concentrations of “down-the-drain” chemicals in China using a global model. Environmental Pollution 165, 233-240.

Wyss, M. and Kaddurah-Daouk, R. (2000) Creatine and creatinine metabolism. Physiological Reviews 80(3), 1107-1213.

Yamada, H., Shimizu, S., Kim, J.M., Shinmen, Y. and Sakai, T. (1985) A novel metabolic pathway for creatinine degradation in pseudomonas-putida-77. Fems Microbiology Letters 30(3), 337-340.

Yu, Y., Huang, Q., Wang, Z., Zhang, K., Tang, C., Cui, J., Feng, J. and Peng, X. (2011) Occurrence and behavior of pharmaceuticals, steroid hormones, and endocrine-disrupting personal care products in wastewater and the recipient river water of the Pearl River Delta, South China. Journal of Environmental Monitoring 13(4), 871-878.

Zhao, G., Yang, L., Zhou, H., Li, K. and Wu, Z. (2011) Pollution status of pharmaceuticals and personal care productions in a certain sewage plant in Beijing. Environ. Monit. China 27, 63-67.

Zuccato, E., Castiglioni, S., Tettamanti, M., Olandese, R., Bagnati, R., Melis, M. and Fanelli, R. (2011) Changes in illicit drug consumption patterns in 2009 detected by wastewater analysis. Drug Alcohol Depend 118(2-3), 464-469.

Zuccato, E., Chiabrando, C., Castiglioni, S., Bagnati, R. and Fanelli, R. (2008) Estimating community drug abuse by wastewater analysis. Environmental Health Perspectives 116(8), 1027-1032.

Appendix 1: Supplemental Material: Degradability of creatinine under sewer conditions affects its potential to be used as a biomarker in sewage epidemiology

SUPPLEMENTAL MATERIAL

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

Phong K. Thai[a *], Jake O'Brien[a], Guangming Jiang[b], Wolfgang Gernjak[b], Zhiguo Yuan[b], Geoff Eaglesham[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

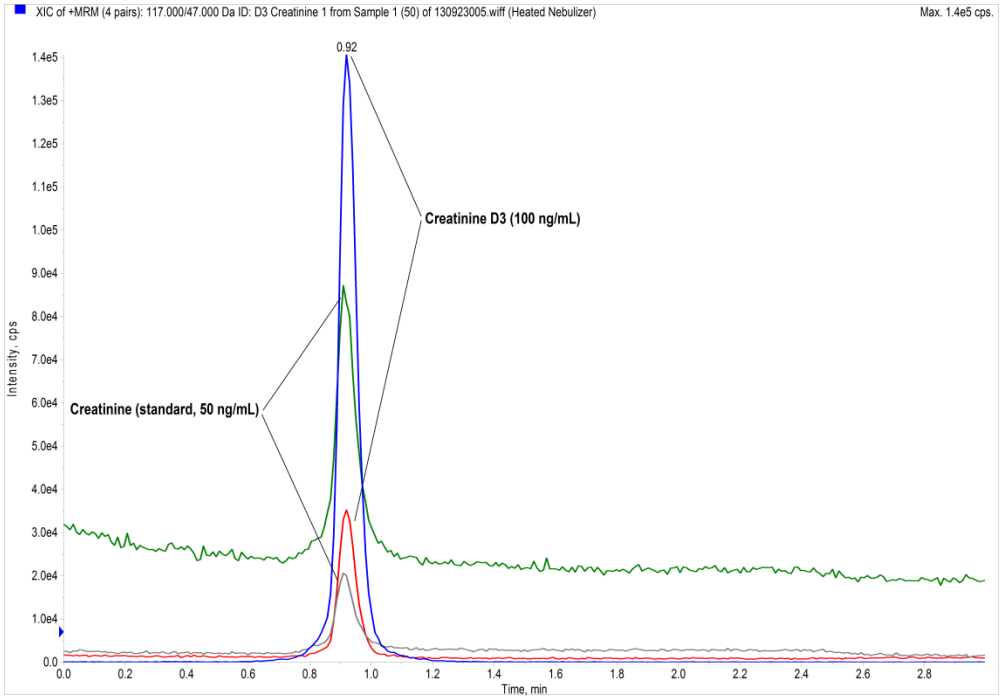
[b]The University of Queensland, Advanced Water Management Centre, St Lucia, QLD 4072, Australia

Table S- 1 Measured and estimated values of creatinine loads in different WWTPs.

WWTP ID	Sampling date	Sampling mode	WWTP estimated population	Census population	Daily flow (ML)	Creatinine load (g/d)	Estimated creatinine excreted (g/d) [a]	Percentage measured (%)[b]
WWTP 1	9/08/2011	Volume proportional	345,000	342,459	80.3	21624	489716	4.42
WWTP 2	10/08/2011	Time proportional	40,000	43,513	10.2	412	62224	0.66
WWTP 3	9/08/2011	Continuous flow proportional	330,000	230,117	57.1	476	329067	0.14
WWTP 4	11/08/2011	Volume proportional	643,000	495,027	127.4	2235	707889	0.32
WWTP 5	10/08/2011	Volume proportional	96,000	92,104	15.6	2901	131709	2.20
WWTP 6	10/08/2011	Volume proportional	100,000	86,882	21.4	19447	124241	15.65
WWTP 7	9/08/2011	Volume proportional	26,600	38,005	4.1	39	54347	0.07
WWTP 8	10/08/2011	Time proportional	3,500	3,682	1.6	11	5265	0.21
WWTP 9	10/08/2011	Time proportional	10,000	8,995	3.8	16	12863	0.12
WWTP 10	11/08/2011	Volume proportional	28,000	25,698	12.6	9875	36748	26.87

[a]Using the average excretion value of 1.1 L of urine/person/day with average creatinine concentration of 1.3 g/L (Brewer et al. 2012)

[b]Percentage of the measured load *vs* the estimated excreted load by method proposed by Brewer et al. (2012) (Brewer et al. 2012).



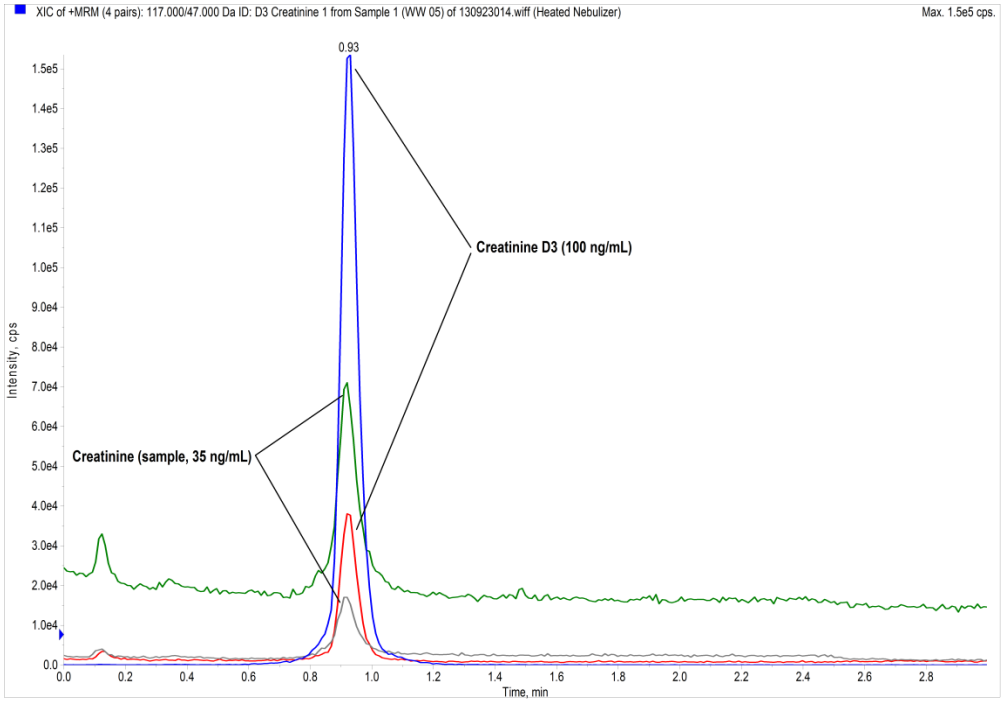


Figure S- 1 Chromatograms of creatinine in a standard and in a real sample.

Appendix 2: Supplemental Information – A model to estimate the population contributing to the wastewater using samples collected on census day

Supporting Information for
A model to estimate the population contributing to the wastewater using samples collected on census day

Jake W. O'Brien*[a], Phong K. Thai[a], Geoff Eaglesham[a], Christoph Ort[b,c], Andreas Scheidegger[c], Steve Carter[d], Foon Yin Lai[a], Jochen F. Mueller[a]

[a]The University of Queensland, The National Research Centre for Environmental Toxicology (Entox), 39 Kessels Road, Coopers Plains, QLD 4108, Australia
[b]The University of Queensland, Advanced Water Management Centre (AWMC), St. Lucia, QLD 4072, Australia
[c]Eawag, Swiss Federal Institute of Aquatic Science and Technology, CH 8600 Dübendorf, Switzerland
[d]Queensland Health Forensic and Scientific Services, 39 Kessels Road, Coopers Plains, QLD 4108, Australia

*j.obrien2@uq.edu.au
* Tel. +61 (0)7 3274 9120, Fax +61 (0)7 3274 9003

Number of pages: 4
Number of figures: 2
Number of tables: 2

SI 1. Total daily wastewater volume versus population

As no rainfall occurred during the sampling period at any of the STPs, the relationship between the total daily volume of wastewater and the census population was assessed with a linear regression (Figure 1). This indicated a strong correlation with an $R^2 = 0.99$ and that each person within a catchment contributes 250 ± 10 L of wastewater per day. However, this method for population estimation is fraught with uncertainties such as the daily variability of water consumption, infiltration/exfiltration of water into/out of the sewer, seasonal variations in water consumption, commercial inputs of wastewater and so on (Editorial 2009, Russo 2009). Discussion with STP operators, however, identified that most of their population estimates are based solely on wastewater volume where a certain volume of water is assigned as a Population Equivalent. Our continuous flow monitoring data from three STPs indicate that wastewater entering the plant during rain events may increase more than 100% and thus this method for assessing population size is not applicable during such events.

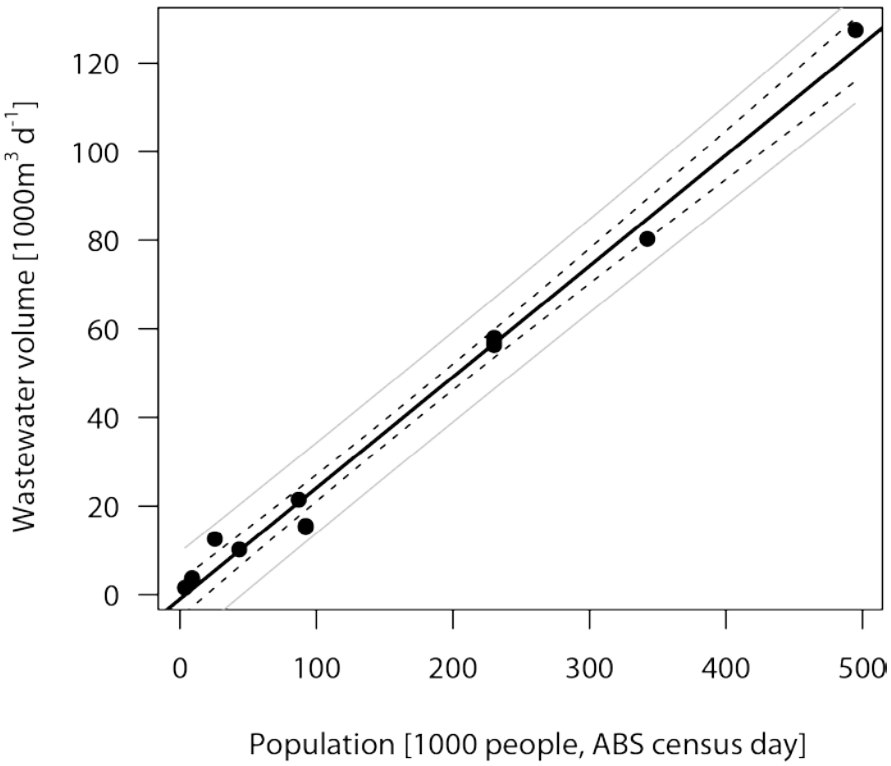


Figure S- 2 Population versus daily wastewater volume for 10 different STPs. The black line is the best fit. Dotted lines represent the 95% confidence interval while continuous grey lines represent the 95% prediction interval.

SI 2. Chemical mass loads for each wastewater catchment

Table S- 2 Chemical mass loads [kg/day] as measured in wastewater samples for each site

STP ID	Date Sampled (D/M/Y)	ABS Census Count	STP Estimate	Chemical Mass Load [kg/day]													
				acesulfame	atenolol	caffeine	carbamaza-epine	codeine	frusemide	gabapentin	hydrochloro-thiazide	ibuprofen	iopromide	naproxen	norfloxacin	paracetamol	salicylic acid
1	10/08/2011	3,682	3,500	0.039428	0.001639	0.055338	0.000233	0.002076	0.001194	0.000468	0.001321	0.002359	0.000753	0.003912	>LOQ	0.267309	0.01939
2	10/08/2011	8,995	10,000	0.049357	0.002168	0.105225	0.000464	0.003291	0.00102	0.010612	0.001553	0.003017	0.000095	0.005254	0.000087	0.358312	0.020684
3	11/08/2011	25,698	28,000	0.146538	0.006955	0.296942	0.002876	0.011609	0.001208	0.084193	0.008862	0.027454	0.002933	0.019417	>LOQ	0.09544	0.098915
4	9/08/2011	38,005	26,600	0.182351	0.006873	0.174698	0.003145	0.00858	0.001619	0.023679	0.005057	0.022425	0.00046	0.009813	0.000857	0.589109	0.082088
5	10/08/2011	43,513	40,000	0.380131	0.012263	0.356932	0.006511	0.021369	0.008245	0.061619	0.010272	0.029262	0.073007	0.017849	0.006816	1.909864	0.1898
6	10/08/2011	86,882	100,000	0.600508	0.043017	1.146587	0.012416	0.043472	0.00328	0.114064	0.024677	0.108056	0.00087	0.045204	0.011923	5.194477	0.355467
6	11/08/2011	86,882	100,000	0.657787	0.043889	1.231807	0.013945	0.042969	0.006051	0.126283	0.026686	0.09267	0.001987	0.050478	0.012604	5.536418	0.36758
7	10/08/2011	92,104	96,000	0.58329	0.028419	0.827786	0.018982	0.029133	0.004335	0.110573	0.020193	0.054892	0.104583	0.025682	0.010298	3.409843	0.341526
7	11/08/2011	92,104	96,000	0.548421	0.045585	0.774905	0.014109	0.038778	0.019386	0.125745	0.021256	0.060503	0.208049	0.022012	0.012997	2.486486	0.279354
8	9/08/2011	230,117	330,000	1.758618	0.087608	1.446619	0.01559	0.084374	0.037025	0.204962	0.054616	0.272773	0.099348	0.116704	0.018165	3.947295	1.715018
8	10/08/2011	230,117	330,000	1.7346	0.079808	1.297264	0.015044	0.080473	0.031916	0.209544	0.056044	0.208183	0.259809	0.109074	0.01958	3.799039	1.339204
8	11/08/2011	230,117	330,000	1.766367	0.082253	1.419766	0.017014	0.089104	0.033944	0.21941	0.060906	0.30746	0.283755	0.12504	0.01647	4.170054	1.348881
9	9/08/2011	342,459	345,000	2.415554	0.048005	4.041362	0.035875	0.197219	0.021713	0.364385	0.078877	0.300027	0.094578	0.162816	0.024872	14.905437	1.260942
10	11/08/2011	495,027	643,000	3.832751	0.153654	7.05338	0.038653	0.175966	0.063137	0.459909	0.086249	0.397188	1.559894 *	0.165895	0.043566	17.267569	2.692283

* measurement considered to be an outlier

LOQ = limit of quantification

SI 3. Population versus daily mass load of iopromide outlier

Linear regressions were plotted for the measured mass loads of all potential chemical population size biomarkers against the population size; it appears that iopromide contains an outlier. Including this outlier changed the slope of the regression from *y = 5.95 e-7* to *y = 2.22e-6* (Figure 2). We therefore decided to exclude this value from our calibration data.

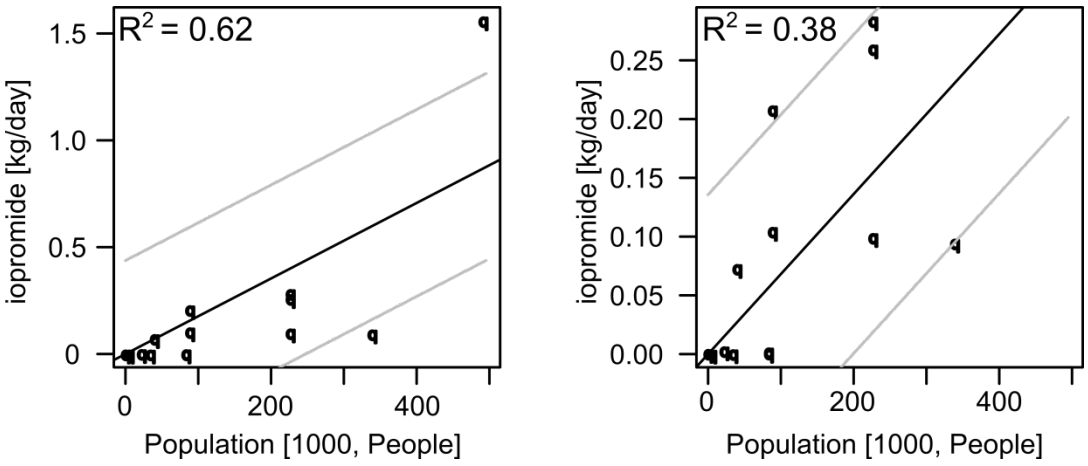


Figure S- 3 Population versus daily mass load of iopromide with and without the outlying value. The black line is the line of best fit. The grey lines indicate the 90% prediction interval.

SI 4. Instrumental parameters for analysis of pharmaceutical and personal care products

Table S- 3 LCMS/MS parameters using an AB/Sciex API 5500Q mass spectrometer (AB/Sciex, Concord, Ontario, Canada) with an electrospray ionization (ESI) interface coupled to a Shimadzu Nexera HPLC system (Shimadzu Corp., Kyoto, Japan). Separation was achieved on a Luna C-18 (2) (3 µm 100 Å LC Column 150 mm x 3 mm, Phenomenex) column using a mobile phase gradient of 1 to 95 % acetonitrile with 0.1% formic acid.

Source	Compound	Retention Time (RT)	Quantitation Ion/Transition					Confirmation Ion/Transition					Supplier
			MRM (Q1 > Q3)	DP	EP	CE	CXP	MRM (Q1 > Q3)	DP	EP	CE	CXP	
ESI +	3,4-Dichloroaniline	7.7	162>127	55	10	30	10	162>74	55	10	70	10	Sigma-Aldrich Australia
	Acetyl-Sulfamethoxazole-d_4	3.4	300.099>138.1	66	10	37	10	300.099>69.1	66	10	67	10	Dr Ehrenstorfer
	Acetyl-Sulfamethoxazole-d_5	3.4	302.3>202.1	60	10	26	9	302.3>138.1	60	10	36	9	Toronto Research Chemicals
	Ametryn	5.85	228.2>186	70	10	28	10	228.2>116	70	10	38	10	Restek
	Atenolol	2.47	267.2>190	60	10	27	9	267.2>145	60	10	38	9	Cerilliant
	Atenolol-d_7	2.47	274.1>145.1	70	10	37	10	274.1>190.1	70	10	28	8	Toronto Research Chemicals
	Atorvastatin	8.85	559.5>440.3	70	10	31	10	559.5>250.2	70	10	62	10	Cerilliant
	Atrazine	7.42	216.1>174	71	10	27	14	216.1>96	71	10	36	12	Restek
	Atrazine-d_5	7.4	221.1>101	60	10	36	6	221.1>179	60	10	27	6	Dr Ehrenstorfer
	Bromacil	6.03	261.2>205	40	10	23	10	263.2>207	40	10	23	10	Restek
	Caffeine	3.09	195.1>138.1	71	10	28	8	195.1>110.1	71	10	32	8	Cerilliant
	Caffeine-d_3	3.08	198.1>138	60	10	27	8	198.1>110	60	10	35	8	CDN Isotopes
	Carbamazepine	6.35	237.2>194	96	10	31	16	237.2>193	96	10	47	12	Cerilliant
	Carbamazepine-d_{10}	6.3	247.2>204.1	65	10	30	8	247.2>202.1	65	10	51	8	CDN Isotopes
	Carbaryl	7.19	202.1>145.1	25	10	13	7	202.1>127.1	25	10	41	7	Restek
	Cephalexin	3.75	348.3>158	45	10	15	10	348.3>140	45	10	38	10	Dr Ehrenstorfer
	Chlorpyriphos	11.56	350.1>198	70	10	27	4	350.1>97	70	10	55	4	Restek
	Ciprofloxacin	2.81	332.3>231.1	61	10	54	12	332.3>288.2	61	10	24	16	Sigma-Aldrich Australia
	Ciprofloxacin-d_8	2.81	340.3>235.2	65	10	55	15	340.3>296.2	65	10	28	20	CDN Isotopes
	Citalopram	4.1	325.3>109	70	10	38	4	325.3>262.2	70	10	28	4	Cerilliant
	Codeine	2.61	300.2>215.1	60	10	37	12	300.2>165.1	60	10	57	10	Cerilliant
	Cyclophosphamide	4.91	261.1>106	70	10	28	10	261.1>120	70	10	33	10	Dr Ehrenstorfer
	Dapsone	4.16	249.2>156	60	10	22	7	249.2>92	60	10	34	14	Dr Ehrenstorfer
	DEET	7.5	192.1>119	86	10	26	10	192.1>91	90	10	44	6	Restek
	Desethyl-Atrazine	4.46	188>146	70	10	26	10	188>104	70	10	37	10	Restek
	Desisopropyl-Atrazine	3.56	174>104	70	10	34	10	174>132	70	10	27	10	Restek
	Desmethyl-Citalopram	4.01	311.3>109	60	10	35	8	311.3>262.2	60	10	25	15	Cerilliant
	Desmethyl-Diazepam	7.05	271.2>140.1	70	10	41	15	271.2>165.1	70	10	41	15	Cerilliant
	Diazepam	8.13	285.2>154.1	76	10	36	12	285.2>193.2	76	10	42	14	Cerilliant
	Diazinon	10.45	305.3>169.1	50	10	35	8	305.3>249.1	50	10	27	8	Restek
	Diclofenac	9.03	296.2>214	40	10	50	10	296.2>250.1	40	10	21	10	Cerilliant
	Diclofenac-d_4	9.01	300.1>219.1	45	10	30	8	300.1>218.1	45	10	46	8	Dr Ehrenstorfer
	Diuron	7.51	235.2>72	70	10	40	10	235.2>46	70	10	38	10	Restek
	Doxylamine	2.74	271.2>182	40	10	24	8	271.2>167.1	40	10	45	7	United States Pharmacopeial Convention
	Erythromycin	4.23	734.7>576.4	50	10	27	8	734.7>158.1	50	10	45	8	Cambridge Isotope Laboratories
	Erythromycin-Hydrate	5	716.7>558.5	66	10	21	18	716.7>158.1	66	10	46	8	Sigma-Aldrich Australia
	Flumeturon	7.25	233.1>72	70	10	38	10	233.1>46	70	10	38	10	Restek
	Fluoxetine	5.41	310.1>44	40	10	42	15	310.1>148	40	10	13	7	Cerilliant
	Fluoxetine-d_5	5.4	315.2>153.1	45	10	14	10	315.2>44	45	10	42	5	Isotec Stable Isotopes
	Gabapentin	2.65	172.1>154	45	10	21	8	172.1>137	45	10	25	8	Cerilliant
	Haloxyfop-Etoyl	11.01	434>316.1	65	10	26	17	434>91	65	10	57	7	Dr Ehrenstorfer
	Haloxyfop-Methly	10.62	376>91	60	10	48	7	376>316.1	60	10	24	17	Accustandard
	Hexazinone	5.73	253.2>171	70	10	24	10	253.2>71	70	10	50	10	Restek
	Ifosfamide	4.71	261.1>92	70	10	37	10	261.1>63	70	10	64	10	Dr Ehrenstorfer
	Indomethacin	9.02	358.3>138.9	66	10	31	10	358.3>75	66	10	107	12	Dr Ehrenstorfer
	Iopromide	2.7	792>573.1	80	10	35	13	792>559.1	80	10	41	13	Dr Ehrenstorfer
	Lincomycin	2.67	407.3>126.1	60	10	44	8	407.3>359.3	60	10	28	20	Dr Ehrenstorfer
	Metolachlor	9.57	284.2>252	76	10	22	18	284.2>176	76	10	38	18	Restek
	Metoprolol	3.07	268.2>116.1	70	10	28	7	268.2>191.1	70	10	27	10	Cerilliant
	Naproxen +ve	7.77	231.2>185.1	64	10	23	10	231.2>170.1	64	10	33	12	Cerilliant
	Norfloxacin	2.78	320.3>276.2	70	10	26	14	320.3>233.2	70	10	35	14	Sigma-Aldrich Australia
	Norfloxacin-d_5	2.78	325.2>281.2	70	10	28	15	325.2>238.2	70	10	36	15	Sigma-Aldrich Australia
	Oxazepam	6.71	287.2>241.2	60	10	32	10	287.2>104	60	10	52	10	Cerilliant
	Oxycodone	2.68	316.2>298.2	65	10	26	16	316.2>241.2	65	10	42	16	Cerilliant
	Paracetamol	2.83	152.1>110	56	10	24	8	152.1>65.1	56	10	42	6	Cerilliant
	Phenytoin	6.37	253.2>182	60	10	26	14	253.2>104	60	10	48	14	Cerilliant
	Praziquantel	7.85	313.3>203.2	70	10	25	10	313.3>55	70	10	72	8	Dr Ehrenstorfer
	Primidone	3.76	219.2>162.1	50	10	18	10	219.2>91	50	10	40	14	Cerilliant
	Prometryn	6.95	242.2>158	70	10	35	10	242.2>200.1	70	10	28	10	Restek
	Propoxur	6.85	210.1>168.1	25	10	12	8	210.1>111	25	10	20	8	Restek
	Propranolol	3.71	260.2>116.1	70	10	28	8	260.2>183.1	70	10	28	8	Cerilliant
	Ranitidine	2.5	315.3>176.1	50	10	26	10	315.3>98.1	50	10	33	10	Cerilliant
	Ranitidine-d_6	2.51	321.2>176.1	55	10	26	8	321.2>130.1	55	10	38	8	Toronto Research Chemicals
	Roxithromycin	5.63	837.6>679.5	10	10	32	9	837.6>158	10	10	52	5	Dr Ehrenstorfer
	Sertraline	5.46	306.3>275.2	35	10	18	12	306.3>159.1	35	10	35	12	Cerilliant
	Simazine	6.28	202.1>132	70	10	29	10	202.1>124	70	10	27	10	Restek
	Simazine-d_{10}	6.2	212>137	60	10	40	10	212>134	60	10	38	10	Dr Ehrenstorfer
	Sulfsalazine	7.07	399.3>223.1	30	10	43	11	399.3>119.1	30	10	63	7	Sigma-Aldrich Australia
	Sulphadiazine	3.16	251.2>92	71	10	37	14	251.2>65	71	10	61	10	Dr Ehrenstorfer
	Sulphamethoxazole	4.6	254.2>92.1	51	10	38	8	254.2>156	51	10	23	8	Cambridge Isotope Laboratories
	Sulphathiazole	3.17	256.2>156.1	51	10	22	10	256.2>92.1	51	10	40	8	Dr Ehrenstorfer
	Tebuthiuron	5.61	229.2>172	70	10	27	10	229.2>116	70	10	40	10	Restek
	Temazepam	7.54	301.2>255.1	55	10	32	8	301.2>283.1	55	10	21	8	Cerilliant
	Temazepam-d_5	7.51	306.2>260.1	56	10	31	14	306.2>288.1	56	10	21	14	Cerilliant
	Terbutryn	6.99	242.2>91.2	46	10	39	6	242.2>71.1	46	10	45	4	Restek
	Tramadol	3.08	264.2>58	45	10	44	8	264.2>42	45	10	125	3	Cerilliant
	Trimethoprim	2.78	291.2>230.1	85	10	35	14	291.2>123.1	85	10	35	8	Dr Ehrenstorfer
	Tylosin	4.66	916.7>174.1	8	10	58	5	916.7>101.1	8	10	72	12	Dr Ehrenstorfer
	Venlafaxine	3.45	278.2>58	45	10	50	7	278.2>121	45	10	40	10	Cerilliant
ESI -	Amoxicilin -ve	1.5	364.1>223	-55	-5	-15	-8	364.1>206	-55	-5	-25	-8	Sigma-Aldrich Australia
	2,4,5-T	8.55	252.9>194.1	-58	-4	-18	-9	254.9>196.1	-58	-4	-18	-9	Restek
	2,4,5-TP	8.8	266.9>194.9	-58	-4	-18	-9	268.9>196.9	-58	-4	-18	-9	Restek
	2,4-D	7.7	219>161	-36	-10	-21	-7	221>163	-36	-10	-22	-7	Restek
	2,4-D 13C6	7.7	225>167	-36	-10	-21	-10	227>169	-36	-10	-21	-10	Restek
	2,4-DB	8.7	247>161	-27	-10	-13	-7	249>163	-27	-10	-13	-7	Restek
	2,4-DP	8.4	233>161	-35	-10	-21	-7	235>163	-35	-10	-21	-7	Restek
	Acesulfame	3.3	162>82	-45	-10	-22	-5	162>78	-45	-10	-45	-5	Dr Ehrenstorfer
	Acesulfame-d_4	3.3	166>86	-45	-10	-22	-5	166>78	-45	-10	-45	-7	Toronto Research Chemicals
	Acetylsalicylic Acid	4.7	178.9>136.9	-35	-10	-9	-11	178.9>92.9	-35	-10	-30	-5	Dr Ehrenstorfer
	Atorvastatin	9.1	557.4>397.2	-70	-10	-39	-16	557.4>278.1	-70	-10	-60	-13	Cerilliant
	Chloramphenicol	5.2	321.9>153	-70	-10	-24	-13	321.9>257.9	-70	-10	-16	-13	Cambridge Isotope Laboratories
	Clopyralid	4	190>146	-47	-4	-13	-6	192>148	-47	-4	-13	-6	Sigma-Aldrich Australia
	Dalapon	3.9	140.9>97	-43	-10	-11	-7	140.9>105	-43	-10	-12	-7	Restek
	DCPA (internal standard)	7.56	205>161	-25	-10	-10	-10	203>159	-25	-10	-10	-7	ChemServices
	Dicamba	6.9	219>175	-25	-10	-10	-7	221>177	-25	-10	-10	-10	Restek
	Diclofenac	9.3	294.1>250.1	-53	-10	-17	-12	294.1>214	-53	-10	-28	-12	Cerilliant
	Diclofenac-d_4	9.3	302.1>258	-50	-10	-16	-8	300.1>256	-50	-10	-16	-8	CDN Isotopes
	Fluroxypyr	6.3	253>195	-35	-10	-17	-10	255>197	-35	-10	-17	-10	Restek
	Fluvastatin	9.1	410.3>348.1	-10	-10	-22	-26	410.3>210.1	-10	-10	-42	-11	United States Pharmacopeial Convention
	Furosemide	6.46	329>285	-57	-10	-21	-13	329>205	-57	-10	-33	-13	Cerilliant
	Gemfibrozol	10.2	249.1>121	-60	-10	-18	-8	249.1>127	-60	-10	-15	-9	Cerilliant
	Haloxyfop	9.6	360>288	-50	-10	-21	-5	362>290	-50	-10	-21	-5	Accustandard
	Hydrochlorothiazide	3.26	296>269	-85	-10	-28	-20	296>205	-85	-10	-34	-12	Cerilliant
	Hydrochlorothiazide-C13d_2	3.24	299>270.1	-85	-10	-28	-12	299>206.1	-85	-10	-35	-12	Toronto Research Chemicals
	Ibuprofen	9.56	205.1>161	-52	-10	-12	-10	205.1>159	-52	-10	-11	-10	Sigma-Aldrich Australia
	Iopromide	2.66	790.001>127	-80	-10	-55	-7	790>127	-80	-10	-55	-7	Dr Ehrenstorfer
	MCPA	7.73	199>141	-45	-10	-22	-7	201>143	-45	-10	-19	-7	Dr Ehrenstorfer
	MCPB	8.73	227>141	-30	-10	-13	-7	229>143	-30	-10	-20	-7	Restek
	Mecoprop	8.4	213>141	-45	-10	-21	-7	215>143	-45	-10	-17	-7	Restek
	Naproxen	8	229.2>185.1	-53	-10	-11	-10	229.2>170.1	-53	-10	-22	-10	Cerilliant
	Picloram	4.4	239>195	-24	-10	-13	-7	241>197	-24	-10	-13	-7	Restek
	Salicylic Acid	5.6	137>93	-45	-10	-24	-6	137>65	-45	-10	-40	-6	Cerilliant
	Triclopyr	8.1	254>196	-35	-10	-19	-7	256>198	-35	-10	-21	-7	Restek
	Triclosan	10.7	287>35	-50	-10	-30	-3	289>35	-50	-10	-30	-3	Sigma-Aldrich Australia
	Warfarin	8.6	307>161	-170	-10	-28	-11	307.01>250	-170	-10	-30	-9	Cerilliant

Notes: MRM = Multiple Reaction Monitoring, DP = Declustering Potential, EP = Entrance Potential, CE = Collision Cell Energy, CXP = Collision Cell Exit Potential
ESI = Electrospray Ionisation

Appendix 3: Supporting Information – Impact of in-sewer degradation of pharmaceutical and personal care products (PPCPs) population markers on a population model

---

Supporting Information for

Impact of in-sewer degradation of pharmaceutical and personal care products (PPCPs) population markers on a population model

Authors:

Jake W. O'Brien*[a]
Andrew Banks[a]
Jochen F. Mueller[a]
Guangming Jiang[b]
Andrew Novic[a]
Christoph Ort[c]
Geoff Eaglesham[a]
Zhiguo Yuan[b]
Phong K. Thai[a,d]

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

[b]The University of Queensland, Advanced Water Management Centre (AWMC), St. Lucia, QLD 4072, Australia

[c]Eawag, Swiss Federal Institute of Aquatic Science and Technology, CH 8600 Dübendorf, Switzerland

[d]International Laboratory for Air Quality and Health, Queensland University of Technology, Brisbane, QLD 4001, Australia

*j.obrien2@uq.edu.au

* Tel. +61 (0)7 3274 9009, Fax +61 (0)7 3274 9003

Number of pages: 13

Number of figures: 2
Number of tables: 3

S1. Comparison of sewer degradation of pharmaceutical and personal care product population markers observed in this study against other literature

Acesulfame

Acesulfame, an artificial sweetener, was considered stable under all sewer conditions as no significant difference from a slope of zero was observed over the study period. This is consistent with the Buerge et al. (2009) finding that acesulfame is not eliminated during wastewater treatment and appears to be stable in the aquatic environment. Acesulfame can thus be used as stable marker to assess the degradation of other chemicals in the environment.

Atenolol

Atenolol, a beta blocker used primarily to treat angina and high blood pressure, was considered stable under all sewer conditions as no significant difference from a slope of zero was observed over the study period. While there are no other studies on aerobic degradation of atenolol, the available data for atenolol degradation under anaerobic pressurized sewer conditions indicate less than 20% removal during a 24 hour pass through the sewer pipe (Jelic et al. 2014).

Caffeine

Under control conditions caffeine, a stimulant, remained stable over the 12 hour study period. However, under gravity sewer conditions, caffeine degraded rapidly fitting the first-order kinetics model with a half-life of 4 hours resulting in only 13% remaining after 12 hours. In the rising main degradation also fitted the first-order kinetics model with a half-life of 1.8 hours and only 1% remaining after the 12 hour study period. For control conditions, our data agree with others that caffeine is stable (Senta et al. 2015). Other studies indicate that caffeine is readily degraded under microbial conditions such as when exposed to activated sludge leading to almost 100% removal during wastewater treatment (Blair et al. 2015).

Carbamazepine

Carbamazepine, an anticonvulsant, was considered stable under all sewer conditions as no significant difference from a slope of zero was observed over the study period. This is consistent with findings that carbamazepine is not removed during wastewater treatment (Jelic et al. 2014, Ryu et al. 2014). Additionally, in some cases effluent concentrations of carbamazepine were noticeably higher than the influent concentrations. Carbamazepine is highly soluble thus it is thought that desorption from solids alone cannot account for the increase and the increase may be a result of metabolites retransforming back to the parent compound (Blair et al. 2015, Rodayan et al. 2014).

Codeine

In wastewater codeine, an opiate analgesic, remained stable with a slight increase (slope = 1.02) over the 12 hour study period. While in gravity and rising main reactors and in the presence of biofilm codeine degraded rapidly fitting the first-order kinetics models better and half-lives of 3.8 and 2.1 hours respectively which meant by 12 hours only 11% remained in the gravity sewer and less than 2% remained in the rising main.

This is contrary to Jelic et al. (2014) who only observed a small amount of formation of codeine during the pass through their pressurised sewer. This is also contrary to other studies which found that removal of codeine during biological treatment was only 13% after 10 hours (Rodayan et al. 2014) however this may not be representative of sewer systems.

Furosemide

Furosemide, a strong diuretic, degraded under all sewer conditions and first-order kinetic models were the best fit for all conditions. Half-lives were consistent between the control and the gravity sewer with 5.7 and 5.8 hours. Under rising main conditions the half-life reduced to 1.9 hours. As a result less than 24% remained in the control and gravity sewers over the 12 hour study period and approximately only 1% remained in the rising main sewer. Jelic et al.(2014) didn’t see any removal of furosemide during a pass through a pressurised sewer and their data indicate that a small amount of furosemide formation may have occurred. Our results however indicate that furosemide undergoes both aerobic and anaerobic degradation. Data for removal during wastewater treatment is in the range of 40 and 80% (Kasprzyk-Hordern et al. 2010).

Gabapentin

Gabapentin, an anticonvulsant, was considered stable under all sewer conditions as no significant difference from a slope of zero was observed over the study period. During wastewater treatment removal, trickling filters resulted in minimal removal of gabapentin but activated sludge resulted in 84% removal in one study (Kasprzyk-Hordern et al. 2010) however only 6.4% in another (Gurke et al. 2015).

Hydrochlorothiazide

Hydrochlorothiazide, a diuretic, was stable in the control with a small increase (linear regression slope = 1.39) and no significant deviation from zero in the gravity sewer. In the gravity sewer no significant difference from zero was observed over the 12 hour study period. In the rising main there was only limited degradation and neither linear regression nor first-order kinetic models fitted the data well. Elimination during wastewater treatment shows little to no removal of hydrochlorothiazide in some studies (Radjenovic et al. 2009, Schroder et al. 2010) but up to 85% in others with no explanation for such large differences in results (Radjenovic et al. 2007).

Ibuprofen

Ibuprofen, a non-steroidal anti-inflammatory, was considered stable under all sewer conditions as no significant difference from a slope of zero was observed over the study period. This is consistent with the finding of Jelic et al. (2014) who found approximately only 5% removal during a single pass through a 7.6 km long pressurised sewer. During wastewater treatment it has been observed that ibuprofen is effectively eliminated through bio-degradation processes in the range of 85 - 95% (Kasprzyk-Hordern et al. 2010).

Iopromide

Iopromide, an imaging contrast agent, degraded under all conditions over the study period with first-order kinetics the best fitting model for all scenarios. In the control the half-life was about 16.8 hours where as in the gravity sewer and rising main it was 10.0 and 10.7 hours respectively. Elimination of iopromide in a conventional treatment plant has been observed at greater than 80% (Kormos et al. 2011).

Naproxen

Naproxen, an analgesic, was found to only be stable in the control reactor with no significant deviation from zero during the study period. Both the gravity sewer and rising main showed linear decay with slopes of -3.15 and -3.26 respectively. This

finding is inconsistent with that of Jelic et al. (2014) who on average saw no removal through a single pass through their 7.6km long sewer pipe, however, their data indicate removal at between -20 and 10%. Removal during wastewater treatment has been observed at more than 90% (Ryu et al. 2014).

Norfloxacin

Norfloxacin, a chemotherapeutic antibacterial agent, remained stable under control conditions with no significant deviation from zero during the 12 hour study period. In the gravity and rising main sewers, norfloxacin rapidly degraded with first-order kinetics indicating half-lives of 0.6. However, a non-zero plateau was observed for norfloxacin at approximately 16% of the initial concentration in the gravity sewer and 13% in the rising main and thus the half-lives given this plateau were 0.3 and 0.4 hours respectively. Data for removal of norfloxacin during wastewater treatment indicate 87-100% removal (Blair et al. 2015, Lindberg et al. 2005, Vieno et al. 2006) with sorption rather than degradation thought responsible for the removal. This may be a potential reason for the plateau observed if the liquid fraction has reached equilibrium with the solids.

Paracetamol

Paracetamol, an analgesic also called acetaminophen, was found to be stable under control conditions with no significant deviation from zero during the 12 hour study period. However, under gravity sewer and rising main conditions first-order kinetic models fitted well with half-lives of 1.5 hours 0.8 hours respectively. This is consistent with removal during wastewater treatment indicating that even though influent concentrations of paracetamol are high, removal in most cases is 100% (Ratola et al. 2012).

Salicylic acid

Salicylic acid, a metabolite of acetyl salicylic acid which is an anti-inflammatory, was unstable under all conditions. For the control linear regression degradation was the best fit with a slope of -8.26 but for the gravity sewer and rising main first-order kinetic models fitted better with halves of 2.6 and 1.3 hours respectively. Studies on salicylic acid removal during wastewater treatment indicates that it is completely eliminated (Lee et al. 2005, Ternes 1998).

Table S- 4 LCMS/MS parameters using an Sciex API 6500Q mass spectrometer (AB/Sciex, Concord, Ontario, Canada) with an electrospray ionization (ESI) interface coupled to a Shimadzu Nexera HPLC system (Shimadzu Corp., Kyoto, Japan). Separation was achieved on a Luna C-18 (2) (3 µm 100 Å LC Column 150 mm x 3 mm, Phenomenex) column using a mobile phase gradient of 1 to 95 % methanol with 0.1% acetic acid.

Source	Compound	Retention Time (RT)	Quantitation Ion/Transition					Confirmation Ion/Transition					Supplier
			MRM (Q1 > Q3)	DP	EP	CE	CXP	MRM (Q1 > Q3)	DP	EP	CE	CXP	
ESI -	Acesulfame	3.3	162>82	-45	-10	-22	-5	162>78	-45	-10	-45	-5	Dr Ehrenstorfer
ESI -	Acesulfame-d_4	3.3	166>86	-45	-10	-22	-5	166>78	-45	-10	-45	-7	Toronto Research Chemicals
ESI +	Atenolol	2.47	267.2>190	60	10	27	9	267.2>145	60	10	38	9	Cerilliant
ESI +	Atenolol-d_7	2.47	274.1>145.1	70	10	37	10	274.1>190.1	70	10	28	8	Toronto Research Chemicals
ESI +	Caffeine	3.09	195.1>138.1	71	10	28	8	195.1>110.1	71	10	32	8	Cerilliant
ESI +	Caffeine-d_3	3.08	198.1>138	60	10	27	8	198.1>110	60	10	35	8	CDN Isotopes
ESI +	Carbamazepine	6.35	237.2>194	96	10	31	16	237.2>193	96	10	47	12	Cerilliant
ESI +	Carbamazepine-d_{10}	6.3	>247.2>204.1	65	10	30	8	>247.2>202.1	65	10	51	8	CDN Isotopes
ESI +	Codeine	2.61	300.2>215.1	60	10	37	12	300.2>165.1	60	10	57	10	Cerilliant
ESI -	Furosemide	6.46	329>285	-57	-10	-21	-13	329>205	-57	-10	-33	-13	Cerilliant
ESI +	Gabapentin	2.65	172.1>154	45	10	21	8	172.1>137	45	10	25	8	Cerilliant
ESI -	Hydrochlorothiazide	3.26	296>269	-85	-10	-28	-20	296>205	-85	-10	-34	-12	Cerilliant
ESI -	Hydrochlorothiazide-$C_{13}d_2$	3.>24	299>270.1	-85	-10	-28	-12	299>206.1	-85	-10	-35	-12	Toronto Research Chemicals
ESI -	Ibuprofen	9.56	205.1>161	-52	-10	-12	-10	205.1>159	-52	-10	-11	-10	Sigma-Aldrich Australia
ESI -	Iopromide	2.66	790.001>127	-80	-10	-55	-7	790>127	-80	-10	-55	-7	Dr Ehrenstorfer
ESI -	Naproxen	8	229.2>185.1	-53	-10	-11	-10	229.2>170.1	-53	-10	-22	-10	Cerilliant
ESI +	Norfloxacin	2.78	320.3>276.2	70	10	26	14	320.3>233.2	70	10	35	14	Sigma-Aldrich Australia
ESI +	Norfloxacin-d_5	2.78	325.2>281.2	70	10	28	15	325.2>238.2	70	10	36	15	Sigma-Aldrich Australia
ESI +	Paracetamol	2.83	152.1>110	56	10	>24	8	152.1>65.1	56	10	42	6	Cerilliant
ESI -	Salicylic Acid	5.6	137>93	-45	-10	->24	-6	137>65	-45	-10	-40	-6	Cerilliant

Notes: MRM = Multiple Reaction Monitoring, DP = Declustering Potential, EP = Entrance Potential, CE = Collision Cell Energy, CXP = Collision Cell Exit Potential, ESI = Electrospray Ionisation

Table S- 5 Octanol/water partition coefficients both calculated using Episuite v4.11 and from experimental database matches.

Chemical	pKa	Log K_{OW} Calculated	Experimental database match Log K_{ow}	Reference
acesulfame	5.67	-1.33		
atenolol	9.6	-0.03	0.16	(Hansch et al. 1995)
caffeine	0.7 and 14	0.16	-0.07	(Hansch et al. 1995)
carbamazepine	13.9	2.25	2.45	(Dal Pozzo et al. 1989)
codeine	10.6	1.28	1.19	(Avdeef et al. 1996)
furosemide	3.8 and 7.5	2.32	2.03	(Sangster 1993)
gabapentin	3.68 and 10.70	-1.37	-1.1	(Sangster 1994)
hydrochlorothiazide	7.9 and 9.2	-0.1	-0.07	(Hansch et al. 1995)
ibuprofen	4.91	3.79	3.97	(Avdeef et al. 1998)
iopromide	<2 and >13	-2.49	-2.05	(Hansch et al. 1995)
naproxen	4.15	3.1	3.18	(Hansch et al. 1995)
norfloxacin	6.32 and 8.47	-0.31	-1.03	(Hansch et al. 1995)
paracetamol	9.38	0.27	0.46	(Sangster 1994)
salicylic acid	2.98	2.>24	2.26	(Hansch et al. 1995)

Log K_{OW} values reported for neutral molecule form and were calculated using the US EPA EPI Suite version 4.11 with KOWWIN version 1.68 (EPA 2012).

pKa values were from SPARC (http://ibmlc2.chem.uga.edu/sparc); values given for -OH, -COOH, or highest NHx groups.

Table S- 6 Pearson correlation analysis between the R^2 for the correlation between mass load and population size from O'Brien et al. (2014) and the time before 10% loss in each of the sewer reactors.

Chemical	Correlation between mass load and population size R^2 (O'Brien et al. 2014)	Time before 10% loss (h)		
		Control	Gravity Sewer	Rising Main
Acesulfame	0.995	>24.00	>24.00	>24.00
Atenolol	0.823	>24.00	>24.00	>24.00
Caffeine	0.869	>24.00	0.61	0.28
carbamazepine	0.849	>24.00	>24.00	>24.00
Codeine	0.908	>24.00	0.57	0.32
furosemide	0.839	0.87	0.88	0.29
gabapentin	0.968	>24.00	>24.00	>24.00
hydrochlorothiazide	0.944	>24.00	>24.00	9.01
ibuprofen	0.919	>24.00	>24.00	>24.00
iopromide	0.377	2.55	1.52	1.63
naproxen	0.912	>24.00	3.17	3.07
norfloxacin	0.929	>24.00	0.09	0.09
paracetamol	0.811	>24.00	0.22	0.12
salicylic acid	0.922	1.21	0.40	0.20
Pearson r Correlation				
R		0.5127	0.3161	0.2721
95% confidence interval		-0.02456 to 0.8202	-0.2577 to 0.7251	-0.3021 to 0
R squared		0.2629	0.9994	0.07404
P value				
P (two-tailed)		0.0608	0.2708	0.3466
P value summary		ns	ns	ns
Significant? (alpha = 0.05)		No	No	No
Number of XY Pairs		14	14	14

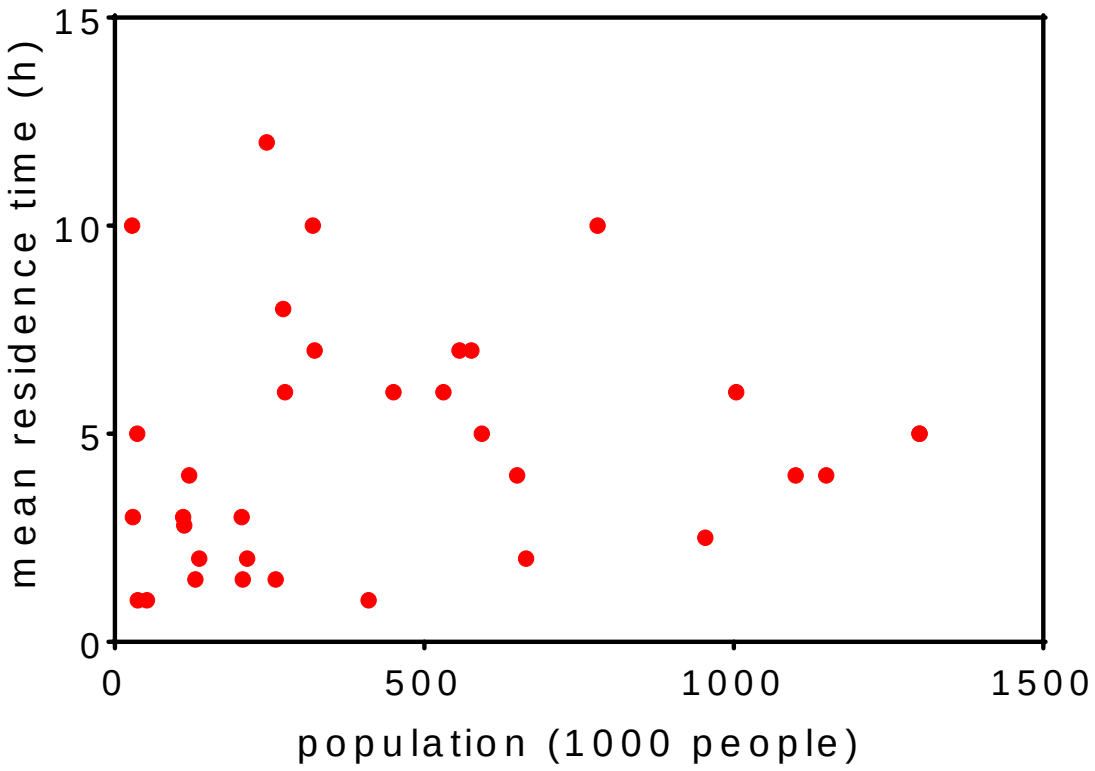


Figure S- 4 Mean residence time versus population size from Ort et al. 2014

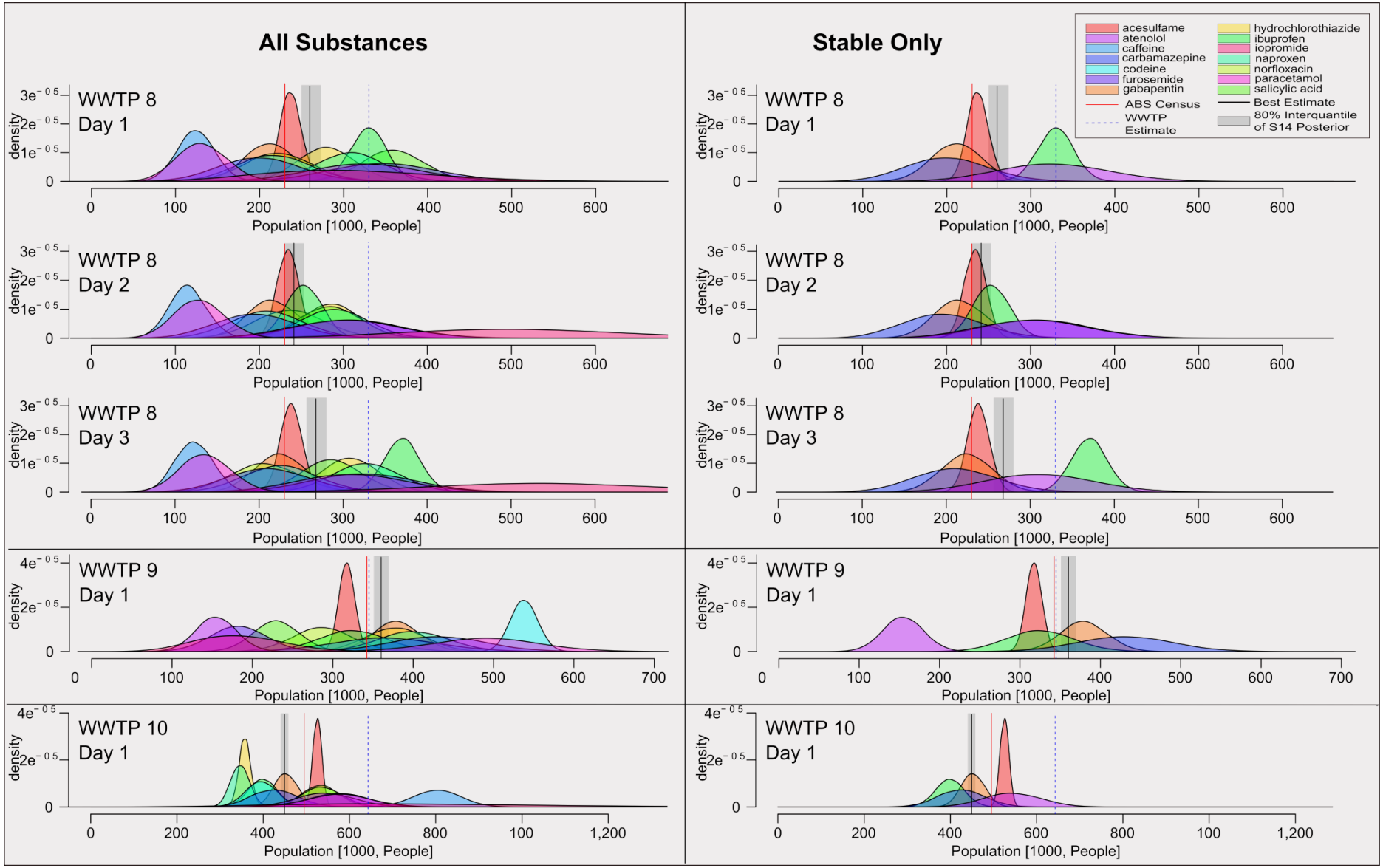


Figure S- 5 Individual substance distributions for estimating population size for all Large WWTPs from (O'Brien et al. 2014).

Appendix 4: Supporting Information – Wastewater analysis of census day samples to investigate per capita input of organophosphorous flame retardants and plasticizers into wastewater

Wastewater analysis of census day samples to investigate per capita input of organophosphorus flame retardants and plasticizers into wastewater

Authors:
Jake W. O'Brien*[a], Phong K. Thai[a,b], Sicco H. Brandsma[c], Pim E.G. Leonards[c], Christoph Ort[d,e], Jochen F. Mueller[a]

Affiliation:
[a]The University of Queensland, The National Research Centre for Environmental Toxicology (Entox), 39 Kessels Road, Coopers Plains, QLD 4108, Australia
[b]Queensland University of Technology, International Laboratory for Air Quality & Health, 2 George Street, Brisbane, QLD 4001, Australia
[c]Institute for Environmental Studies, VU University, De Boelelaan 1087, 1081 HV Amsterdam, The Netherlands
[d]Eawag, Swiss Federal Institute of Aquatic Science and Technology, CH 8600 Dübendorf, Switzerland
[e]The University of Queensland, Advanced Water Management Centre (AWMC), St. Lucia, QLD 4072, Australia

***Corresponding author:** Tel.: +61 3274 9009 Fax: +61 (0)7 3274 9003 Email: j.obrien2@uq.edu.au

Table S- 7 Daily concentration (µg/L) of OPEs in wastewater collected from 11 different Australian STPs

Site (2011)	EHDPP (µg/L)	TPHP (µg/L)	TBOEP (µg/L)	TCEP (µg/L)	∑TClPP (µg/L)	TDCIPP (µg/L)	TIBP (µg/L)	TNBP (µg/L)	∑OPEs (µg/L)
STP 1 10th Aug	<LOD	<LOD	0.4	0.2	1.2	0.1	<LOD	<LOD	2.0
STP 2 10th Aug	<LOD	<LOD	4.1	<LOD	1.3	0.05	<LOD	<LOD	5.5
STP 3 11th Aug	<LOD	<LOD	3.6	0.2	1.3	0.1	<LOD	<LOD	5.2
STP 4 9th Aug	<LOD	<LOD	4.8	0.3	4.1	0.2	1.2	<LOD	10.6
STP 5 10th Aug	<LOD	<LOD	5.1	0.6	2.6	0.2	1.6	<LOD	10.2
STP 6 10th Aug	<LOD	<LOD	4.2	0.3	3.7	0.3	1.1	<LOD	9.5
STP 6 11th Aug	<LOD	<LOD	4.4	0.5	3.2	0.2	1.4	<LOD	9.7
STP 7 10th Aug	0.1	<LOD	5.7	0.4	3.0	0.2	1.4	<LOD	10.7
STP 8 10th Aug	<LOD	<LOD	6.6	0.3	3.0	0.2	<LOD	<LOD	10.1
STP 8 11th Aug	<LOD	<LOD	5.4	0.3	2.6	0.2	<LOD	<LOD	8.4
STP 9 9th Aug	<LOD	<LOD	1.7	0.2	1.8	0.1	<LOD	<LOD	3.8
STP 9 10th Aug	<LOD	<LOD	3.8	0.2	2.5	0.1	<LOD	<LOD	6.6
STP 9 11th Aug	<LOD	<LOD	6.2	0.3	2.0	0.1	<LOD	<LOD	8.7
STP 10 9th Aug	<LOD	<LOD	4.2	0.2	2.3	0.1	<LOD	<LOD	6.9
STP 11 11th Aug	<LOD	<LOD	5.8	0.2	2.4	0.1	<LOD	<LOD	8.5
Median	<LOD	<LOD	4.4	0.3	2.5	0.1	1.4	<LOD	8.6
Limit of Detection (LOD)	0.1	0.2	0.1	0.2	1.1	0.03	0.8	3.0	

Table S- 8 Per capita mass load of OPFRs across different catchments and days for eleven Australian STPs

Site (2011)	EHDPP (µg/person/day)	TPHP (µg/person/day)	TBOEP (µg/person/day)	TCEP (µg/person/day)	∑TCIPP (µg/person/day)	TDCIPP (µg/person/day)	TIBP (µg/person/day)	TNBP (µg/person/day)	∑OPFRs (µg/person/day)
STP 1 10th Aug	NA	NA	187	102	501	60	NA	NA	850
STP 2 10th Aug	NA	NA	1704	NA	559	21	NA	NA	2283
STP 3 11th Aug	NA	NA	1778	94	654	40	NA	NA	2567
STP 4 9th Aug	6	NA	514	35	444	24	133	NA	1157
STP 5 10th Aug	14	46	1198	151	614	48	371	NA	2442
STP 6 10th Aug	12	NA	1042	62	902	70	263	NA	2350
STP 6 11th Aug	12	NA	1067	110	773	59	343	NA	2364
STP 7 10th Aug	12	NA	969	62	510	31	231	NA	1815
STP 8 10th Aug	NA	NA	1343	69	601	45	123	NA	2186
STP 8 11th Aug	NA	NA	1091	58	534	35	NA	NA	1719
STP 9 9th Aug	NA	NA	427	47	436	24	NA	NA	934
STP 9 10th Aug	NA	NA	928	57	603	33	NA	NA	1620
STP 9 11th Aug	NA	NA	1567	77	503	33	NA	NA	2181
STP 10 9th Aug	NA	NA	997	53	540	34	NA	NA	1624
STP 11 11th Aug	NA	NA	1504	53	611	32	NA	NA	2200

NA = Mass loads could not be calculated as the concentration within the sample was below the limit of detection (LOD)

Table S- 9 Average procedural blank concentration of each OPE measured by GC/MS in EI mode

	EHDPP (µg/L)	TPHP (µg/L)	TBOEP (µg/L)	TCEP (µg/L)	∑TCIPP (µg/L)	TDCIPP (µg/L)	TIBP (µg/L)	TNBP (µg/L)
Blank	0.02	0.08	0.03	0.06	0.37	0.01	0.29	1.0

Table S- 10 Coefficient of variation for daily per capita input of each OPE mass load into wastewater for eleven STP catchments in Australia.

	TBOEP	ΣTCIPP	TIBP	TCEP	TDCIPP	EHDPP	TPHP
Number of Values	11	11	5	10	11	4	1
Minimum	0.187	0.444	0.123	0.034	0.02	0.005	0.046
25% Percentile	0.969	0.51	0.1275	0.052	0.03	0.0065	0.046
Median	1.054	0.558	0.23	0.062	0.033	0.0115	0.046
75% Percentile	1.504	0.614	0.336	0.096	0.047	0.0135	0.046
Maximum	1.778	0.837	0.37	0.151	0.064	0.014	0.046
Mean	1.099	0.5772	0.2314	0.0755	0.0381	0.0105	0.046
Std. Deviation	0.4726	0.1048	0.107	0.0338	0.0138	0.0039	0
Std. Error of Mean	0.1425	0.0316	0.0479	0.0107	0.0042	0.0019	0
Coefficient of Variation (%)	43.003	18.157	46.24	44.781	36.335	36.886	0
Lower 95% CI of mean	0.7818	0.5068	0.0985	0.0513	0.0288	0.0043	0
Upper 95% CI of mean	1.417	0.6476	0.3643	0.0997	0.0474	0.0167	0
Sum	12.09	6.349	1.157	0.755	0.419	0.042	0.046

Appendix 5: Measuring selected PPCPs in wastewater to estimate the population in different cities in China

The following publication is incorporated as Appendix 5:

Gao, J., **O'Brien, J.W.,** 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 10.1016/j.scitotenv.2016.05.216

Peer–reviewed and published by Science of the Total Environment
Accepted author manuscript

Measuring selected PPCPs in wastewater to estimate the population in different cities in China

Jianfa Gao[a, 1], Jake O'Brien[a, 1], Peng Du[b], Xiqing Li[b], Christoph Ort[c], Jochen F. Mueller[a], Phong K. Thai[a, d, *]

[a] The University of Queensland, National Research Center for Environmental Toxicology, Brisbane, QLD 4108, Australia

[b] College of Urban and Environmental Sciences, Peking University, 100871 Beijing, China

[c] Eawag, Swiss Federal Institute of Aquatic Science and Technology, CH 8600 Dübendorf, Switzerland

[d] International Laboratory for Air Quality and Health, Queensland University of Technology, Brisbane, QLD 4001, Australia

*Corresponding author:
Phong K. Thai, Queensland University of Technology
(e) phong.thai@qut.edu.au;

[1] These authors contributed equally to this article

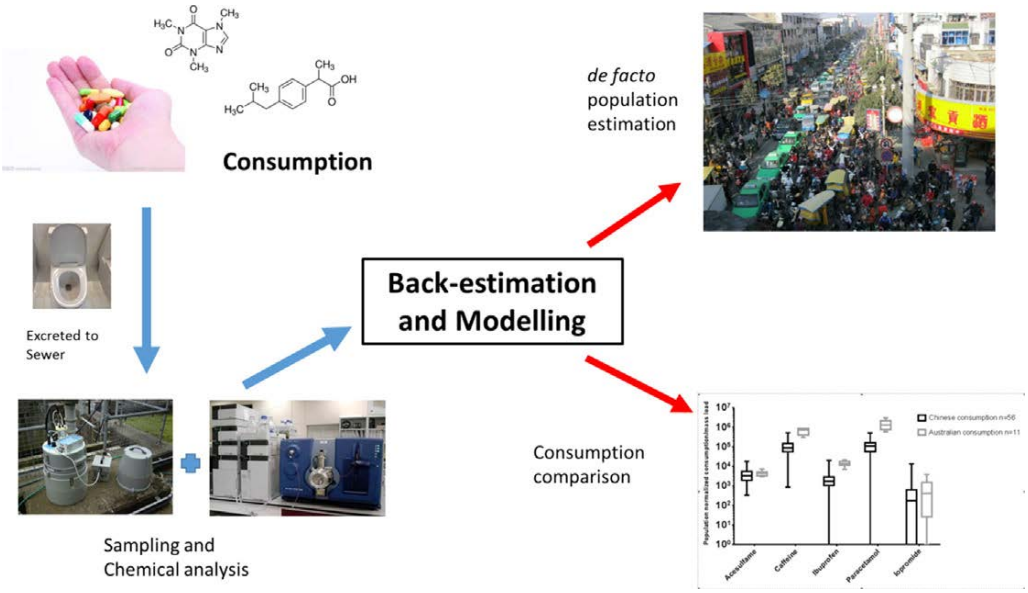
App. 5 Abstract

Sampling and analysis of wastewater from municipal wastewater treatment plants (WWTPs) has become a useful tool for understanding exposure to chemicals. Both wastewater based studies and management and planning of the catchment require information on catchment population in the time of monitoring. Recently, a model has been developed and calibrated using selected pharmaceutical and personal care products (PPCPs) measured in influent wastewater for estimating population in different catchments in Australia. The present study aimed at evaluating the feasibility of utilizing this population estimation approach in China. Twenty-four hour composite influent samples were collected from 31WWTPs in 17 cities with

catchment sizes from 200,000–3,450,000 people representing all seven regions of China. The samples were analyzed for 19 PPCPs using liquid chromatography coupled to tandem mass spectrometry in direct injection mode. Eight chemicals were detected in more than 50% of the samples. Significant positive correlations were found between individual PPCP mass loads and population estimates provided by WWTP operators. Using the PPCP mass load modeling approach calibrated with WWTP operator data, we estimated the population size of each catchment with good agreement with WWTP operator values (between 50–200% for all sites and 75–125% for 23 of the 31 sites). Overall, despite much lower detection and relatively high heterogeneity in PPCP consumption across China the model provided a good estimate of the population contributing to a given wastewater sample. Wastewater analysis could also provide objective PPCP consumption status in China.

Keywords: PPCP consumption, population estimation, wastewater treatment plant, LC-MS/MS

Graphical Abstract



Highlights

• Large scale monitoring of selected PPCPs in 17 cities across China

• Model using selected PPCPs to estimate population provided reasonably good result.

• More accurate population data are needed to calibrate the model to be used in China.

• Consumption of some popular PPCPs varied across different regions of China.

App. 5 Introduction

Wastewater analysis (WWA), or wastewater-based epidemiology, is a valuable approach to evaluate chemical consumption and exposure in the population and complements other consumption data such as surveys and production/sales statistics. For chemicals that are both excreted and measurable in the wastewater, WWA can provide objective and quick estimation about their use in a given wastewater treatment plant (WWTP) catchment. Until now, one of the largest sources of uncertainty of WWA is the estimation of *de facto* population during the monitoring period (Castiglioni et al. 2013).

Accurate estimation of *de facto* population is of great importance not only for management and planning purposes, but also for reliable back-estimation of per capita chemical consumption using WWA in a given wastewater catchment because population may change due to commuting, events, tourism, and migrating labor forces in a given catchment (Castiglioni et al. 2013). For a given WWTP catchment, population estimates could be obtained from direct methods such as Census or indirect methods such as daily flow or biological oxygen demand. Census data however are only accurate for the day on which they were collected and are extremely high cost limiting the frequency in which they can be carried out. Indirect methods could be more representative of the population on a given day potentially cost effective, but so far the most commonly used indirect methods, such as flow, have relatively large uncertainties, e.g. the fluctuation of flow due to rainfall. Alternative approaches have been proposed to estimate the *de facto* population including the use of several biomarkers to estimate the population contributing to a

wastewater treatment plant for a given period (Been et al. 2014, Chen et al. 2013, Daughton 2012a). But until now only a set of commonly consumed pharmaceuticals and personal care products (PPCPs) have been evaluated as useful to estimate the population in different catchments in Australia (Lai et al. 2011, O'Brien et al. 2014). To date, the model was calibrated using samples collected during the Australian census which allowed accurate head counts to be obtained. This model was applied and proved able to provide more robust population normalized drug consumption in a WWA study in Australia (Lai et al. 2015).

China is the world's largest producer and consumer of PPCPs (Liu and Wong 2013). The rapid economic growth and the increasing number of aging population in the society contribute to the rising consumption of PPCPs. The increased consumption of PPCPS in China provides a good opportunity to apply population estimation model with PPCPs mass load in the influent of a catchment.

Additionally, over-use and misuse of both prescribed and over-the-counter pharmaceuticals causes considerable harm to the community sustainability in China (Mao et al. 2015). There is thus a need for a tool to effectively monitor the consumption of PPCPs in the population to provide information for developing public health policies and evaluating their performance. Until now, WWA studies in China have focused only on estimating the consumption of recreational illicit drugs (Du et al. 2015, Khan et al. 2014, Li et al. 2014). Although many studies have monitored the occurrence of a wide variety of PPCPs in wastewater and other environmental compartments (Bu et al. 2016, Chen et al. 2012b, Peng et al. 2008, Sim et al. 2010, Wang et al. 2015, Yu et al. 2011), few studies in China have attempted to link the load measured in the influent with consumption in the population (Sui et al. 2015). WWA has been used to assess the geographical consumption patterns of illicit drugs (Been et al. 2016) and has been proposed as a tool to assess consumption of PPCPs (Thomas and Reid 2011). Therefore WWA may be a useful tool to assess PPCPs consumption in China (Gao et al. 2015).

The aims of the present study were to investigate the feasibility of utilizing a previously established model for *de facto* population estimation using daily PPCPs

mass loads in wastewater influent from multiple WWTPs in China and to assess the consumption of these PPCPs.

App. 5.2 Materials and Methods

App. 5.2.1 Sampling

A sampling campaign was carried out in 31 WWTPs from 17 cities including densely populated and developed cities like Beijing, Shanghai and Shenzhen across seven regions of China (Du et al. 2015). Details about the samples and sampling sites are provided in Table S- 13. Briefly, twenty-four hour composite influent samples were collected from early July to early October 2014. The treatment capacity of the investigated WWTPs range from 25,000 m^3/day to 910,000 m^3/day and the total estimated population in the investigated catchment area is 32.9 million. All the samples were acidified to pH 2 on site using 2M hydrochloric acid immediately after the sampling. The samples were then immediately frozen before being shipped to Peking University on ice in thermal insulation boxes, and then stored frozen (at -20 ℃). All the samples were aliquoted (~60 ml) to small clean bottles, re-frozen and shipped on ice to The University of Queensland by air within 20 hours and kept at -20 ℃ until analysis.

App. 5.2.2 Standards and Reagents

Analytical grade formic acid was purchased from Sigma Aldrich (Castle Hill, Australia). Analytical standards were purchased from various suppliers ((O'Brien et al. 2014)-SI). Water was purified with a Milli-Q system (Millipore, 0.2 µm filtered, 18.2 MΩ cm^{-1}). Liquid chromatography grade acetonitrile was purchased from Merck (Darmstadt, Germany). Mobile phases were filtered using Sartorius Stedim 0.45 µm RC filters (Goettingen, Germany).

App. 5.2.3 Instrumental Analysis

The analytical method was previously described by O'Brien et al. (2014)(O'Brien et al. 2014). We focused on PPCPs which could be directly measured in filtered wastewater using LC-MS/MS without the need for solid phase extraction.

In brief, wastewater samples were thawed and filtered through 0.45 µm reconstituted cellulose filter disk followed by internal standards spiking. An AB Sciex 5500 QTrap mass spectrometer (AB Sciex, Concord, Ontario, Canada) with an electrospray ionization (ESI) interface coupled to a Shimadzu Nexera HPLC system (Shimadzu Corp., Kyoto, Japan) was used to analyze target chemicals. A Luna C-18 column (3 µm, 100 Å, LC Column 150 × 3 mm, Phenomenex) was used with 1-95% acetonitrile (0.1% formic acid) as mobile phase. Both positive and negative ionization mode was applied to analyze the selected PPCPs (see Table S- 14). Quantitation was performed using the isotope dilution method. The most abundant transition ion for each compound was used as the quantification ion with the less abundant transition as the qualifier ion. An acceptable threshold of 30% for the ratio between quantification ion to qualifier ion was used to confirm results.

App. 5.2.4 Quality assurance and quality control

Rigorous quality assurance and quality control (QA/QC) was implemented to ensure the accurate quantification of the selected PPCPs. All the standards and samples were analyzed in duplicate and the relative difference of all replicated samples was less than 15%. Three MilliQ water laboratory blanks were analyzed identically to the wastewater samples. No analytes were detected above the limit of detection in any of the blank samples. A calibration standard curve was run at the beginning and the end of the sequence with additional calibration standards after every tenth sample. Deuterated internal standards were used to minimize the influence of matrix effects.

App. 5.2.5 Population estimation using PPCPs mass load in the influent

To estimate the *de facto* population for each WWTP catchment during the sampling period, the model developed by O'Brien et al. (2014)(O'Brien et al. 2014) was used. This model uses Bayesian inference to estimate *de facto* population based on linear regressions of daily chemical mass loads of high use PPCPs against population size. We used the best estimates that considered the mass load of selected PPCPs of significant correlation with population. To estimate the population for each WWTP catchment, the model was calibrated with the measured mass loads of PPCPs and

the population provided by the WWTP operators of 30 catchments, the 31st catchment population was estimated by the model (the leave-one-out approach). These estimates were then compared with the estimates provided by WWTP operators for each catchment. To the best of our knowledge the population estimates provided by WWTP operators were based on catchment characteristics and the most recent census data (2010). To assess whether regional consumption of PPCPs could be differentiated, the model was also applied using a North-South split where only calibration data for catchments in the North were used to estimate the population for WWTP catchments in the North and only calibration data for catchments in the South were used to estimate the population for WWTP catchments in the South.

App. 5.2.6 Estimation of PPCPs consumption

As is standard practice in WWA studies for illicit drug consumption, the population normalized consumption of PPCPs was calculated from the equation:

$$\text{Consumption of PPCPs}\left(\frac{\frac{\text{mg}}{1000\ \text{inh}}}{\text{day}}\right) = \frac{C_i * \text{F}}{\text{P} * E_i} * \frac{\text{MW}_{\text{par}}}{\text{MW}_{\text{met}}}$$

where C_i is the concentration of a chemical i in the sample (mg/L), F is the daily flow on the sampling day (L), P is the population size in the catchment area of the WWTP provided by the operator (1000 inhabitants) and $E_i$is the excretion factor of the selected biomarker (%), MW_{par} is the molecular weight of the parent compound, MW_{met} is the molecular weight of metabolites. In the present study, we only used the parent compound to back-calculate the consumption.

App. 5.3 Results and discussion

To the best of our knowledge, this study is the first attempt to measure selected PPCPs in influent wastewater from all the seven regions of China. The utilization of these monitoring data for population estimation and the consumption of five PPCPs representing different indicators are discussed in the following sections.

App. 5.3.1 PPCPs profiles in Chinese wastewater

Fourteen out of 19 chemicals included in the analysis were detected in the wastewater samples. Salicylic acid, acesulfame, caffeine, ibuprofen and paracetamol were detected in more than 95% of the samples. Frequent detection of these compounds is in good agreement with previous studies that reported the widespread occurrence of these compounds in wastewater and environmental matrices in China (Bu et al. 2013, Duan et al. 2013, Sun et al. 2016, Zhao et al. 2011). Hydrochlorothiazide, iopromide and gabapentin were detected in more than half of the samples. Carbamazepine, citalopram, naproxen, tramadol, atenolol and temazepam were detected in less than 20 % of the samples and five compounds were not detected (i.e. venlafaxine, atorvastatin, furosemide, fluoxetine and norfloxacin). Previous studies have identified that the occurrence of PPCPs could have significant geographical patterns. The analyte concentrations in the present study are comparable with data reported in other studies with different geographical pattern (Beijing, Xiamen and Shanghai, data not shown) (Sui et al. 2010, Sui et al. 2015, Sun et al. 2016).

App. 5.3.2 Population estimation using PPCPs mass loads in the influent

Concentration of PPCPs were converted to daily mass loads by multiplying by the daily flow (Table S- 13). Ten PPCPs recommended by O'Brien et al (2014)(O'Brien et al. 2014) were evaluated for the correlation between their daily mass loads and the population estimates provided by the WWTP operators. All the PPCPs except carbamazepine demonstrated significant statistical correlation with population estimates ($P < 0.01$, see Table S- 14).

Using the data from the 31 WWTPs across China to calibrate the model, we obtained estimates of the *de facto* population for the sampling period in each catchment. For 21 out of 31 WWTPs the modelled population was within 20 % of the population estimate provided by the WWTP operators and the remaining 10 WWTPs estimates were consistently within a factor of 2 (see Table S- 12). The largest variations were found for BJ-1 (200%) and HZ-2 (51%). It appeared that the model

was biased towards overestimating populations for primarily catchments in the northern part of China and underestimating catchments mostly locate in the southern part of China. Since it is entirely feasible that systematic differences in PPCPs use exist between the northern and southern part of China we re-calibrated the model where we divided the WWTPs into Northern and Southern China catchments. However, the North-South grouping did not improve the estimation significantly (the ratio is from 54%-200% in north group and 65%-183% in the south group, see Table S- 16 and Table S- 17).

App. 5.3.3 Consumption of selected PPCPs in China

The population normalized mass load (PNML) and population normalized consumption (PNC) of selected PPCPs were also assessed (Table S- 11 and Table S- 15). Consumption was calculated by dividing the PNML by the excretion factor as shown in Table S- 14. Those PPCPs are commonly used substances that could be easily measured in wastewater and could also be associated with population life style, behavior and health status. Previous studies have observed geographical PPCPS consumption differences (Bu et al. 2013, Cox et al. 2003, Wangia and Shireman 2013). These differences were observed for some compounds in this study such as acesulfame and caffeine.

App. 5.3.4 Consumption of acesulfame and caffeine

Acesulfame, an artificial sweetener, and caffeine, a stimulant, are commonly found in soft drinks and snacks. Consumption of acesulfame and caffeine could reflect the expenditure on a wide range of soft drinks and snack food in the population similar to lifestyle markers. As can be seen in and Figure S- 10 and Figure S- 11, the consumption of caffeine is much higher than acesulfame. The difference could be even larger considering caffeine could degrade more significantly than acesulfame in different sewer conditions (Buerge et al. 2008, Seiler et al. 1999). The much higher use of caffeine to acesulfame is reasonable because caffeine is the key stimulant found in tea, coffee, energy drinks and lot of pharmaceuticals while there are several artificial sweeteners in addition to acesulfame, such as aspartame and saccharin in the food and beverage industry.

Population in JN-1 had significantly higher PNC of caffeine than all the other sites investigated during the sampling period and two northwest sites YC-1 and XA-1 had relatively higher PNC of acesulfame than the remaining catchments. Population in SZ-1 and SZ-2 had considerable high PNC of both caffeine and acesulfame. As it is shown in Table S- 11 that population in the catchment of north China had higher PNC of acesulfame than south China population. Consumption of caffeine ranged from 840 mg/day/1000 inh in GY-2 to 42,000 mg/day/1000 inh in JN-1 with 18 sites lower than 10,000 mg/day/1000 inh and another 12 sites ranged from 10,000 to 20,000 mg/day/1000 inh. Although significant geographical differences could be observed, most catchments investigated in the study have comparable consumption of caffeine. It is worthy to note that the most popular drink in China is tea, which could have lower and more variable content of caffeine than coffee (Barone and Roberts 1996). Previous studies have indicated that the economic status could influence the consumption behavior of PPCPS in different places (Bu et al. 2016, Hodges et al. 2012, Whelan et al. 2012). It was observed that caffeine and acesulfame consumption in China have good agreement with this hypothesis. For example, several developed catchments with more developed social-economical characteristics (SZ-1, SZ-2, HZ-1, 2, BJ-2 and SH-2) had considerable higher consumption of acesulfame and caffeine.

App. 5.3.5 Consumption of paracetamol and ibuprofen

Paracetamol, also known as acetaminophen, and ibuprofen are commonly used non-narcotic analgesic and anti-inflammatory agents. These two drugs are highly metabolised by humans with only 2% of paracetamol and 9% of ibuprofen excreted as parent drugs (see Table S- 14). The highest paracetamol consumption was observed in HRB-1 with 18, 000 mg/day/1000 inh followed by JN-1 (16,000 mg/day/1000 inh) and LZ-1(10,000 mg/day/1000 inh). Thirteen out of thirty-one sites are in the range of 1000-4000 mg/day/1000 inh with another thirteen sites from 4000 to 8000 mg/day/1000 inh. The paracetamol concentrations in NJ-1 and GY-2 samples were below the detection limit possibly due to the low consumption during the sampling period and high in sewer loss in the catchments.

The production of ibuprofen in China was reported to be approximately 8000 ton in 2013 with 5600 ton exported. Assuming that the remaining 2,400 ton was all consumed, this equates to about 4700 mg/day/1000 inh of ibuprofen consumed in China ((MIIT) 2013). All the WWTPs but JN-1 and XA-1 had lower ibuprofen PNC compared with the national average based on production figures. This could possibly attribute to the large inter-individual variation regarding the excretion factor. Five sites had relatively low consumption (less than 1000 mg/day/1000 inh) with another eighteen sites with medium consumption between 1000 to 3000 mg/day/1000 inh, seven catchments had high consumption of ibuprofen (> 3000 mg/day/1000 inh). The coefficient of variance (CV) of ibuprofen consumption among all the investigated catchments was 141% while the CV of paracetamol consumption was 87%. It indicated that the consumption of paracetamol could have more geographic variance than ibuprofen across the country.

App. 5.3.6 Consumption of iopromide

Iopromide was detected in most samples except those from GY-1, SJZ-3, WH-1, WH-2, KM-2 and NN-1. It is noteworthy that the iopromide in SJZ-3 was below the method detection limit but it had relatively high PNC of other PPCPS investigated. Half of the investigated catchments consumed 50 to 500 mg/day/1000 inh of iopromide while eight catchments had higher consumption ranging from 500 to 3000 mg/day/1000 inh. Considering that iopromide is not the sole contrast media in clinical practice, the observed geographical consumption variance could be attributed to the prescription habit and the iopromide producer’s marketing strategy. As shown in Figure S- 7, in general the iopromide consumption indicated that the Eastern China population had higher PNC than the Western population which may indicate that the Eastern population receives more radiographic checks than the Western population. The higher check rates could reveal the fact that population in the more developed East China with higher income and better health care insurance are highly likely to have more preventative and diagnostic health checks than the population in the developing western region.

App. 5.3.7 Comparison with Australia

PPCPS consumption could correlate with demographic characteristics of a given population which was proposed in previous studies (Hodges et al. 2012, Whelan et al. 2012) and observed in China. It is interesting to compare the data from China with the data in Australia reported by O'Brien et al. (2014)(O'Brien et al. 2014) to verify whether demographic and economic characteristics affect the consumption behavior of PPCPS.

In general the PNC/PNML of PPCPS in Australia is higher than the corresponding values in China (see Figure S- 8). Moreover, the Australian inter-WWTPs variation is smaller than the Chinese counterparts. The higher PNC in Australia supports the hypothesis that population with higher income is more likely to consume more PPCPS in daily life. The inter-WWTPs variation of PNC of selected PPCPS in Australia is relatively smaller than China, probably due to the more homogenous consumer behavior of the investigated PPCPS in Australia. Another noteworthy observation is that the PNML for the two anti-inflammatory drugs (ibuprofen and paracetamol) in Australia are much higher than in China. The observation could be explained by the fact that Chinese population use considerable amount of traditional herbal medicines and other substitute therapies whereas the Australian population could possibly tend to consume these two cheap over-the-counter drugs for many minor treatments. The per capita consumption of acesulfame for China and Australia is comparable. Caffeine consumption, however, was higher in Australia than in China which may indicate that the Australian population consume more caffeinated drinks (coffee mainly) than the Chinese population (green tea and black tea that have lower caffeine than coffee).

App. 5.3.8 Uncertainties

One of the main factors contributing to the uncertainty of the population model prediction in China is the quality of the population data provided by the WWTP operators that were used to calibrate the model. The accuracy of the model prediction not only relies on the accuracy of the measurement of selected PPCPS and accuracy of flow data, but also the accuracy of the population data for each of

the WWTP catchments in the calibration data. Therefore systematic overestimation or underestimation may happen with poor quality calibration data leading to relatively large uncertainties. The latest census in China was conducted 4 years before the sampling during which there could be significant catchment development, immigration and emigration. Due to a number of socio-economic reasons, the population in the big cities keep growing dramatically during the past ten years. This is in contrast with the Australian study where the population used in the model calibration was exceptionally reliable since the sampling was synchronized with the Census Day at which *de facto* population was obtained and mapped for geographical boundaries of WWTP catchments. The Chinese national census is only conducted at low frequency (approx. every 10 years), it therefore may be favorable to compare the PPCPS model estimation with other methods such as regional population survey or catchment cellphone signal (Ran et al. 2013) to evaluate the uncertainty of the method.

The most abundant and homogenous consumed PPCPS could vary from region to region due to a number of factors including the population health status, prescription habits, availability and price. The PPCPS selected in the Australian model could be less popular and have more temporal fluctuation and geographical variation in China. Hence selection of more homogenous consumed PPCPS as potential population biomarkers could improve the reliability of model predictions. The possible contribution from industrial wastewater, especially pharmaceutical wastewater discharge from manufacturing and hospitals could also affect the model and associated population estimates in a given catchment, hence more detailed catchment survey could be considered in future studies. Variation of overuse and misuse of selected PPCPS could also contribute to the overall uncertainty in the population estimation.

We acknowledge that the excretion factor identified in literature is from studies with limited numbers of participants which could compromise the accuracy of consumption estimation in WWA applications. Due to the limited resources, the present study only sampled WWTPs located in big cities over a short period of time. Future sampling campaigns should cover both urban and rural areas across the

country to provide better understanding of the distribution of PPCPS consumption in China. In addition, a longer period of sampling could also contribute to the insight of the temporal consumption pattern of PPCPS.

App. 5.4 Conclusion

The population estimation model utilizing the PPCPS mass loads in the influent produced reasonable results in China considering the uncertainty related to the calibration data. Further calibration and validation of the model is needed with samples from WWTPs with different characteristics (sub-rural and rural areas) during the census period ideally to improve on the accuracy of the calibration population. Wastewater analysis could also reveal the consumption status of PPCPS in the population. Per capita consumption of the PPCPS included in this study was lower in China than in Australia. Within China, East China has higher consumption than the West and the catchments in North China have larger per capita consumption than South China. Multiple factors including but not limited to socio-economical characteristics of the catchment, life style, population health status, prescription habits, availability and price need to be further evaluated to better understand the PPCPS consumption behavior in the population.

App. 5 Acknowledgements

The National Research Centre for Environmental Toxicology (Entox) is a joint venture of The University of Queensland (UQ) and Queensland Health Forensic Scientific Services (QHFSS). Jianfa Gao receives a scholarship from the ARC Discovery (DP150100645). Jake O'Brien receives an APA PhD scholarship. Phong K. Thai is funded by the QUT VC Fellowship. Jochen F. Mueller is funded by the ARC Future Fellowship (FT120100546). The authors wish to thank all the people from the sampling WWTPs who assisted the sampling. We also give our sincere thanks to Andreas Scheidegger who contributed to the initial development of the model.

App. 5 Figures:

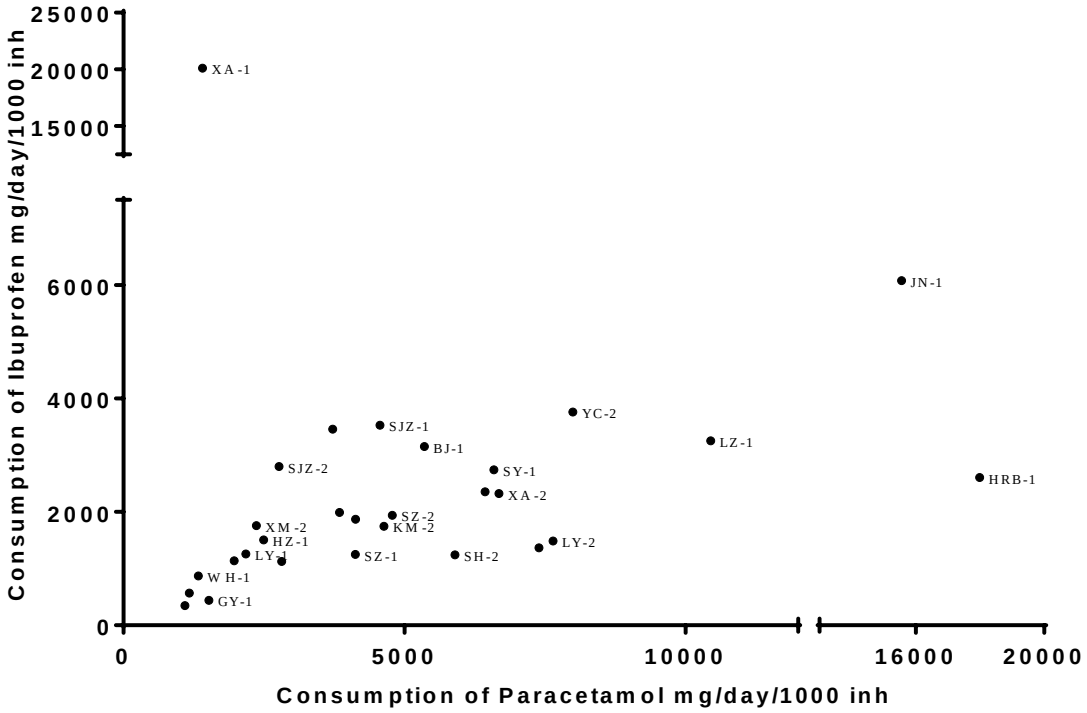


Figure S- 6 Estimated consumption of paracetamol and ibuprofen in China

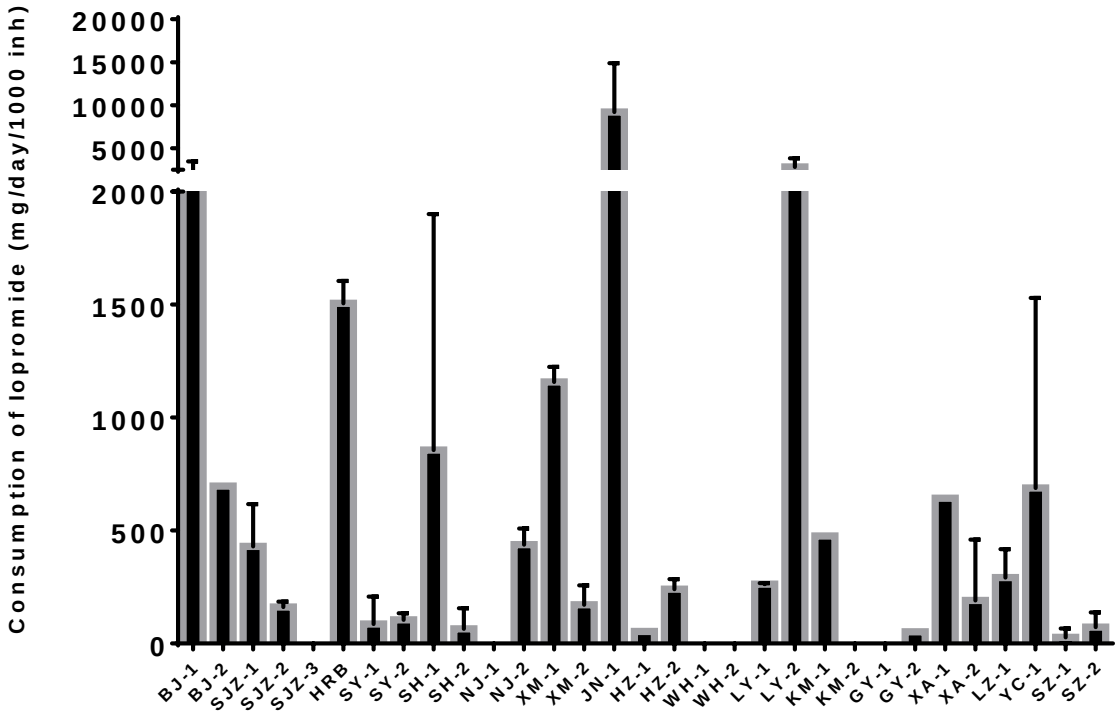


Figure S- 7 Iopromide consumption in China.

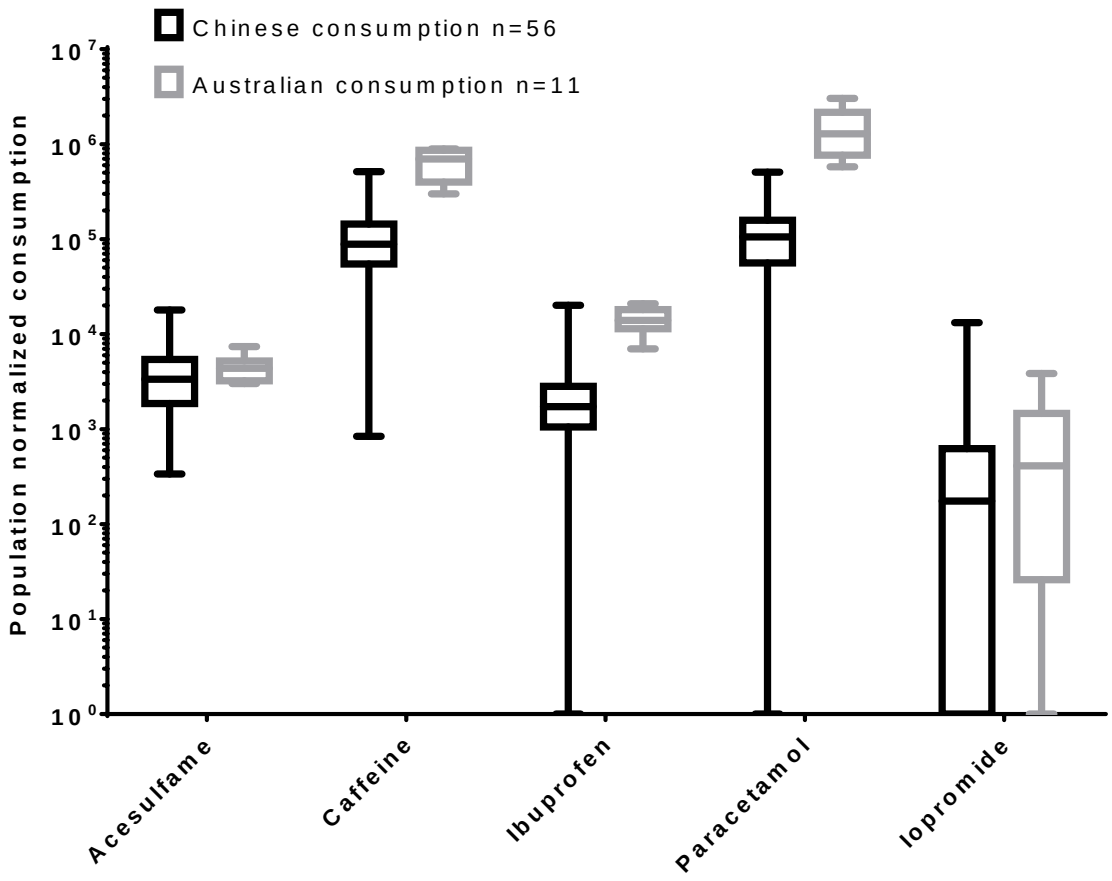


Figure S- 8 PNC of selected PPCPs in China and Australia

App. 5 Tables:

Table S- 11 Consumption estimation of selected PPCP in China (mg/day/1000 inh) (the human excretion factor accounted)

WWTP	Acesulfame	Caffeine	Ibuprofen	Iopromide	Paracetamol
BJ-1	5534	132250	3150	2097	5353
BJ-2	3393	132723	3456	697	3720
SJZ-1	5520	66961	3527	435	4562
SJZ-2	3995	37832	2800	163	2767
SJZ-3	2727	61502	1990	<28	3842
HRB-1	6967	142390	2607	1521	17991
SY-1	5754	59116	2741	87	6587
SY-2	4368	49846	1869	105	4128
SH-1	1442	107254	1367	865	7392
SH-2	1350	161759	1241	65	5898
NJ-1	1009	12738	567	<30	<2900
NJ-2	1687	78572	1125	442	2815
XM-1	2262	85177	567	1169	1171
XM-2	2633	86082	1757	174	2363
JN-1	7734	424232	6078	9315	15560
HZ-1	3411	125819	1504	54	2494
HZ-2	2288	131642	1138	243	1970
WH-1	2619	29358	870	<62	1330
WH-2	1170	19567	346	<34	1092
LY-1	4128	88748	1256	265	2175
LY-2	5190	105220	1487	2871	7640
KM-1	2335	78533	2353	481	6431
KM-2	2241	66132	1747	<47	4634
GY-1	740	23283	438	<9	1519
GY-2	337	841	<200	53	<1838
XA-1	17941	157744	20113	649	1405

XA-2	4310	94902	2323	192	6677
LZ-1	5689	115757	3253	295	10444
YC-2	14719	157906	3759	696	7993
SZ-1	2724	186244	1250	28	4125
SZ-2	3510	179257	1939	73	4783

Table S- 12 Population estimation from WWTP operators and PPCP model

WWTP	WWTP est.	Model Est.	Model/WWTP %
BJ-1	2420000	4840000	200
BJ-2	3450000	2798795	81
SJZ-1	1600000	2236145	140
SJZ-2	600000	592771	99
SJZ-3	760000	494458	65
HRB-1	550000	1033735	188
SY-1	800000	963855	120
SY-2	1200000	1445783	120
SH-1	200000	205622	103
SH-2	320000	336707	105
NJ-1	200000	197590	99
NJ-2	1560000	1491084	96
XM-1	1200000	1137349	95
XM-2	1200000	1214458	101
JN-1	410000	678394	165
HZ-1	2890000	2506988	87
HZ-2	2740000	1386506	51
WH-1	510000	499759	98
WH-2	740000	624096	84
LY-1	600000	616868	103
LY-2	670000	909478	136
KM-1	690000	786988	114
KM-2	670000	667309	100
GY-1	300000	291566	97
GY-2	1360000	1026827	76
XA-1	290000	321446	111
XA-2	1000000	1172691	117

LZ-1	600000	746988	124
YC-1	250000	259036	104
SZ-1	1500000	1192771	80
SZ-2	2000000	1044177	52

App. 5 Supporting Information

Table S- 13 Sewage treatment plants and sampling information

City	WWTP	Population served (million)	Sampling dates	Wastewater flow (m^3/d) (average flow)
Harbin	HRB-1	0.55	2014-10-12 (-3- 2014-10-13 (-2-	200000
Shenyang	SY-1	0.8	2014-07-13 (18- 2014-07-14 (17-	400000
	SY-2	1.2	2014-07-12 (18- 2014-07-14 (17-	385000
Yinchuan	YC-1	0.25	2014-08-03 (22- 2014-08-04 (18-	100400
Xi'an	XA-1	0.29	2014-09-12 (19-	170000
	XA-2	1	2014-09-14 (15- 2014-09-16 (15-	380000
Lanzhou	LZ-1	0.6	2014-09-10 (16- 2014-09-11 (15-	160000
Beijing	BJ-1	2.42	2014-07-22 (26- 2014-07-27 (25-	650000
	BJ-2	3.45	2014-07-27 (25- 35℃)	910000
Shijiazhuang	SJZ-1	1.6	2014-08-24 (21- 2014-08-25 (21-	493700
	SJZ-2	0.6	2014-08-24 (21- 2014-08-25 (21-	157800
	SJZ-3	0.76	2014-08-24 (21- 2014-08-25 (21-	202100

City	WWTP	Population served (million)	Sampling dates	Wastewater flow (m^3/d) (average flow)
Wuhan	WH-1	0.51	2014-08-03 (25- 2014-08-04 (25-	300000
	WH-2	0.74	2014-08-05 (27- 2014-08-11(21-	240000
Luoyang	LY-1	0.6	2014-09-11 (16- 2014-09-13 (17-	164300
	LY-2	0.67	2014-09-09 (20- 2014-09-10 (18-	190550
Jinan	JN-1	0.41	2014-09-18 (13- 2014-09-20 (17-	341100
Nanjing	NJ-1	0.2	2014-08-10 (23- 2014-08-11 (23-	58000
	NJ-2	1.56	2014-08-10 (23- 2014-08-11 (23-	640000
Hangzhou	HZ-1	2.89	2014-08-04 (26-	600000
	HZ-2	2.74	2014-08-03 (26- 2014-08-04 (26-	570000
Shanghai	SH-1	0.2	2014-09-05 (23- 2014-09-06 (24-	52200
	SH-2	0.32	2014-09-05 (23- 2014-09-06 (24-	75000
Xiamen	XM-1	1.2	2014-08-06 (26- 2014-08-08 (26-	262600
	XM-2	1.2	2014-08-06 (26- 2014-08-08 (26-	190000
Shenzhen	SZ-1	1.5	2014-09-21 (25- 2014-09-22 (24-	288500

City	WWTP	Population served (million)	Sampling dates	Wastewater flow (m^3/d) (average flow)
	SZ-2	2	2014-09-24 (25- 2014-09-29 (26- 2014-10-04 (21-	752000
Kunming	KM-1	0.69	2014-08-26 (17-	210000
	KM-2	0.67	2014-08-27 (16-	300000
Guiyang	GY-1	0.3	2014-08-31 (20-	25000
	GY-2	1.36	2014-08-31 (20-	250000

Table S- 14 Characteristics of selected PPCP

PPCP	Class	EF	IM	LOR (ppb)	DF (%)	CP	P value with population
Acesulfame	Artificial sweetener	more than 99% (Buerge et al. 2009)	Neg	0.05	100	0.63	<0.0001
Atenolol	Antagonist	88% (Reeves et al. 1978)	Pos	0.05	1.5	NA	NA
Atorvastatin	Antihyperlipidemic agent	less than 2% (Jernal et al. 1999)	Pos	0.1	0	NA	NA
Caffeine	Stimulant	2.4% (Blanchard et al. 1985)	Pos	0.1	100	0.77	<0.0001
Carbamazepine	Anticonvulsant	less than 1% (Morselli and Frigerio 1975)	Pos	0.05	14	-0.36	0.1182
Citalopram	Antidepressant	12% (Dalgaard and Larsen 1999)	Pos	0.1	1.5	NA	NA
Fluoxetine	Antidepressant	less than 10% (Lemberger et al. 1985)	Pos	0.1	0	NA	NA
Furosemide	Diuretic and antihypertensive agent	90% (Beermann et al. 1975)	Neg	0.2	0	NA	NA
Gabapentin	Anticonvulsant and analgesic	86% - 99% (Vollmer et al. 1986)	Pos	0.1	55	0.73	<0.0001
Hydrochlorthiazide	Diuretic and antihypertensive agent	65-72% (Beermann and Groschinsky-Grind 1977)	Neg	0.1	88	0.48	<0.0001
Ibuprofen	Analgesic and Anti-inflammatory	9% (Mills et al. 1973)	Neg	0.1	96	0.55	<0.0001
Iopromide	Contrast media	higher than 95%	Neg	0.1	73	0.06	0.0019
Naproxen	Anti-inflammatory and analgesic antipyretic agent	10% (Segre 1975)	Neg	0.1	14	0.92	<0.0001
Norfloxacin	Antibiotic	26-32% (R.Wise 1984)	Pos	0.2	0	NA	NA
Paracetamol	Antipyretic	2% (Davis et al. 1976)	Pos	0.2	95	0.40	<0.0001
Salicylic Acid	Metabolite	3%(Lehmann et al. 1973)	Neg	0.1	100	0.31	<0.0001
Temazepam	Antianxiety/hypnotic drug	1.5% (Schwarz 1979)	Pos	0.15	1.5	NA	NA
Tramadol	Narcotic analgesic	29% (Lintz et al. 1981)	Pos	0.1	9	NA	NA
Venlafaxine	Antidepressant	5% (Muth et al. 1991)	Pos	0.1	0	NA	NA

EF: Excretion Factor; IM: Ionization Mode; DF: Detection Frequency; Pos: Positive; Neg: Negative; CP: Correlation with Population (R^2); NA: Not Applicable

Table S- 15 Population normalized mass load (mg/day/1000 inhabitant) of selected PPCPs in the influent

WWTP	Acesulfame	Caffeine	Carbamazepine	Gabapentin	Hydrochlorothiazide	Ibuprofen	Iopromide	Naproxen	Paracetamol	SA	Tramadol
BJ-1	5479	3174	18	78	142	283	2076	< LOD	2677	6013	< LOD
BJ-2	3359	3185	15	59	132	311	697	28	1860	4138	< LOD
SJZ-1	5464	1607	79	56	170	317	430	39	2281	2578	181
SJZ-2	3955	908	35	33	113	252	161	< LOD	1384	1353	45
SJZ-3	2700	1476	< LOD	16	77	179	< LOD	14	1921	37812	51
HRB-1	6897	3417	< LOD	< LOD	181	235	1506	< LOD	8996	6058	< LOD
SY-1	5696	1419	< LOD	31	133	247	86	< LOD	3294	4228	< LOD
SY-2	4324	1196	< LOD	44	93	168	104	< LOD	2064	3259	< LOD
SH-1	1428	2574	< LOD	115	168	123	857	20	3696	4520	< LOD
SH-2	1337	3882	< LOD	87	132	112	65	< LOD	2949	2311	< LOD
NJ-1	999	306	< LOD	< LOD	< LOD	51	< LOD	< LOD	< LOD	224	< LOD
NJ-2	1670	1886	< LOD	46	98	101	437	< LOD	1407	1674	< LOD
XM-1	2240	2044	< LOD	< LOD	29	51	1157	< LOD	585	100	< LOD
XM-2	2607	2066	< LOD	< LOD	9	158	172	< LOD	1182	2419	< LOD
JN-1	7657	10182	< LOD	236	260	547	9222	47	7780	3885	< LOD
HZ-1	3377	3020	< LOD	54	45	135	53	< LOD	1247	8338	< LOD
HZ-2	2265	3159	6	53	40	102	241	< LOD	985	2058	< LOD
WH-1	2592	705	< LOD	< LOD	< LOD	78	< LOD	< LOD	665	755	< LOD
WH-2	1158	470	< LOD	< LOD	20	31	< LOD	< LOD	546	1199	< LOD

WWTP	Acesulfame	Caffeine	Carbamazepine	Gabapentin	Hydrochlorothiazide	Ibuprofen	Iopromide	Naproxen	Paracetamol	SA	Tramadol
LY-1	4087	2130	< LOD	< LOD	104	113	263	< LOD	1087	1176	< LOD
LY-2	5138	2525	< LOD	34	124	134	2842	< LOD	3820	2983	< LOD
KM-1	2312	1885	< LOD	74	100	212	476	< LOD	3215	4345	< LOD
KM-2	2219	1587	< LOD	46	114	157	< LOD	< LOD	2317	2542	< LOD
GY-1	733	559	< LOD	< LOD	14	39	< LOD	< LOD	759	1054	< LOD
GY-2	333	20	< LOD	< LOD	< LOD	< LOD	52	< LOD	< LOD	42	< LOD
XA-1	17762	3786	< LOD	< LOD	126	1810	643	< LOD	702	15432	< LOD
XA-2	4267	2278	< LOD	< LOD	72	209	191	< LOD	3338	1410	< LOD
LZ-1	5632	2778	< LOD	61	133	293	292	41	5222	4427	< LOD
YC-2	14572	3790	< LOD	51	179	338	689	< LOD	3996	10049	< LOD
SZ-1	2697	4470	< LOD	< LOD	24	112	27	< LOD	2063	3004	< LOD
SZ-2	3475	4302	< LOD	13	26	175	73	< LOD	2392	4997	< LOD

Table S- 16 Population estimates with North China group calibration

WWTP	WWTP est.	Model est.	Model/WWTP %
BJ-2	3450000	1856627	54
SJZ-3	760000	494458	65
SJZ-2	600000	515663	86
LY-1	600000	520482	87
XA-2	1000000	867470	87
SY-1	800000	777510	97
LZ-1	600000	597590	100
YC-1	250000	251004	100
XA-1	290000	333092	115
LY-2	670000	791084	118
SJZ-1	1600000	1940562	121
JN-1	410000	655341	160
BJ-1	2420000	4315181	178
SY-2	1200000	2361446	197
HRB-1	550000	1100000	200

Table S- 17 Population estimates with South China group calibration

WWTP	WWTP est.	Model est.	Model/WWTP %
SZ-2	2000000	1301205	65
GY-2	1360000	1015904	75
SZ-1	1500000	1337349	89
WH-2	740000	659759	89
HZ-2	2740000	2684980	98
GY-1	300000	296386	99
NJ-1	200000	199197	100
SH-1	200000	212048	106
WH-1	510000	540723	106

SH-2	320000	354699	111
KM-2	670000	791084	118
XM-1	1200000	1455422	121
HZ-1	2890000	3597992	124
NJ-2	1560000	2343133	150
KM-1	690000	1219277	177
XM-2	1200000	2197590	183

Table S- 18 Instrumental parameters for analysis of selected PPCP

ESI Positive													
		Quantification Transition						Confirmation Transition					
Compound	Retention time	Q1	Q3	DP	EP	CE	CXP	Q1	Q3	DP	EP	CE	CXP
Tebuconazole	6.46	308	70	50	10	45	10	308	125	50	10	55	15
Fluroxypyr	5.08	255	209	50	10	22	20	255	181	50	10	31	17
Pendimethalin	7.3	282.1	212.1	25	10	15	25	282.1	194.1	25	10	26	22
Trifluralin	7.14	336.3	220.1	30	10	23	27	336.3	206.1	30	10	25	27
Fluazipop	6.12	328.2	282.2	70	10	27	28	328.2	254.1	70	10	35	27
Paraxanthine	3.85	181	124	40	10	27	14	181	69	40	10	42	8
3,4 DiCl Aniline	5.39	162	127	55	10	28	12	162	74	55	10	68	12
5-HIAA	3.2	192.1	146.1	40	10	21	13	192.1	117.1	40	10	48	13
Ametryn	5.9	228.2	186	70	10	26	16	228.2	116	70	10	36	14
Asulam	3.65	231	156	65	10	14	13	231	108	65	10	27	10
Atenolol	3.13	267.2	190	50	10	25	17	267.2	145	50	10	36	12
Atorvastatin	6.6	559.5	440.3	70	10	27	30	559.5	250.2	70	10	58	24
Atrazine	5.66	216.1	174	71	10	25	16	216.1	96	71	10	34	12
Bromacil	5.31	261.2	205	35	10	21	18	263.2	207	35	10	21	18
Caffeine	4.58	195.1	138.1	71	10	26	16	195.1	110.1	71	10	30	14
Carbamazepine	5.88	237.2	194	86	10	29	14	237.2	193	86	10	45	12
Carbofuran	5.65	222.1	165.2	50	10	15	6	222.1	123	50	10	27	6
Chlorpyriphos	7.16	350.1	198	70	10	23	18	350.1	97	70	10	51	11
Citalopram	5.4	325.3	109	70	10	36	14	325.3	262.2	70	10	26	24
Clopyralid	2.25	192	110	75	10	45	10	192	146	75	10	27	16
Codeine	4.02	300.2	215.1	60	10	35	19	300.2	165.1	60	10	57	16
Cotinine	3.66	177.1	80	90	10	33	12	177.1	98	90	10	28	12
DEET	6.04	192.1	119	86	10	24	14	192.1	91	90	10	42	11
Desethyl Atrazine	4.6	188	146	70	10	24	17	188	104	70	10	35	12

ESI Positive													
		Quantification Transition						Confirmation Transition					
Desisopropyl Atrazine	3.87	174	104	70	10	32	10	174	96	70	10	25	9
Desmethyl Citalopram	5.4	311.3	109	60	10	33	10	311.3	262.2	60	10	23	22
Desmethyl Diazepam	6.34	271.2	140.1	70	10	39	16	271.2	165.1	70	10	37	18
Diazinon	6.65	305.3	169.1	50	10	27	15	305.3	249.1	50	10	25	22
Dichlorvos	5.3	221	109	50	10	19	10	221	127	50	10	18	12
Diuron	5.65	233.1	72	70	10	40	12	233.1	46	70	10	38	12
Fenamiphos	6.49	304.2	217.1	86	5.5	31	18	304.2	202.1	86	5.5	45	18
Flumeturon	5.37	233.1	72	70	10	36	12	233.1	46	70	10	36	8
Fluoxetine	5.6	310.1	44	40	10	42	12	310.1	148	40	10	11	15
Gabapentin	3.26	172.1	154	45	10	19	14	172.1	137	45	10	23	14
Hexazinone	5.86	253.2	171	70	10	22	16	253.2	71	70	10	40	8
Imazapic	5.12	276.1	231.1	80	10	26	22	276.1	163	80	10	33	16
Imazethapyr	5.5	290.1	177.1	80	10	35	20	290.1	106	80	10	57	14
Imidacloprid	5	256.1	209.1	60	10	23	14	256.1	175	60	10	23	12
Iopromide	4.02	792	573.1	80	10	33	34	792	559.1	80	10	43	34
Malathion	6.47	331.1	99	40	10	30	12	331.1	127	40	10	18	17
Methiocarb	6.17	226.1	169.2	45	10	14	12	226.1	121	45	10	23	11
Methomyl	4.18	163.1	88.1	35	10	11	10	163.1	106	35	10	13	10
Metolachlor	6.65	284.2	252	76	10	20	18	284.2	176	76	10	36	15
Metribuzin	5.49	215.1	187	70	10	23	16	215.1	47	70	10	70	6
Metsulfuron-Methyl	6.05	382.1	167	50	10	26	15	382.1	199	50	10	25	17
Naproxen +ve	6.17	231.2	185.1	64	10	21	15	231.2	170.1	64	10	35	11
Paracetamol	1.45	152.1	110	56	10	22	14	152.1	65.1	56	10	40	8
Picloram	3.31	243	197	80	10	31	13	243	143	80	10	59	17
Prometryn	6.25	242.2	158	70	10	33	18	242.2	200.1	70	10	26	20
Propiconazole	6.79	342	159	100	10	37	14	342	41	100	10	58	6
Propoxur	5.5	210.1	168.1	25	10	10	18	210.1	111	25	10	18	14
Simazine	5.33	202.1	132	70	10	25	15	202.1	124	70	10	25	14
Tebuthiuron	5.48	229.2	172	70	10	25	18	229.2	116	70	10	38	14
Temazepam	6.47	301.2	255.1	55	10	30	24	301.2	283.1	55	10	19	24
Terbuthylazine	6	230.1	174	80	10	23	16	230.1	104	80	10	45	13
Terbuthylazine Des ethyl	5.3	202	146	40	10	21	15	202	104	40	10	37	12
Terbutryn	6.31	242.2	91.2	46	10	37	8	242.2	71.1	46	10	43	10

ESI Positive													
		Quantification Transition						Confirmation Transition					
Tramadol	4.78	264.2	58	40	10	42	8	264.2	42	40	10	123	5
Venlafaxine	5.17	278.2	58	40	10	48	9	278.2	121	40	10	38	14
Atenolol D7	3.06	274.1	145.1	60	10	35	12	274.1	190.1	60	10	26	17
Caffeine 13C3	4.6	198.3	140.1	60	10	24	17	198.3	112.1	60	10	33	14
D2 5HIAA	3.16	194.1	148	60	10	21	17	194.1	120	60	10	38	14
Codeine D3	4.04	303.3	152	96	10	89	15	303.3	115	96	10	103	14
Cotinine-D4	3.36	181.2	84	85	10	32	10	181.2	98	85	10	28	10
D4 Acetyl Sulfamethoxazole	4.91	300.1	138.1	61	10	35	17	300.1	69.1	61	10	65	8
D5 Atrazine	5.64	221.1	179	60	10	25	14	221.1	101	60	10	34	12
D10 Carbamazepine	5.85	247.2	204.1	65	10	28	18	247.2	202.1	65	10	49	17
D6 Fluoxetine	5.6	316.2	44	46	10	40	12	316.2	154.2	46	10	11	16
D10Simazine	5.29	212	137	60	10	30	14	212	134	60	10	28	14
Temazepam D5	6.45	306.2	260.1	56	10	29	22	306.2	288.1	56	10	19	26
Tadalafil	6.38	390.2	268.1	90	10	16	25	390.2	204	90	10	78	19
Sildenafil	5.73	475.2	58	130	10	110	8	475.2	283.1	130	10	50	26
Simazine hydroxy	3.66	184.1	114	50	10	27	12	184.1	69	50	10	37	10
Simazine amine	4.14	183.1	113	50	10	27	12	183.1	68	50	10	42	10
mCPDMU	5.35	199.0	72	45	10	32	10	199.0	46	45	10	29	9
DCPU	5.25	205.03	127	45	10	38	13	205.01	162	45	10	21	15
DCPMU	5.44	219.01	127	45	10	35	13	219.02	162	45	10	20	15
Hexazinone oxy	4.88	267.1	171	50	10	27	16	267.1	71	50	10	42	8
Hexazinone hydroxy	5.12	269.1	171	50	10	27	16	269.1	71	50	10	42	8
Hexazinone desmethyl	5.59	239.1	157	50	10	27	16	239.1	71	50	10	42	8
Ametryn desethyl	5.26	200.1	158	50	10	27	12	200.1	68	50	10	50	12
Ametryn desisopropyl	4.76	186.1	144	50	10	27	14	186.1	116	50	10	32	10
Ametryn hydroxy	4.2	198.1	156	50	10	25	14	198.1	86	50	10	32	10
Norfloxacin	4.27	320.3	276.2	70	10	24	24	320.3	233.2	70	10	33	18
Norfloxacin D5	4.25	325.2	281.2	70	10	26	25	325.2	238.2	70	10	34	24
ESI Negative													

ESI Positive													
		Quantification Transition						Confirmation Transition					
		Quantification Transition						Confirmation Transition					
Compund	Retention time	Q1	Q3	DP	EP	CE	CXP	Q1	Q3	DP	EP	CE	CXP
Salicylic acid	4.11	137	93	-25	-10	-22	-8	137	65	-25	-10	-38	-8
Acesulfame	0.87	162	82	-25	-10	-18	-9	162	78	-25	-10	-43	-9
MCPA	5.72	199	141	-25	-10	-18	-12	201	143	-25	-10	-17	-12
Ibuprofen	6.33	205.1	161	-32	-10	-9.5	-15	205.1	159	-32	-10	-9	-15
Mecoprop	5.95	213	141	-25	-10	-17	-9	215	143	-25	-10	-15	-9
24 D	5.63	219	161	-16	-10	-19	-12	221	163	-16	-10	-15	-12
Dicamba	4.79	219	175	-25	-10	-10	-7	221	177	-15	-10	-8	-12
245T	5.97	252.9	194.9	-30	-10	-16	-17	254.9	196.9	-30	-10	-16	-17
Triclopyr	6.02	254	196	-15	-10	-12	-17	256	198	-15	-10	-14	-17
Bromoxynil	5.65	273.8	78.9	-60	-5	-48	-6	275.8	78.9	-70	-5	-54	-6
Triclosan	6.74	287	35	-30	-10	-33	-15	289	35	-30	-10	-30	-15
Hydrochlorthiazide	2.6	296	269	-90	-10	-26	-20	296	205	-90	-10	-31	-18
Furosemide	5.5	329	285	-37	-10	-19	-23	329	205	-37	-10	-29	-25
Haloxyfop	6.45	360	288	-30	-10	-19	-25	362	290	-30	-10	-19	-25
Acesulfame D4	0.86	166	86	-25	-10	-20	-9	166	78	-25	-10	-43	-9
13C6 24D	5.63	225	167	-16	-10	-19	-15	227	169	-16	-10	-16	-15
DCPA	5.75	203	159	-20	-10	-10	-7	205	161	-15	-10	-8	-7
Hydrochlorthiazide ^{13}CD2	2.55	298.9	269.9	-115	-10	-27	-21	298.9	205.9	-115	-10	-32	-15
Ibuprofen D3	6.3	208.1	164	-30	-10	-10	-15	208.1	161	-30	-10	-9	-13
Isoxaflutole	5.14	358.2	79	-60	-10	-21	-8	358.2	64	-60	-10	-75	-10
Fluroxypyr	5.07	253	195	-40	-10	-11	-17	253	233	-40	-10	-21	-22
2,4 DB	6.27	247	161	-35	-10	-20	-15	249	163	-35	-10	-21	-15

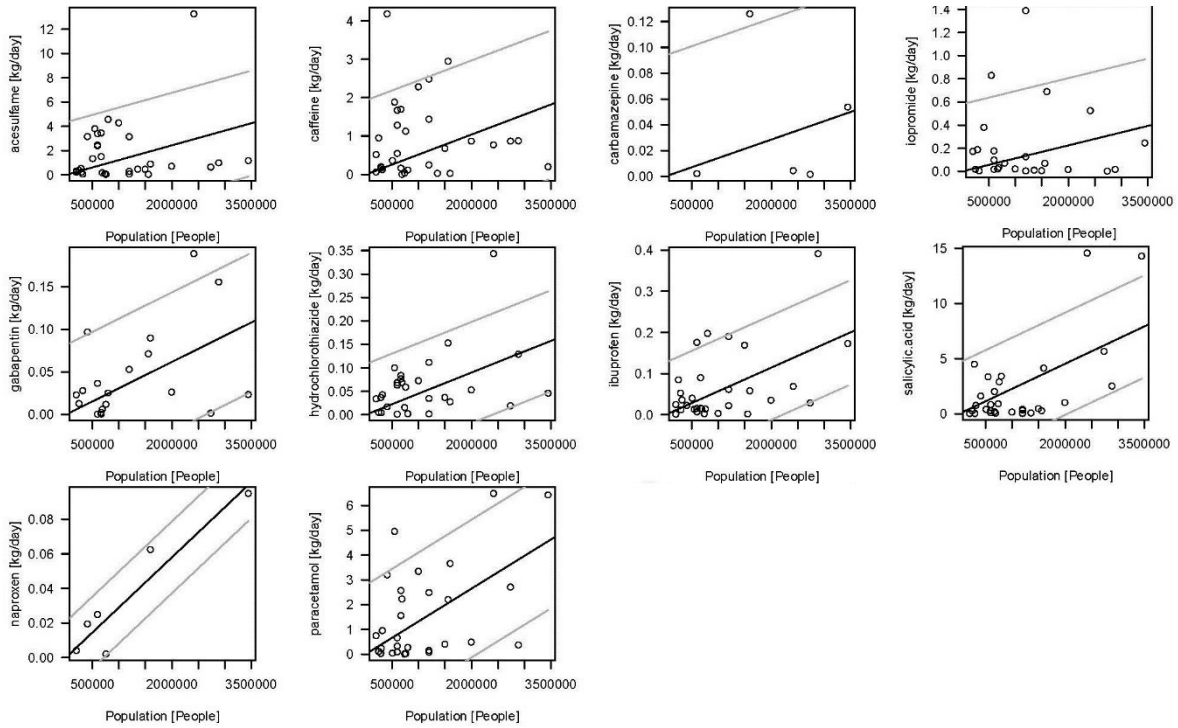


Figure S- 9 Official population versus daily mass load of selected PPCP. The black line is the line of best fit. The gray lines indicates the 90% prediction interval

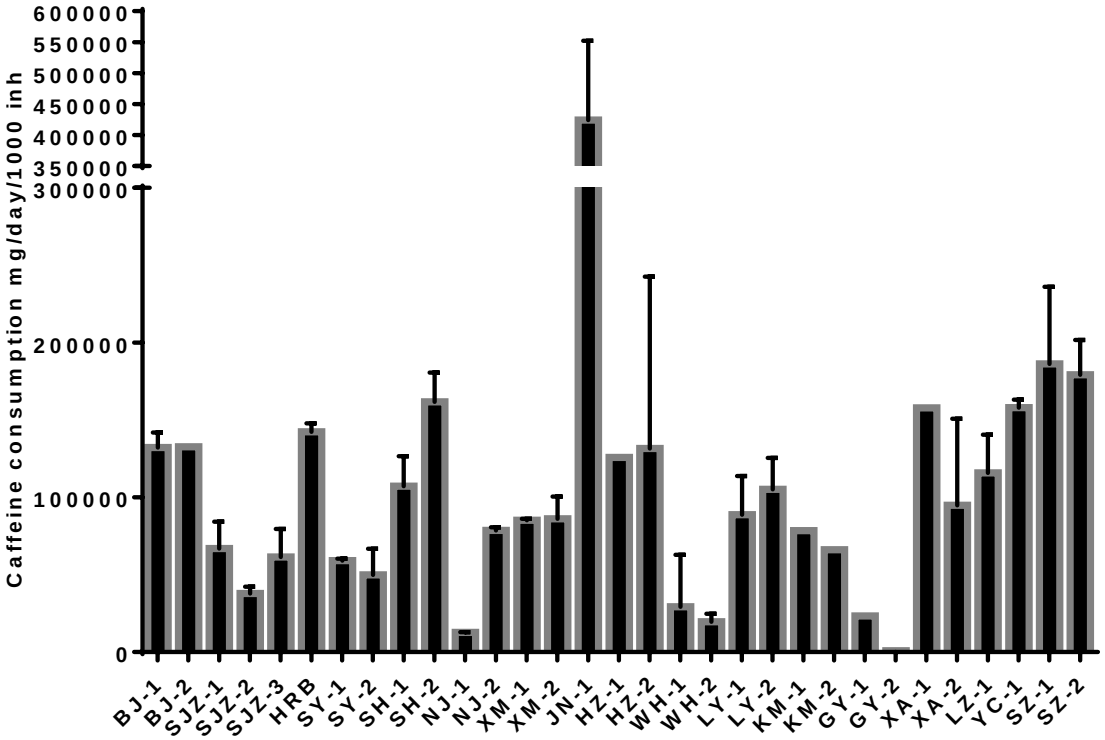


Figure S- 10 Caffeine consumption in selected STP catchment area

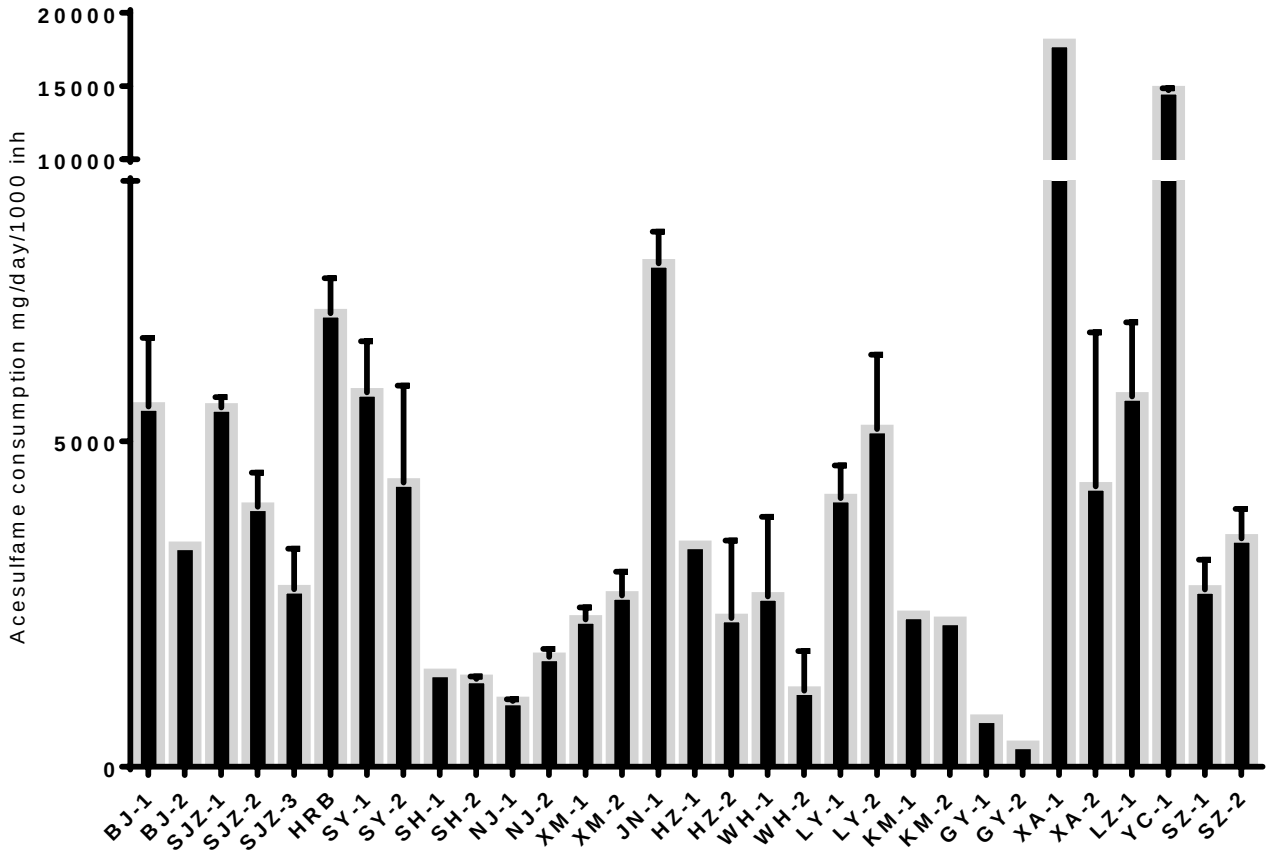


Figure S- 11 Acesulfame consumption in China

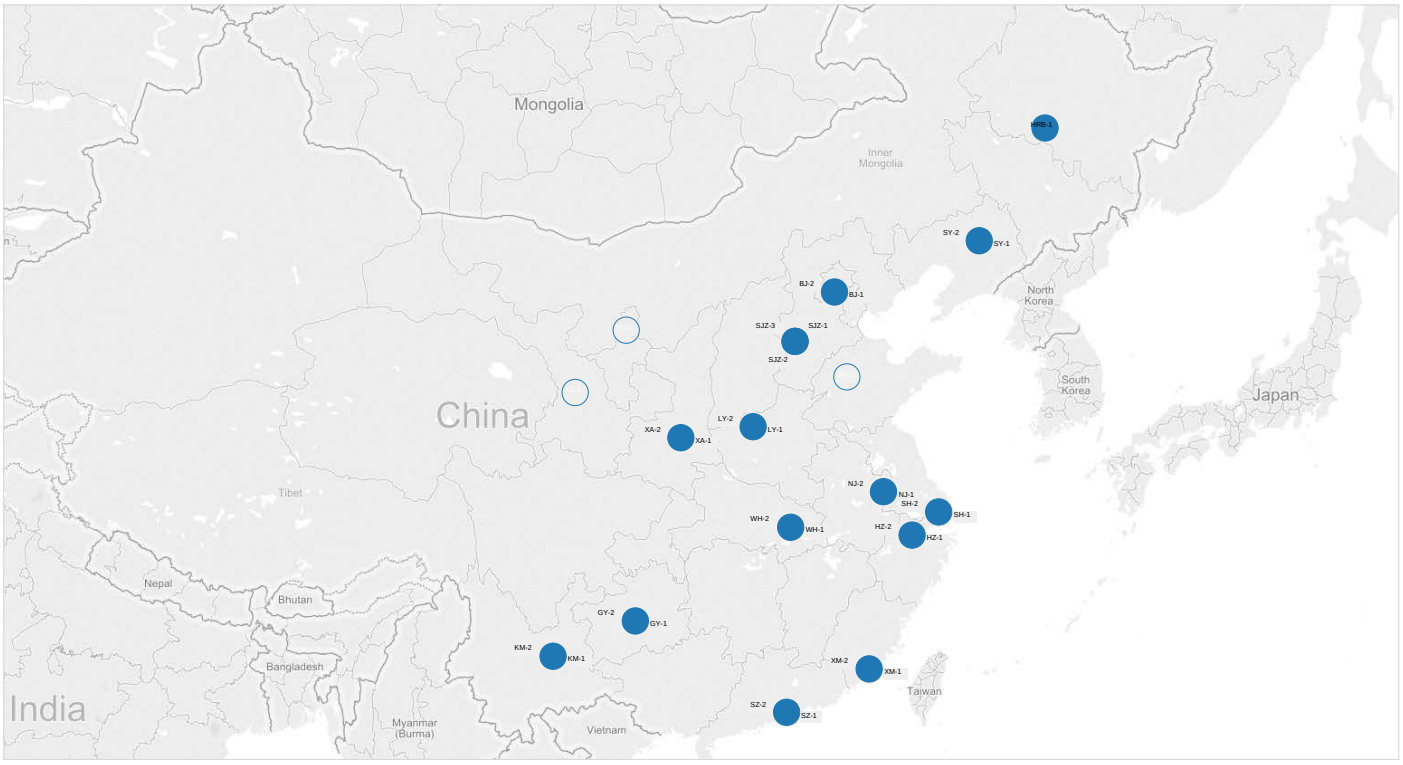


Figure S- 12 Map of investigated WWTPs

Appendix 6: Systematic and Day-to-Day Effects of Chemical-Derived Population Estimates on Wastewater-Based Drug Epidemiology

The following publication is incorporated as Appendix 6:

Lai, F.Y., Anuj, S., Bruno, R., Carter, S., Gartner, C., Hall, W., Kirkbride, K.P., Mueller, J.F., **O'Brien, J.W.,** Prichard, J., Thai, P.K., Ort, C., 2014. Systematic and Day-to-Day Effects of Chemical-Derived Population Estimates on Wastewater-Based Drug Epidemiology. Environ Sci Technol. 49, 999−1008.

Peer–reviewed and published by Environmental Science and Technology
Accepted author manuscript

Systematic and day-to-day effects of chemical-derived population estimates on wastewater-based drug epidemiology

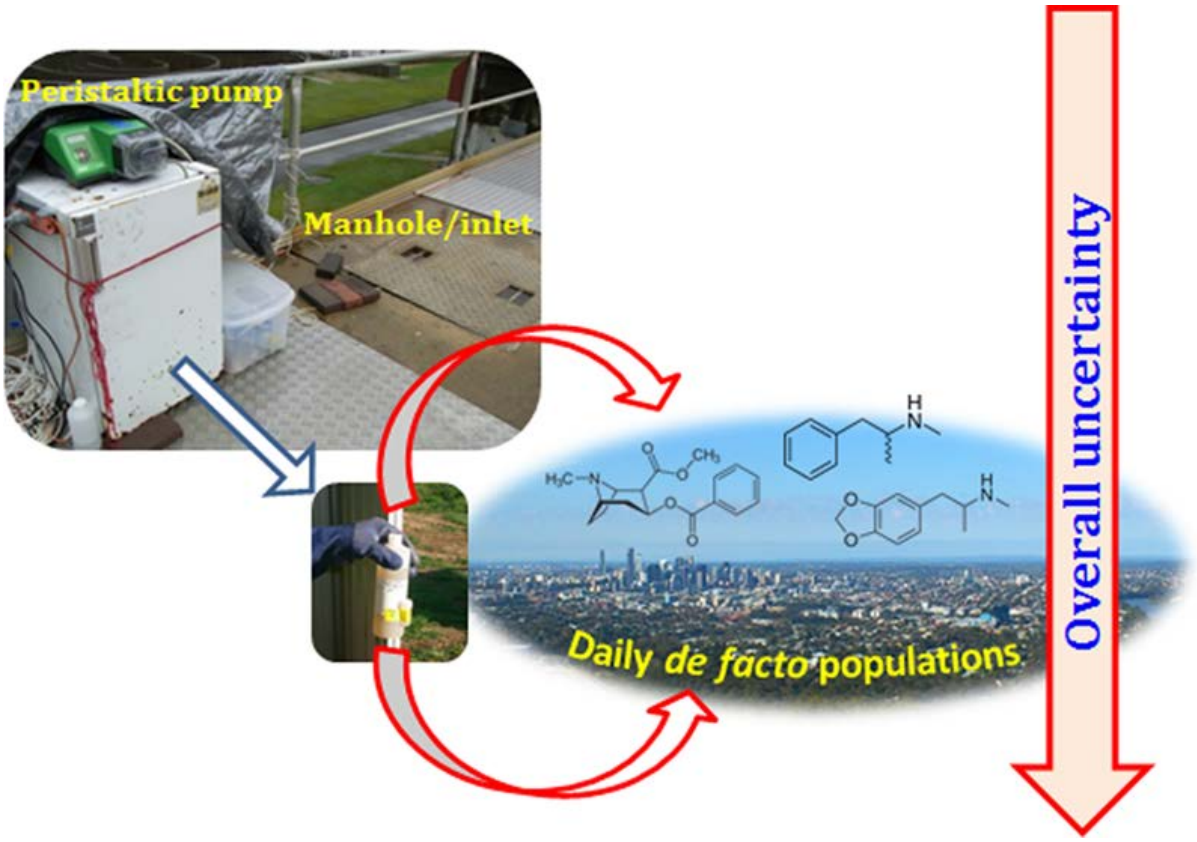
Foon Yin Lai [a,*], Shalona Anuj [a,b], Raimondo Bruno [c], Steve Carter [b], Coral Gartner [d], Wayne Hall [e], K. Paul Kirkbride [f], Jochen F. Mueller [a], Jake W. O'Brien [a], Jeremy Prichard [g], Phong K. Thai [a], Christoph Ort [h,i,*]

a. The University of Queensland, The National Research Centre for Environmental Toxicology, 39 Kessels Road, Coopers Plains, QLD 4108, Australia.
b. Queensland Health Forensic Scientific Services, Queensland Government, 39 Kessels Road, Coopers Plains, QLD 4108, Australia.
c. School of Psychology, University of Tasmania, Private Bag 30, Hobart, TAS 7001, Australia.
d. The University of Queensland, Centre for Clinical Research, Royal Brisbane and Women's Hospital, Herston, QLD 4029, Australia.
e. The University of Queensland, Centre for Youth Substance Abuse Research, Royal Brisbane and Women's Hospital, Herston, QLD 4029, Australia.
f. School of Chemical and Physical Sciences, Flinders University, Adelaide, SA 5001, Australia.
g. Faculty of Law, University of Tasmania, Private Bag 89, Hobart, TAS 7001, Australia
h. Swiss Federal Institute of Aquatic Science and Technology (Eawag) Urban Water Management, Ueberlandstrasse 133, CH 8600, Duebendorf, Switzerland.
i. The University of Queensland, Advanced Water Management Centre (AWMC), Brisbane, QLD 4072, Australia.

*Corresponding authors' contact details:
Dr. Foon Yin LAI: The University of Queensland, The National Research Centre for Environmental Toxicology (Entox), 39 Kessels Road, Coopers Plains, QLD 4108, Australia; Tel.: +61 (0)4 06714601; Fax.: +61 (0)7 3274 9003; E-mail: foon.lai@uqconnect.edu.au
Dr. Christoph ORT: Swiss Federal Institute of Aquatic Science and Technology, Eawag, Urban Water Management, Ueberlandstrasse 133, CH-8600, Duebendorf, Switzerland; Tel.: +41 (0)58 765 52 77; E-mail: christoph.ort@eawag.ch

Abstract: Population size is crucial when estimating population- normalized drug consumption (PNDC) from wastewater-based drug epidemiology (WBDE). Three conceptually different population estimates can be used: de jure (common census, residence), de facto (all persons within a sewer catchment), and chemical loads (contributors to the sampled wastewater). De facto and chemical loads will be the same where all households contribute to a central sewer system without wastewater loss. This study explored the feasibility of determining a de facto population and its effect on estimating PNDC in an urban community over an extended period. Drugs and other chemicals were analyzed in 311 daily composite wastewater samples. The daily estimated de facto population (using chemical loads) was on average 32% higher than the de jure population. Consequently, using the latter would systemically overestimate PNDC by 22%. However, the relative day-to-day pattern of drug consumption was similar regardless of the type of normalization as daily illicit drug loads appeared to vary substantially more than the population. Using chemical loads population, we objectively quantified the total methodological uncertainty of PNDC and reduced it by a factor of 2. Our study illustrated the potential benefits of using chemical loads population for obtaining more robust PNDC data in WBDE.

Abstract Art:



App. 6.1 Introduction:

Measuring drug residues in raw sewage−subsequently referred to as wastewater-based drug epidemiology (WBDE) has become an important tool to estimate illicit drug use worldwide. The final estimate is typically presented on a basis of per capita consumption (i.e., consumed mass of a drug per capita per day). The back-estimation methodology of WBDE relies on a number of parameters, including concentrations of drug residues, wastewater volumes, excretion fractions, and population size.(Zuccato et al. 2008) There is uncertainty in each of these parameters. One key component concerns the number of people in a catchment, which can substantially affect the accuracy of the final estimate and comparisons of data across different communities.(Castiglioni et al. 2014, Zuccato et al. 2008) The variability and accuracy of this parameter cannot be easily estimated. In most WBDE studies, the population parameter has been obtained either from the design capacity of wastewater treatment plants (WWTPs) or the most recently available census data.(van Nuijs et al. 2011a) Design capacity usually refers to population equivalents including industrial pollutant loads and also refers to a certain (long-term) planning horizon. For these two

reasons, usually less people contribute to the wastewater than indicated by design capacity. In exceptional circumstances, WWTPs may also operate above design capacity to receive wastewater from more people than expected. However, this is difficult to assess and quantify objectively. Census data, taken on a specific day, provide only a single estimate of a population size; typically, it refers to a de jure population, which is counted according to home address, but does not provide important information on whether people are actually within the WWTP catchment under investigation or elsewhere on a specific day. Relying on such a fixed population size may not be practical to WBDE.(Zuccato et al. 2008)

Researchers have recommended that attempts should be made to estimate the number of people effectively contributing to a wastewater sample; e.g., see refs (Zuccato et al. 2008), (van Nuijs et al. 2011a) and (Daughton 2012a). A few indicators have been proposed to estimate de facto populations by means of chemical loads measured in wastewater. Theoretically, if all households in the catchment of a WWTP are connected to a central sewer system, and assuming that no wastewater losses occur - e.g., leaky sewers, wastewater bypassing the WWTP during rainy periods, or abnormal operational situations - the population estimated from chemical loads in wastewater is a fair approximation of *de facto* population. Therefore, *de facto* subsequently refers to both *de facto* population and population estimated from chemical loads.(Daughton 2012a) For practical applicability in WBDE it appears important that (i) selected chemicals are human-specific; (ii) the method is applied over an extended period of consecutive days (to reveal day-to-day variation); (iii) methodological uncertainties can be quantified objectively; and (iv) the relevance of the parameter populations size is determined in the context of the overall methodological uncertainty of WBDE. Human-specific chemicals were proposed but not validated in five studies, namely, creatinine,(Chiaia et al. 2008),(Brewer et al. 2012) coprostanol,(Daughton 2012a) cholesterol, cotinine, and a neurotransmitter metabolite, 5- hydroxyindoleacetic acid,(Chen et al. 2013) and commonly prescribed pharmaceuticals and artificial sweeteners.(Lai et al. 2011) Only two studies were found in which the measurement was over an extended period: one covered 235 days in a 1 year period using hydrochemical parameters (nitrogen, phosphorus, biological oxygen demand, and chemical oxygen demand), and another one covered 13 days in an 8 month period using ammonium;(van Nuijs et al. 2011c),(Been et al. 2014) those selected chemicals are not human-exclusive. Only one recent publication provides an approach to quantify objectively the methodological uncertainty of the population estimates.(O'Brien et al. 2014) Lastly, the two studies addressing population size estimation in the context of overall uncertainty were review-type reports that did not provide new methods.(Castiglioni et al. 2014),(Castiglioni et al. 2013) In summary, to the best of our knowledge, no study to date has addressed all four aspects listed previously in a comprehensive manner that is pertinent to their applicability in WBDE.

Furthermore, most studies rely on a day-specific excretion fraction by one person, which does not allow an objective quantification of associated uncertainty if these values are either missing or unreliable.

Recently, a multisubstance model to estimate *de facto* population was developed and calibrated with wastewater samples collected on a Census Day.(O'Brien et al. 2014) Applying this model facilitates the examination of the variation in daily *de facto* populations over time in large catchments and consequently its effect on the estimation of population-normalized illicit drug loads in WBDE particularly when compared to using a constant population number such as that from census data. The aims of our study were to (a) estimate a day-specific *de facto* population using the recently developed model in a large catchment on consecutive days over an extended period, (b) compare the estimated *de facto* populations with the de jure population and the effects of using the different population estimates on temporal patterns and levels of illicit drug use, and (c) evaluate whether the overall methodological uncertainty of WBDE can be reduced and better quantified using day-specific estimated *de facto* populations.

App. 6.2 Materials and Methods

App. 6.2.1 Wastewater Sampling.

The sampling was set up at the inlet of a wastewater treatment plant that served a mainly urban catchment in South East Queensland (Australia) with a de jure population of 211,340 people and a de facto population of 230,117 on Census Day (Aug. 11, 2011) according to the Australian Bureau of Statistics.(Australian Bureau of Statistics (ABS) 2012) Samples were collected between June 2011 and June 2012 (n = 311 days; see Table S- 20 in the Supporting Information for missing data (n = 33 days) due to logistical or technical reasons). A continuous flow- proportional sampling technique was applied to ensure collection of representative daily composite samples (from 6 AM to 6 AM the next day).(Ort et al. 2010b),(Ort et al. 2010a) The samples were refrigerated at 4 °C during collection, acidified on site to pH 2 using 2 M hydrochloric acid, and then frozen until analysis. This preservation method has been commonly used and can stabilize the targeted residues of illicit drugs in wastewater during storage; e.g., see refs (Chiaia et al. 2008) and (van Nuijs et al. 2012). Data on daily wastewater volumes were recorded by the WWTP.

App. 6.2.2 Analysis of Targeted Compounds.

Samples were analyzed for the targeted illicit drug residues and high-use chemicals using liquid chromatography (Shimadzu, Nexera UHPLC system, Kyoto, Japan) coupled with tandem mass

spectrometry (AB SCIEX QTRAP5500, Ontario, Canada) (LC-MS/MS). The analytical method applied in this study has
been validated and described previously.(Lai et al. 2011),(O'Brien et al. 2014) Briefly, an electronic robot (Tecan Genesis Workstation 200, Australia) was used to transfer filtered samples into a vial which were then spiked with mixtures of carbon-labelled and deuterium-labelled chemical standards (Table S- 21 of the Supporting Information) for compensating potential instrumental variability and matrix effects during analysis. Separation of the targeted analytes was performed on a C18 LC analytical column using gradient mobile phases (Table S- 21 of the Supporting Information). Together with the calibration standards, concentrations of the targeted analytes were measured using mass spectrometry with a multireaction monitoring (MRM) scheme. Two MRM transitions were used for identification and quantification of each analyte. (Lai et al. 2011),(O'Brien et al. 2014) Concentrations of the targeted analytes have taken the recovery of the spiked mass-labelled standards into account (i.e., isotope dilution method). In every batch of analysis, Milli-Q water samples (i.e., procedural blanks), duplicate samples, and samples spiked with native chemicals were included as quality assurance and control (QA/QC) of the analysis. The QA/QC results are summarized in Table S- 22 of the Supporting Information. Briefly, no contamination was found in the blank samples. The difference (coefficient of variance, CV (%)) between duplicate samples was on average 4.8−7.9% for the illicit drug residues and 4.2− 9.4% for the high-use chemicals. Recovery of the native chemicals spiked in the samples was 75−81% for the illicit drug residues and 99−113% for the high-use chemicals. Interday variation across 3 days was 2.3−11% for the illicit drug residues and 4.7−21% for the high-use chemicals.

App. 6.2.3 Estimating Daily de Facto Populations.

Day-specific *de facto* populations and confidence intervals were estimated with the multisubstance model.(O'Brien et al. 2014) Details on the calculation (calibration, Bayesian inference, and validation) can be found in O'Brien et al.(O'Brien et al. 2014) Briefly, we calibrated the model with mass loads of 14 chemicals in the influent of 10 WWTPs catchment sizes ranging from approximately 3,500 to 500,000 people) and *de facto* populations, both determined on or around the last Australian Census Day. It should be noted that normally census data refer to *de jure* populations; however, in Australia, *de facto* populations are also determined on Census Day.(O'Brien et al. 2014) In this study, we only used eight chemicals that can be readily measured in the samples without pre-concentration, consistent with the measurement of the targeted illicit drug residues. These eight high-use chemicals included acesulfame, atenolol, caffeine, carbamazepine, codeine, hydrochlorothiazide, naproxen, and salicylic acid. Model calculations indicated that the use of eight chemicals provided *de facto* population estimates consistent with

using 14 chemicals (Table S- 23 of the Supporting Information). It should be noted that any variability that is unknown or cannot be quantified explicitly was implicitly accounted for when calibrating the model with mass loads of the high-use chemicals in wastewater from various catchments and accurate population sizes. The uncertainties encompass systematic or random effects due to, e.g., disposals of unused chemicals, unknown absolute excretion rates, day-to-day variations of actual consumption, and transformations of chemicals in sewer systems, sampling, and storage (see O'Brien et al. for more explanation).(O'Brien et al. 2014) It is a fair assumption that the system under investigation (e.g., catchment and sewers), and the relevant processes (e.g., average consumption habits, pharmacokinetics, and transformation in sewers) did not change substantially between the Census Day and our monitoring period.

App. 6.2.4 Population-Normalized Drug Consumption.

The back-estimation method follows the previously proposed equation (see Supporting Information).(Zuccato et al. 2008) Briefly, the estimation involves three main steps: (a) the mass loads (mg/day) of drug residues are obtained by multiplying concentrations (μg/L) with total wastewater flow (ML/day); (b) the estimated mass loads are then extrapolated to the consumed amount with a correction factor taking the average excretion rate and the ratio of molecular weight between the parent drug to its metabolite into account (Table S- 24 of the Supporting Information); and (c) the consumed amount is normalized to a population size to result in the collective consumption of the population ((mg/ day)/(1000 people)). We chose, when analytically possible, two drug residues (i.e., dual tracers, the unchanged parent drug and metabolite) to back-estimate consumption of the parent drug (Table S- 22 of the Supporting Information): cocaine and benzoylecgonine, respectively, for estimation of cocaine; methamphetamine and amphetamine, respectively, for estimation of methamphetamine (as illicit amphetamine use is rare in Australia);((ACC) 2013) and only MDMA (3,4-(methylenedioxy)-methamphetamine) itself for estimation of MDMA. These targeted residues have been demonstrated to be adequate for back-estimating consumption of the corresponding parent drug; see, e.g., refs (Zuccato et al. 2008), (van Nuijs et al. 2011a), and (Khan and Nicell 2011, 2012, Zuccato et al. 2011). Consistent patterns and consumption rates obtained from the two residues allow verification of the reliability of the estimate of the parent drug.(Lai et al. 2011)

App. 6.2.5 Uncertainty Analysis.

Previous studies have revealed the uncertainty components associated with the back- estimation methodology of WBDE.(Castiglioni et al. 2014),(Lai et al. 2011) Five components were considered for contribution to the overall uncertainty (U_{tot}) for the final estimate of population-normalized drug consumption (PNDC; (mg/day)/(1000 people)); we refer to the U_{tot}*(de jure)* as the total uncertainty

of the *de jure*-PNDC and U_{tot}*(de facto)* as the total uncertainty of the *de facto*-PNDC. These encompass uncertainties related to sampling (U_S), chemical analysis (U_C), flow measurements (U_F), excretion rates (U_E), and population size (U_P). The magnitude of each uncertainty component is presented as relative standard deviations (RSD, %)8 as determined from modelling results for U_S and U_P, interday variability of instrumental analysis for U_C, literature data for U_E, and estimates from WWTP operators for U_F (Table S- 19). These uncertainty components are independent from each other, because they arise from individual aspects of studies and the methods of calculating them. As the primary metabolite chosen for back-estimating consumption of the three illicit drugs is relatively stable under typical sewer conditions,(Thai et al. 2014a) we therefore consider the uncertainty of in-sewer chemical biodegradation as negligible. This study used Monte Carlo simulation to estimate U_{tot} as described previously.(Jones et al. 2014, Ort et al. 2014a) Linear error propagation as suggested earlier, see, e.g., ref (Lai et al. 2011), only provides a good approximation of U_{tot} if U_P is small and also other uncertainty components do not exceed a certain value. Otherwise the linear error propagation tends to systematically underestimate U_{tot}.

App. 6.2.6 Statistics.

Nonparametric Mann−Whitney tests (unpaired) were used to examine significant differences between weekdays and weekends for the estimated *de facto* populations and for illicit drug consumption. Nonparametric Wilcoxon tests (paired) were used to assess significant differences between population-normalized consumption based on *de jure* and *de facto* populations. The statistics were performed using GraphPad Prism (version 6.00, GraphPad Software Inc.).

App. 6.3 Results and Discussion

App. 6.3.1 Estimated Day-Specific *de Facto* Populations.

The *de facto* population for individual days was estimated in a range between 96,400 and 466,000 (minimum−maximum; 90% interquantile, 96,400−304,000 (25% of all days were outside this range)), with an average of 280,000 persons and a variation (CV) of 15.6% throughout 311 monitoring days (Figure S- 13A). The variation of our data is similar to that reported in Brussels over 235 days (CV, 18%; 90% interquantile, 495,000− 1,040,000 (25% of all days outside this range)).(van Nuijs et al. 2011c) We observed a mild seasonal variation in the estimated *de facto* population in the catchment: it gradually increased from approximately 264,000 to 284,000 persons (monthly average; Table S- 25 of the Supporting Information) between July and September, remained at about 300,000 persons from October to December 2011, was recorded as the highest estimate of 310,000 persons in January 2012, and then slowly decreased from 294,000 persons in February to 243,000 persons in June 2012. The yearly average *de facto*

population on the weekends (~280,000 persons) was similar to that during the weekdays (~278,000 persons) over the study period, as were most of the months (Figure S- 13A). The variation (CV) of the estimated *de facto* population among individual week days was relatively lower for Monday (13%), Friday (14%), and Sunday (13%) but slightly higher for Saturday (18%) and from Tuesday to Thursday (16−17%). There was also a significant difference in the estimated de facto populations between Sundays and Wednesdays−Saturdays ($p = 0.005−0.03$) and between Mondays and Tuesdays−Saturdays ($p < 0.0001−0.005$).

App. 6.3.2 Comparison between Two Different Population Estimates.

The estimated *de facto* population (~280,000 people) is on average about 32% higher than the de jure population (i.e., ~211,000 people on the Census Day) (Figure S- 13B). For almost all days, the estimated de facto population was higher than the *de jure* population (Figure S- 13A). The difference ranged from +2.1 to +120% (Figure S- 13B). On only 17 days was the estimated *de facto* population lower than the *de jure* population, ranging from −2.8 to −54% (Figure S- 13B). The overall differences between the estimated *de facto* population and the *de jure* population appear reasonable because the studied catchment is one of the most urbanised areas in South East Queensland and it is a popular destination for domestic and international visitors with many resorts, music festivals, theme parks, and tourist attractions that operate throughout the year. High commuting flow is thus common in the catchment area. While the difference between the estimated de facto population and the de jure population is in a range of 22− 44% (25−75 percentile) for half of the monitoring days (Figure S- 16 of the Supporting Information), there were some days on which there was a substantial increase or decrease in the estimated *de facto* population compared to the *de jure* population (Figure S- 13B). For example, an elevated de facto population was estimated on Nov. 28, 2011 (+101%, Figure S- 13B), Jan. 26, 2012 (+120%, Figure S- 13B) and Jan. 30, 2012 (+94%, Figure S- 13B). We noticed a coincidence between a high rainfall level recorded from Jan. 23, 2012 to Jan. 30, 2012 (peak rainfall on Jan. 25, 2012 with ~230 mm)(Government) and an increase in the *de facto* population on Jan. 26, 2012 and Jan. 30, 2012. However, it should be noted that the *de facto* population, estimated based on the daily mass loads of the high-use chemicals, cannot be driven by the increase in wastewater volumes and is independent of the dilution factor, as long as the lowered concentrations of the chemicals are still above the quantification limits. This can be seen from the data, for example, on Mar. 4, 2012 and Mar. 29, 2012, when increased wastewater volumes due to rainfall around these days (Figure S- 17A) did not result in an increase in estimated de facto population (Figure S- 13A,B). Higher *de facto* population estimates may reflect either an increase in per capita consumption of the high-use chemicals or more people being present who consume the usual per

capita amount of the chemicals. The observed elevated *de facto* population appears reasonable because (a) every November is a special period when young teenagers gather in this urban catchment to celebrate their completion of secondary school and (b) the 26th of January is the Australia Day public holiday, so the catchment attracts a lot of people for holidays and celebration activities. On some of the monitoring days, the estimated *de facto* population was substantially lower than the *de jure* population (Figure S- 13A), for example, the data on Jan. 24, 2012 (−48%, Figure S- 13B) and Apr. 28, 2012 (−54%, Figure S- 13B). Heavy rainfall events were recorded in the catchment around those 2 days. This may have led to a diversion of wastewater (combined sewer overflows), resulting in substantially decreased mass loads of high-use chemicals entering the WWTP. However, PNDC is still thought to be unbiased as explained at the end of the next section.

App. 6.3.3 Effects on PNDC Using Different Population Estimates.

The deviation between the de facto-PNDC and the de jure-PNDC is calculated as the following:

$$\text{deviation}/\% = \frac{\frac{\text{drug loads}}{de\ facto\ \text{population}} - \frac{\text{drug loads}}{de\ jure\ \text{population}}}{\frac{drug\ loads}{de\ jure\ \text{population}}}$$

$$= \left(\frac{de\ jure\ \text{population}}{de\ facto\ \text{population}} - 1\right) \times 100$$

This gives negative deviations (−2 to −54.6%) for most of the monitoring days (Figure S- 13C) since the PNDC is reduced when the drug load is normalized to the estimated *de facto* population which is greater than the *de jure* population. By contrast, the deviation becomes positive (+2.9 to +119%) when the drug load is normalized to an estimated *de facto* population that is smaller than the *de jure* population (Figure S- 13C). There were significant differences ($p < 10^{-4}$) between the *de facto* population normalized and *de jure* population normalized consumption of the three illicit drugs. Over the monitoring period, the PNDC based on the estimated *de facto* populations was systematically lower by 22% (Figure S- 13C) because the average *de facto* population was 32% higher than the *de jure* population (Figure S- 13B). This indicates that the model of estimating the *de facto* populations allows for the assessment of how systematic differences in the population drug consumption are related to changes in the number of people who contributed in the sampled wastewaters in the studied catchment. While there were absolute differences in the PNDC estimated using the two different population estimates, the relative day-to-day pattern of consumption for the three illicit drugs did not significantly change over the monitoring period (Figure S- 19 of the Supporting Information). The variation of PNDC was very similar, regardless of whether one used the estimated *de facto* or *de jure* population (*de facto*/*de jure*: for cocaine, 55%/59%; for methamphetamine, 38%/37%; for MDMA, 132%/138%) (CV, in Table S- 26 of the

Supporting Information). The same was true for variations between weekdays and weekends over the year (Table S- 26 of the Supporting Information). Our data are in line with those of a recent study(Ort et al. 2014a) which summarized and reported that only a small variation in a PNDC was observed between estimates made using *de jure* and *de facto* populations. For example, in Brussels, using hydrochemical parameters for *de facto* populations and census for *de jure* populations (census = 1.1 million people), the CVs for PNDC differed only slightly (*de facto*/*de jure*: for cocaine, 33%/31%; for methamphetamine, 40%/41%; for MDMA, 180%/186%).(van Nuijs et al. 2011c) Our data also follow the result of another recent study which revealed consistent patterns of per capita illicit drug use between using census data (*de jure* 220,000 persons) and estimated population numbers using ammonium ions in the urban area of Lausanne, Switzerland.(Been et al. 2014) These coherent findings among international studies in large catchments indicate that the consumed mass of illicit drugs varies more substantially than the population, and consequently, relative temporal changes of population drug consumption usually persist irrespective of the type of population estimates used for normalization. This may not be the case for small catchments and days with special events. Despite the absence of large variations in the overall day-today patterns, we observed 19 days with substantially higher or lower differences between the population's drug consumption using the two different population estimates (Figure S- 20 of the Supporting Information). Those exceptional days indicate that a day-specific *de facto* population estimate is essential when estimating a PNDC on a single day. Our data suggest that it is hard to predict when substantial variations in *de facto* populations happen since those exceptional days were randomly detected and not simply related to specific events in the catchment. The deviations in population drug consumption on these 19 days may have been due to (a) the "normal number" of people increasing or decreasing their overall consumption of the eight high-use chemicals, (b) a substantial increase or a decrease in the number of people consuming the "average per capita amount" of the high-use chemicals, or (c) unusual operational conditions in the sewer network affecting influent loads in the WWTP. Unfortunately, it is not possible to differentiate reasons (a) and (b). However, the values observed outside the "normal range of *de facto* population" indicate that the results of these few days should be interpreted cautiously. When using the *de jure* population, such "caution indicators" are missing. An example for reason (c) with "unusual operational conditions" was observed by van Nuijs et al.:(van Nuijs et al. 2011c) a *de facto* population which was less than 10% of the "normal" *de facto* population was estimated because an unreported amount of wastewater did not enter the influent of the WWTP where samples were taken. This does not imply that only 10% of the population were in the catchment but simply that only 10% of the normal wastewater volume was sampled and used to calculate a *de facto* population. Since the illicit drug loads were also

quantified for 10% of the wastewater only, the PNDC was not affected. However, if the *de jure* population had been used, a substantial underestimation during the days with unusual operational conditions would have resulted; unless one had corrected for the missing wastewater volume, assuming that the non-sampled wastewater volumes showed similar concentrations (for both illicit drugs and substances to estimate *de facto* population). In a similar way, faulty flow measurements do not affect PNDC when the *de facto* population is estimated from chemical loads based on the same sample and wastewater volumes as illicit drug residues.(Lai et al. 2011)

App. 6.4 Temporal Variations.

App. 6.4.1 Cocaine.

Both cocaine and its major urinary metabolite, benzoylecgonine, were measured in all samples and used to back-estimate cocaine consumption in the catchment. The yearly average consumption (25−75 percentile; median) of cocaine was estimated at 264 (mg/day)/(1000 people) (162−315; 230) using cocaine itself and 193 (mg/day)/(1000 people) (108−249; 167) using benzoylecgonine. This is equal to, on average, approximately two doses in a day among 1000 people, assuming that the reference dose of cocaine is 100 mg.(Lai et al. 2013a) Benzoylecgonine has been recommended as the key biomarker for back-estimating cocaine consumption because it is exclusively excreted by humans and more persistent in wastewater than cocaine.(Castiglioni et al. 2011, Thai et al. 2014a, van Nuijs et al. 2009a, van Nuijs et al. 2012) We thus used benzoylecgonine to assess the temporal pattern of cocaine consumption in the studied catchment. The overall variation of cocaine consumption in this study (55%, Table S-26 of the Supporting Information) was about twice that reported in Brussels.(van Nuijs et al. 2011c) Cocaine consumption showed a gradual increasing pattern over the monitoring period (Figure S- 14A, Table S- 25 of the Supporting Information). Epidemiological indicators of cocaine use during this period are scant but generally suggest that there was limited use and stable markets since there was little change in hospital admissions, information calls, or self-reported substance use (among injecting drug and club drug consumers) in surveillance studies.(Hickey et al. 2011, McIlwraith et al. 2012a, McIlwraith et al. 2012b) However, analyses of the small number of cocaine seizures suggested that purity increased during April−June 2012 compared with previous months in this period.((ACC) 2013) The weekly pattern of cocaine consumption was consistent across the time period (Figure S- 14A). The average consumption of cocaine on the weekends (299 (mg/day)/(1000 people)) was approximately two times higher than that during the weekdays (149 (mg/day)/(1000 people)) ($p < 10^{-4}$). This implies that, on the weekends, there was either a larger amount of cocaine consumed by the same number of regular users and/or an increase in the number of consumers who used a similar amount/dose within the catchment. Drug

purity was irrelevant to the weekly pattern because it is highly unlikely that there would be marked variations in drug purity within the week and the weekly pattern is clear regardless of the general level of consumption. Given the urban location of the studied site, there were a number of specific events, such as car races, sport competitions, music concerts/festivals and New Year celebrations, during the monitoring period. Elevated consumption of cocaine coincided with some of the major events in the catchment (Figure S- 14A). The effect of the major events on cocaine consumption was more pronounced than the weekend effect; for example, the level of cocaine use on the second-last weekend of October 2011 and on the 2012 New Year's Day (Sunday) was well above the levels seen for weekends in most of the months (Figure S- 14A). This is also observed for another sampling day which was Sunday on the first weekend of May 2012. It is noteworthy that the overall patterns of cocaine consumption estimated from cocaine itself and benzoylecgonine were very similar, except for a spike in cocaine consumption estimated from cocaine itself in one Thursday sample in February 2012 and one Tuesday sample in May 2012 (Figure S- 21A of the Supporting Information). This suggests that there may have been direct release of cocaine into the wastewater system on these 2 days. Environmental inputs of cocaine can arise from, for example, disposal of cocaine, which provides amounts of cocaine in sewage systems well above those from human consumption and results in overestimation of cocaine consumption using wastewater samples.e.g.(Brewer et al. 2012),(Castiglioni et al. 2011) This can also potentially explain the observed difference (an average relative difference of 30%) between two estimates in this study (higher consumption when using cocaine itself rather than benzoylecgonine) and also slightly higher cocaine/benzoylecgonine ratio in the wastewater samples (an average of 0.34) than that in human pharmacokinetic data (0.21 from 7.5%/ 35%; see Table S- 24 of the Supporting Information). Variations in metabolism among individuals and mode of administration can also lead to changes in excretion profiles of cocaine, and therefore affect the difference in the cocaine/benzoylecgonine ratio between the population in the studied catchment and that in pharmacokinetic studies; see, e.g., refs (van Nuijs et al. 2009b) and (van Nuijs et al. 2009c). As noted elsewhere,(Prichard et al. 2012) the use of such a dual tracer approach (i.e., both parent drugs and metabolites) gives additional confidence in the results and may serve as a quality-control check providing information about potential breakdown/transformation of the compounds in the sewers (i.e., higher estimate from metabolites) or releases of unconsumed drugs (i.e., higher estimates using the parent compound).

App. 6.4.2 Methamphetamine.

As the primarily excreted metabolite, methamphetamine itself was chosen to back-estimate methamphetamine consumption. The average yearly consumption (25−75 percentile; median) of methamphetamine was estimated at 440 (mg/day)/(1000 people) (312− 549; 391).This is

equivalent to approximately 14 (doses/day)/(1000 people), assuming that a reference dose of methamphetamine of 30.5 mg.(Lai et al. 2013a) The use of methamphetamine appeared more prevalent than that of cocaine in the catchment, and the variation in methamphetamine consumption (38%) was smaller than that of cocaine consumption over the whole monitoring period (Table S- 26 of the Supporting Information). The yearly consumption pattern of methamphetamine (Figure S- 22A and Table S- 25 of the Supporting Information) was similar to that of cocaine (Figure S- 14A). Most epidemiological data in relation to methamphetamine are only compiled in annual aggregates but are generally in keeping with these trends: police seizures increased during 2011/-12 in both number and weight, as did the number of clandestine laboratories identified;((ACC) 2013) self-reported frequency of use among club drug consumers increased and so did the number of calls made about methamphetamine to information lines and hospital admissions.(Hickey et al. 2011, McIlwraith et al. 2012a) Analysis of police seizures demonstrated steady increases in purity over the first three-quarters of the 2011/12 period. ((ACC) 2013) There was higher consumption of methamphetamine on the weekends (average, 523 (mg/day)/(1000 people)) than the
weekdays (406 (mg/day)/(1000 people)) throughout the year (Figure S- 14B) ($p < 10^{-4}$); the average difference was only about 1.3 times which is less than that for cocaine consumption. A few days of elevated methamphetamine consumption were captured around specific events in the catchment (Figure S- 22A of the Supporting Information). As for cocaine consumption, we also analyzed amphetamine as another drug residue for the estimation of methamphetamine consumption. Both amphetamine and methamphetamine itself estimated a similar pattern of methamphetamine consumption (an average relative difference of 17%) over the monitoring period (Figure S- 21B of the Supporting Information). The consistency of estimating methamphetamine consumption from both amphetamine and methamphetamine itself supports other evidence that illicit use of amphetamine is negligible in Australia;((ACC) 2013) hence, amphetamine measured in the samples was primarily from the metabolism of methamphetamine consumption.

App. 6.4.3 MDMA.

For MDMA, only one excreted residue can be analytically identified with confidence; thus, estimating MDMA consumption relies solely on MDMA itself as described in the literature.e.g.(Thomas et al. 2012) The average consumption (25−75 percentile; median) of MDMA was estimated at about 229 (mg/day)/ (1000 people) (75−277; 145) over the year. This is equivalent to an average of three doses a day per 1000 people, assuming a reference dose of 80.5 mg.(Lai et al. 2013a) Two discrete rising consumption periods of MDMA were observed: June 2011−January 2012 and February−May 2012 (Figure S- 22B and Table S- 25 of the

Supporting Information). Epidemiological indicators of MDMA consumption increased in this period, with self- reported frequency of use among club drug consumers increasing along with perceived purity.(Hickey et al. 2011, McIlwraith et al. 2012a) Analysis of police seizures demonstrated that purity increased between the third and fourth quarters of 2011, declining in the first quarter of 2012 and increasing in the second quarter of 2012.((ACC) 2013) While these purity data are based on a restricted number of seizures, the general pattern is consistent with the fluctuations detected in the wastewater samples in this study (Figure S- 22B of the Supporting Information). A strong weekend effect was observed on MDMA consumption similar to that for cocaine. It was approximately two times higher on weekends (yearly average, 347 (mg/day)/(1000 people); Table S- 25 of the Supporting Information) than on weekdays (181 (mg/day)/(1000 people)) ($p < 10^{-4}$). This pattern of use is consistent with trends reported in consumer studies. (Hickey et al. 2011, Hopper et al. 2006, McIlwraith et al. 2012a) A few spikes were identified in the quantities of MDMA consumed over the study period (Figure S- 22B of the Supporting Information). Some of these spikes coincided with major events in the catchment, confirming previous findings of links between MDMA use and musical entertainment events.(Sindicich 2013, Van Havere et al. 2011)

App. 6.5 Overall Methodological Uncertainty.

Three different evaluations were performed to calculate the total uncertainty (U_{tot}) of PNDC (Table S- 19). First, the components U_C, U_S, U_F, and U_E are typically considered for U_{tot}. Often, U_P is unfortunately excluded because it is unknown and obtaining a realistic, independent, and site-specific estimate is difficult. Applying Monte Carlo simulation, the average U_{tot} was estimated at approximately 22% for the three illicit drugs over the monitoring period (see Table S- 19 for details and range), in which U_F is the most dominant uncertainty component. This estimate was similar to our previous finding for this urban catchment.(Lai et al. 2011) Second, when considering a conservative value of 50% (range 7−55%, according to Castiglioni et al.)(Castiglioni et al. 2013) for U_P-analogue to the uncertain knowledge of population (wide prior) used for Bayesian updating in the population estimation model(O'Brien et al. 2014)-U_{tot}(*de jure*) would be approximately 57% for the three illicit drugs, in which U_P becomes the dominating uncertainty factor. Third, as mentioned previously, the daily *de facto* population is estimated from chemical mass loads which are calculated with the same daily flow data as the daily illicit drug loads. Thus, U_F affects both daily *de facto* population estimates and illicit drug loads in the same way and cancels out when calculating PNDC. For calculating *de facto*-PNDC, the U_{tot}(*de facto*) is, therefore, based on U_S, U_C, U_E, and U_P. For U_P, the multi-substance model estimated an average of 3.5% over the monitoring period (posterior of population estimation(O'Brien et al. 2014)). The average U_{tot}(*de facto*) is then estimated at approximately 10% for all compounds (Table S- 19).

There was a distinctive difference among a typical U_{tot}, U_{tot}(*de jure*), and U_{tot}(*de facto*) for the three illicit drugs over the monitoring period (Figure S- 14 and Figure S- 23 and Figure S- 24 of the Supporting Information). The levels of U_{tot} for estimating cocaine consumption was clearly diminished in a decreasing order as follows: U_{tot}(*de jure*) (Figure S- 14C) > a typical U_{tot} (Figure S- 14B) > U_{tot}(*de facto*) (Figure S- 14A). The same holds true for estimating methamphetamine and MDMA consumption (Figure S- 23 and Figure S- 24 of the Supporting Information). Our data clearly revealed a substantial reduction in the U_{tot} when a dayspecific *de facto* population was estimated from chemicals in the same sample as used to measure illicit drug residues. A European-wide study estimated about 26% (a typical U_{tot}) as the uncertainty of estimating cocaine consumption.(Castiglioni et al. 2013) A similar level was also estimated in our study, but our data reflected a much lower level when using the daily estimated *de facto* populations. This study showed an objective quantification of the total methodological uncertainty and achieved a substantial reduction thereof. In our case, the total uncertainty of the *de facto*-PNDC was reduced by a factor of 2 because of (i) eliminating the uncertainty of flow measurements and (ii) performing a unique assessment of uncertainty related to the population size. Whether or not the effort to reduce the total methodological uncertainty from about 20% to 10% for a daily value is justified, there remains an open discussion and it depends on the specific application and setting. However, it appears that avoiding a systematic over- or underestimation of PNDC - which normally remains undiscovered - is highly desirable, particularly when comparing consumption data across different locations. With a dataset that covers a much shorter duration and does not cover consecutive days, we could have not derived these findings and substantiated our conclusions. This also holds true for the identification of monitoring days that require more attention for interpretation. In combination with previously summarized long-term studies,(Ort et al. 2014a) our study offers guidance to optimize future monitoring campaigns where financial or logistic reasons limit the numbers of sampling days for evaluation. A remaining challenge is to interpret an estimated population of, e.g., 100,000 people, as different settings could have led to this result: (1) 100,000 people all being present in a catchment over 24 h and having used the toilet an average of five times; or (2) 500,000 people in transit through the catchment area using the toilet an average of only once in the day, as an extreme opposite. Both situations would result in approximately 500,000 toilet flushes.

App. 6.6 Supporting Information

Text describing calculation information for back-estimation of illicit drug consumption, tables listing dates of missing representative samples, a summary of LC-MS/MS analysis data, quality assurance, and control samples for illicit drug residues and high-use chemicals, estimated *de*

facto populations, pharmacokinetic data of three conventional illicit drugs, population and consumption data for cocaine, methamphetamines, and MDMA, and CVs of consumption between *de jure* and estimated *de facto* populations, and figures showing Mann−Whitney test comparison between weekends and weekdays for estimated *de facto* populations and drug consumption, percentage differences between estimated *de facto* and *de jure* populations, daily concentrations of high-use chemicals for estimating *de facto* population and wastewater volume, high-use chemical concentrations vs flow rate, and various drug consumption comparisons.

App. 6.7 Acknowledgements

Entox is a joint venture of UQ and QHFSS. We thank the Regional Council for the sampling opportunity. We also thank Andrew Banks and Kristie Thompson for assisting with sample preparation. We acknowledge the financial support from QHFSS/Entox Collaborative Research Funds, Australia Crime Commission, and Crime and Misconduct Commission of Queensland. The LC-MS/MS was funded by the ARC LIEF grant. C.G. is funded by a National Health and Medical Research Council Early Career Research Fellowship. W.H. is funded by a National Health and Medical Research Council Australia Fellowship. J.M. is funded through the Australian Research Council Future Fellowship. P.K.T. is funded through a UQ Postdoctoral Research Fellowship.

App. 6. Figures:

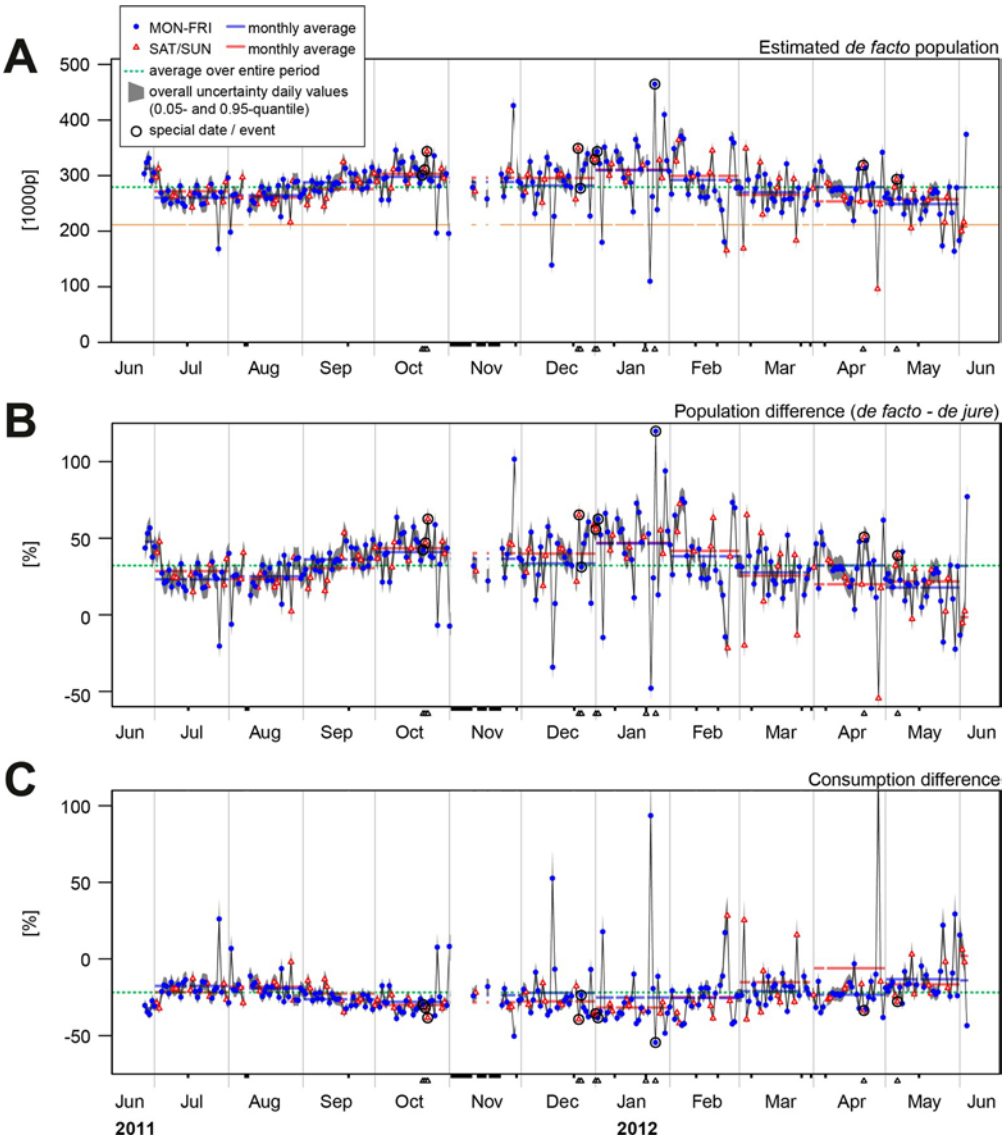


Figure S- 13 (A) Estimated daily *de facto* populations from the wastewater samples over the monitoring period (orange solid line, the de jure population of 211,340 persons). (B) Percentage differences between the daily *de facto* population and the *de jure* population (i.e., differences of the two estimates divided by the *de jure* population). (C) Deviations (percentage differences) of per capita consumption rates between normalization to the estimated *de facto* population and the *de jure* population. This panel is different from that of panel B due to the fact that the population estimate influences the population-normalized estimates by (*de jure*/*de facto* − 1) (see section 3.3 for details). Black bars along the X-axis = missing date (see Table S- 20 of the Supporting Information); triangles along the X-axis = days with special dates/events. Figure S- 15 of the Supporting Information shows a direct comparison of data between the weekends and weekdays for panel A (see Table S- 25 of the Supporting Information for the numerical data of the monthly average and the average over the entire monitoring period).

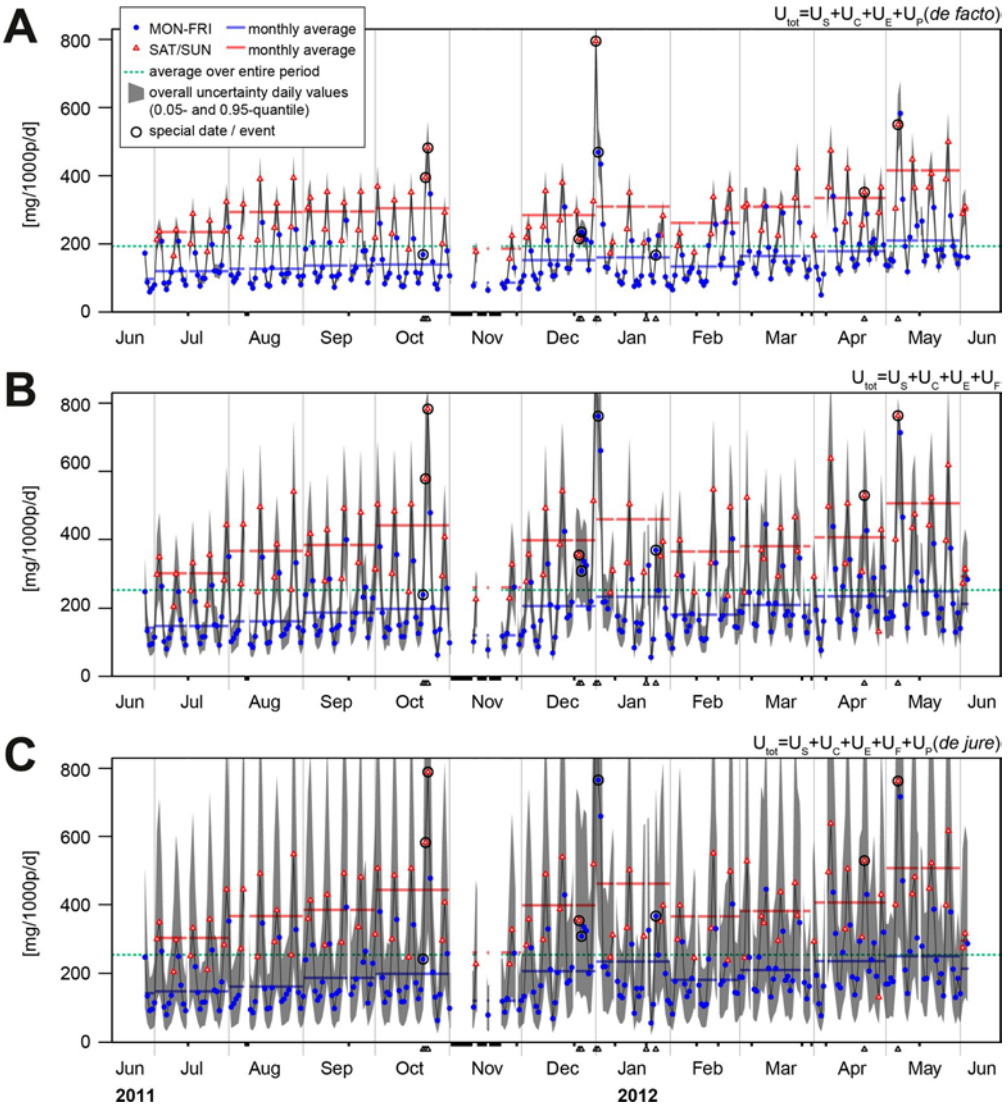


Figure S- 14 Estimated population-normalized consumption for cocaine considering different population estimates and uncertainty components: (A) *de facto* population normalized consumption with U_{tot}(*de facto*) = U_S + U_C + U_E + U_P(*de facto*); (B) *de jure* population normalized consumption with a typical U_{tot} = U_S + U_C + U_E + U_F; (C) *de jure* population normalized consumption with U_{tot}(*de jure*) = U_S + U_C + U_E + U_F + U_P(*de jure*). Black bars along the X-axis = missing dates (see Table S- 20 of the Supporting Information); triangles along the X-axis = days with special dates/events. Figure S- 15 of the Supporting Information shows a direct comparison of data between the weekends and weekdays for A (see Table S- 25 of the Supporting Information for the numerical data of the monthly average and the average over the entire monitoring period).

App. 6. Tables:

Table S- 19 Evaluation of the Total Methodological Uncertainty (RSD %) for Estimating Illicit Drug Consumption between Normalization to the de Jure Population and the Estimated *de Facto* Population Using Monte Carlo Simulation

	cocaine		methamphetamine		MDMA	
with *de jure* populations	U_P ignored	U_P considered	U_P ignored	U_P considered	U_P ignored	U_P considered
U_S	5	5	5	5	5	5
U_C	7.1	7.1	5.1	5.1	9.8	9.8
U_F	20	20	20	20	20	20
U_E	1.9	1.9	0.82	0.82	2.3	2.3
U_P	n.a.	50	n.a.	50	n.a.	50
$u$tot	22 (22−23)	57 (54−61)	21 (21−22)	56 (53−62)	23 (23−24)	57 (55−62)
with *de facto* populations						
U_S		5		5		5
U_C		7.1		5.1		9.8
U_F		−		−		−
U_E		1.9		0.82		2.3
U_P		3.5 (2.1−9.7)		3.5 (2.1−9.7)		3.5 (2.1−9.7)
$u$tot		9.6 (9.1−13)		8.0 (7.5−12)		12 (11−15)

[a]n.a., not applicable; −, uncertainty of flow cancels out for population-normalized drug loads since *de facto* population estimate is affected in the same way as illicit drug loads. See Lai et al.[8] for the details of quantifying the uncertainty components.

App. 6. Supplementary information

Systematic and day-to-day effects of chemical-derived population estimates on wastewater-based drug epidemiology

Foon Yin Lai [a,*], Shalona Anuj [a,b], Raimondo Bruno [c], Steve Carter [b], Coral Gartner [d], Wayne Hall [e], K. Paul Kirkbride [f], Jochen F. Mueller [a], Jake W. O'Brien [a], Jeremy Prichard [g], Phong K. Thai [a], Christoph Ort [h,i,*]

a. The University of Queensland, The National Research Centre for Environmental Toxicology, 39 Kessels Road, Coopers Plains, QLD 4108, Australia.
b. Queensland Health Forensic Scientific Services, Queensland Government, 39 Kessels Road, Coopers Plains, QLD 4108, Australia.
c. School of Psychology, University of Tasmania, Private Bag 30, Hobart, TAS 7001, Australia.
d. The University of Queensland, Centre for Clinical Research, Royal Brisbane and Women's Hospital, Herston, QLD 4029, Australia.
e. The University of Queensland, Centre for Youth Substance Abuse Research, Royal Brisbane and Women's Hospital, Herston, QLD 4029, Australia.
f. School of Chemical and Physical Sciences, Flinders University, Adelaide, SA 5001, Australia.
g. Faculty of Law, University of Tasmania, Private Bag 89, Hobart, TAS 7001, Australia
h. Swiss Federal Institute of Aquatic Science and Technology (Eawag) Urban Water Management, Ueberlandstrasse 133, CH 8600, Duebendorf, Switzerland.
i. The University of Queensland, Advanced Water Management Centre (AWMC), Brisbane, QLD 4072, Australia.

*Corresponding authors' contact details:

Dr. Foon Yin LAI: The University of Queensland, The National Research Centre for Environmental Toxicology (Entox), 39 Kessels Road, Coopers Plains, QLD 4108, Australia; Tel.: +61 (0)4 06714601; Fax.: +61 (0)7 3274 9003; E-mail: foon.lai@uqconnect.edu.au

Dr. Christoph ORT: Swiss Federal Institute of Aquatic Science and Technology, Eawag, Urban Water Management, Ueberlandstrasse 133, CH-8600, Duebendorf, Switzerland; Tel.: +41 (0)58 765 52 77; E-mail: christoph.ort@eawag.ch

The equation of the back-estimation of illicit drug consumption (mg/day/1000p):

$$\frac{Drug\ residue\ concentration\left(\frac{\mu g}{L}\right) \times Waterwater\ flow\ volumn\left(\frac{ML}{day}\right) \times Correction\ factor}{Population\ sizes\ in\ the\ catchment\ of\ interest}$$

Concentrations of drug residue in the samples are analysed using validated methods with LC-MS/MS.(Lai et al. 2011) Daily wastewater flow volume is measured and provided by the wastewater treatment plant. Correction factors are related to the average and molar excretion fraction of the parent drug or the metabolite (see details in Lai et al. (2013)(Lai et al. 2013a)). Day-specific populations are estimated through a multi-substance model (see details in O'Brien et al. (2014)(O'Brien et al. 2014)).

Table S- 20 Dates of missing representative samples due to logistic or technical reasons

15/07/2011	18/11/2011
8/08/2011	19/11/2011
9/08/2011	20/11/2011
20/09/2011	21/11/2011
2/11/2011	22/11/2011
3/11/2011	29/11/2011
4/11/2011	23/12/2011
5/11/2011	9/01/2012
6/11/2011	22/01/2012
7/11/2011	12/02/2012
8/11/2011	21/02/2012
9/11/2011	6/03/2012
10/11/2011	27/03/2012
13/11/2011	31/03/2012
14/11/2011	6/04/2012
15/11/2011	15/05/2012
16/11/2011	

Table S- 21 Summary of LC-MS/MS information on analysing illicit drug residues and high-use chemicals

	Illicit drug residues	High-use chemicals
LC system	Shimadzu, Nexera UHPLC system (Kyoto, Japan)	
LC column[a]	Luna C18, 150X3 mm, 3 µm	Kinetics C18, 100X4 mm, 2.6µm
Mobile phase	1% acetonitrile in 0.1% formic acid (A) 95% acetonitrile in 0.1% formic acid (B)	Milli-Q water in 0.1 % formic acid (A) 95% acetonitrile in 0.1 % formic acid (B)
Flow rate (mL/min)	0.3	0.7
LC gradient	Start at 8% B, ramped up to 35% at 3.5 min, increased to 100% B at 7 min, held 100% B for 4 mins, declined to 8% B, equilibrate for 3 mins	Start at 0.2% B, ramped up to 100% B at 5 min, held 100% B for 0.25 mins, declined to 0.2% B, equilibrate for 3 mins
Injection volume (µL)	8	2
Mass spectrometry	AB SCIEX QTRAP®5500 (Ontario, Canada)	
Calibration standards	0.05, 0.5, 1, 5, 10, 50 ng/mL	0.05, 0.1, 0.5, 1, 5, 10, 50 ng/mL
Ionisation modes	Positive ESI	Switching positive and negative ESI
Monitoring modes	MRM with two transitions (Scheduled)	MRM with two transitions (full scan)

[a]Phenomenex, Torrance CA, USA.

Table S- 22 Quality assurance and control samples for analysis of illicit drug residues and high-use chemicals

	Blank samples[a] (n=15)	Duplicate samples (n=29)	Matrix spike recovery (%) (n=28)			Inter-day variability (n=2, 3 days)
	Concentrations (µg/L)	Average CV (%)	Mean	SD	CV (%)	CV (%)
Illicit drug resides						
Cocaine	<0.01	4.8	76	15	20	2.3
Benzoylecgonine	<0.01	5.7	75	16	21	7.1
Amphetamine	<0.01	7.9	81	10	13	11
Methamphetamine	<0.01	5.7	80	13	16	5.1
MDMA	<0.01	5.7	81	11	14	9.8
	Blank samples[a] (n=15)	Duplicate samples (n=40)	Matrix Spike recovery % (n=35)			Inter-day variability (n=1, 3 days)
	Concentrations (µg/L)	Average CV (%)	Mean	SD	CV (%)	CV (%)
High-use chemicals						
Atenolol	<0.1	9.4	108	17	16	8.6
Codeine	<0.5	8.6	103	15	15	7.6
Caffeine	<0.5	7.2	112	32	28	21
Hydrochlorothiazide	<1.0	9.4	103	13	13	9.0
Acesulfame	<5.0	5.9	113	26	23	4.7
Salicylic acid	<1.0	6.6	99	14	14	16
Carbamazepine	<0.05	4.2	99	6.8	6.8	6.8
Naproxen	<1.0	6.4	102	10	10	13

[a] Milli-Q water as procedural blank samples; SD: standard deviation; CV: coefficient variation
Internal standards used: cocaine-D_3 (for cocaine), benzoylecgonine-D_3 (for benzoylecgonine), amphetamine-D_6 (for amphetamine), methamphetamine-D_9 (for methamphetamine), MDMA-D_5 (for MDMA), atenolol-D_7 (for atenolol), codeine- D_3 (for codeine), caffeine-^{13}C (for caffeine), hydrochlorothiazide-^{13}C-D_2 (for hydrochlorothiazide), acesulfame-D_4 (for acesulfame), acetyl sulfamethoxazole-D_4 (for salicylic acid and naproxen) and carbamazepine-D_{10} (for carbamazepine).

Table S- 23 Estimated de facto populations using 8 chemicals or 14 chemicals.

	8 chemicals			14 chemicals		
	Estimated population	Standard derivation	Relative standard deviation (%)	Estimated	Standard	Relative
24/07/2011	280964	9541	3.40	280964	9437	3.36
28/07/2011	169639	9441	5.57	169639	9411	5.55
2/08/2011	198795	9465	4.76	198795	9484	4.77
26/08/2011	280964	9398	3.35	280964	9446	3.36
27/08/2011	214699	9427	4.39	214699	9462	4.41
22/09/2011	280964	9535	3.39	280964	9576	3.41
27/10/2011	196145	9431	4.81	196145	9404	4.79
1/11/2011	196145	9422	4.80	196145	9470	4.83
28/11/2011	426747	9461	2.22	426747	9590	2.25
14/12/2011	137831	9355	6.79	137831	9305	6.75
4/01/2012	180241	9444	5.24	180241	9402	5.22
24/01/2012	111325	9308	8.36	111325	9324	8.38
26/01/2012	463855	9508	2.05	463855	9407	2.03
24/02/2012	180241	9397	5.21	180241	9487	5.26
25/02/2012	164337	9335	5.68	164337	9336	5.68
3/03/2012	169639	9442	5.57	169639	9340	5.51
25/03/2012	182892	9384	5.13	182892	9478	5.18
28/04/2012	95422	9234	9.68	95422	9239	9.68
12/05/2012	206747	9416	4.55	206747	9520	4.60
25/05/2012	172289	9329	5.41	172289	9449	5.48
26/05/2012	217349	9366	4.31	217349	9422	4.33
30/05/2012	164337	9424	5.73	164337	9276	5.64
1/06/2012	182892	9521	5.21	182892	9352	5.11
2/06/2012	198795	9427	4.74	198795	9483	4.77
3/06/2012	214699	9407	4.38	214699	9532	4.44

Table S- 24 Pharmacokinetic data of three conventional illicit drugs

Parent drug	Drug residue	Average excretion	Molecular weight	Correction
Cocaine	Cocaine Benzoylecgonine	$_{7.5}$c–f $_{35}$c–f	1.0	13
Methamphetamine		$_{39}$g–i $_{5.5}$g–i		2.6
MDMA		$_{15}$j, k		6.7

[a]Ratio of molecular weight of the parent drug to the drug residue; [b]Molecular mass ratio divided by average excretion; [c]Ambre

Table S- 25 Results (monthly average and average over entire period) of the estimated de facto population, population difference, consumption difference, cocaine consumption, methamphetamine consumption and MDMA consumption. Note: 'mg' in this table refers to the average amount per 1000 people per month and the average amount per 1000 people per the whole monitoring period.

		Estimated *de facto* population					Cocaine consumption (mg)		
		Monthly	Monthly	Monthly			Monthly	Monthly	Monthly
2011	June	312,273	NA	312,273	2011	June	97	NA	97
	July	263,983	271,601	260,174		July	158	235	120
	August	263,237	260,547	264,261		August	173	293	127
	Septembe	284,441	275,785	287,738		Septembe	180	295	137
	October	299,501	303,133	297,771		October	193	305	139
	November	290,628	296,259	288,516		November	113	186	86
	December	286,098	295,609	282,022		December	192	284	152
2012	January	309,942	310,636	309,678	2012	January	202	310	160
	February	293,921	299,360	292,017		February	167	261	133
	March	268,496	265,282	269,782		March	206	309	165
	April	271,283	253,438	279,314		April	227	335	178
	May	251,095	257,323	248,831		May	265	416	210
	June	243,418	208,048	278,788		June	230	298	162
	Entire	279,522	278,286	280,026		Entire	193	299	149
		Population difference (%)					Methamphetamine consumption		
		Monthly	Monthly	Monthly			Monthly	Monthly	Monthly
2011	June	48	NA	48	2011	June	272	NA	272
	July	25	29	23		July	360	445	317
	August	25	23	25		August	389	481	354
	Septembe	35	30	36		Septembe	393	487	358
	October	42	43	41		October	373	469	328
	November	38	40	37		November	290	344	270
	December	35	40	33		December	325	396	294
2012	January	47	47	47	2012	January	327	367	312
	February	39	42	38		February	423	494	398
	March	27	26	28		March	562	573	558
	April	28	20	32		April	593	690	549
	May	19	22	18		May	707	844	657
	June	15	-2	32		June	684	799	568
	Entire	32	32	33		Entire	440	523	406
		Consumption difference (%)					MDMA consumption (mg)		
		Monthly	Monthly	Monthly			Monthly	Monthly	Monthly
2011	June	-32	NA	-32	2011	June	57	NA	57
	July	-19	-22	-18		July	92	135	71
	August	-19	-18	-19		August	129	242	86
	Septembe	-25	-23	-26		Septembe	148	266	103
	October	-29	-30	-28		October	212	325	158
	November	-25	-28	-24		November	199	360	138
	December	-24	-28	-22		December	257	396	197
2012	January	-27	-32	-25	2012	January	393	398	391
	February	-25	-25	-25		February	161	301	112
	March	-19	-15	-21		March	214	367	153
	April	-18	-6	-23		April	276	477	186
	May	-14	-17	-13		May	431	580	377
	June	-6	2	-14		June	282	388	176
	Entire	-22	-21	-22		Entire	229	347	181

Table S- 26 Coefficient variation (CV %) of illicit drug consumption between normalisation to the de jure population and the estimated de facto population.

	Cocaine	Methamphetamine	MDMA
With de jure populations			
Weekdays	54%	38%	177%
Weekends	38%	30%	78%
Whole period	59%	37%	138%
With de facto populations			
Weekdays	50%	39%	172%
Weekends	33%	32%	70%
Whole period	55%	38%	132%

Weekdays: Monday–Friday; Weekends: Saturday–Sunday.

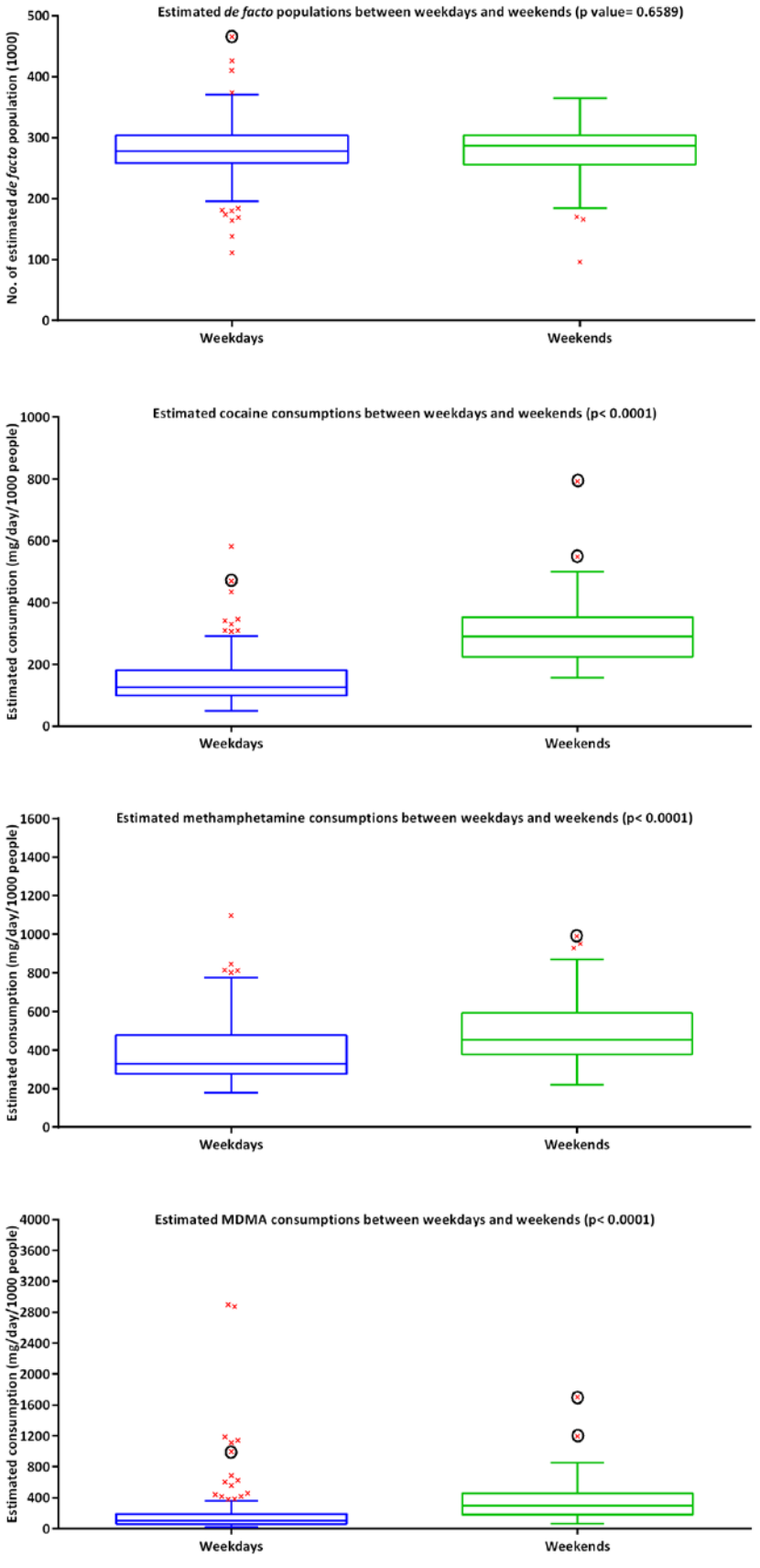
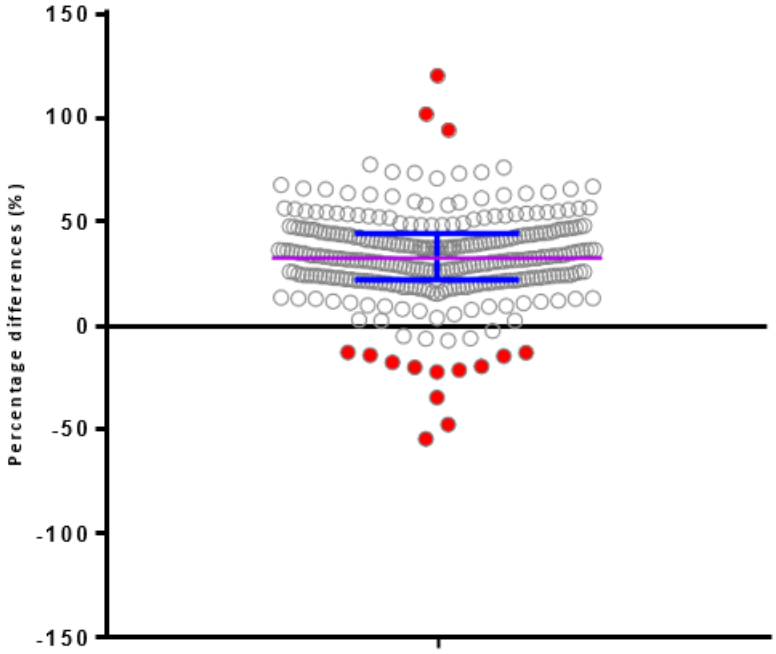


Figure S- 15 A box plot for data comparison (Mann-Whitney test) between weekends and weekdays for the estimated *de facto* populations and drug consumption. A red cross indicates outliers which were defined as above or below three times of the interquartile range of the dataset. A circle shows an outlier data which represents the data of specific date/event.



Outliers		
Date	Day	% difference
28/07/2011	Thursday	-20.1
28/11/2011	Monday	101
14/12/2011	Wednesday	-34.6
4/01/2012	Wednesday	-14.8
24/01/2012	Tuesday	-47.5
26/01/2012	Thursday	120
30/01/2012	Monday	93.8
24/02/2012	Friday	-14.4
25/02/2012	Saturday	-21.5
3/03/2012	Saturday	-19.7
25/03/2012	Sunday	-13.1
28/04/2012	Saturday	-54.4
25/05/2012	Friday	-17.8
30/05/2012	Wednesday	-22.4
1/06/2012	Friday	-13.0

Figure S- 16 Percentage differences between the estimated de facto population and the de jure population. Purple line represents the median. Blue line represents the inter-quartile range. Red circles represent outliers which were defined as above or below three times of the interquartile range of the dataset.

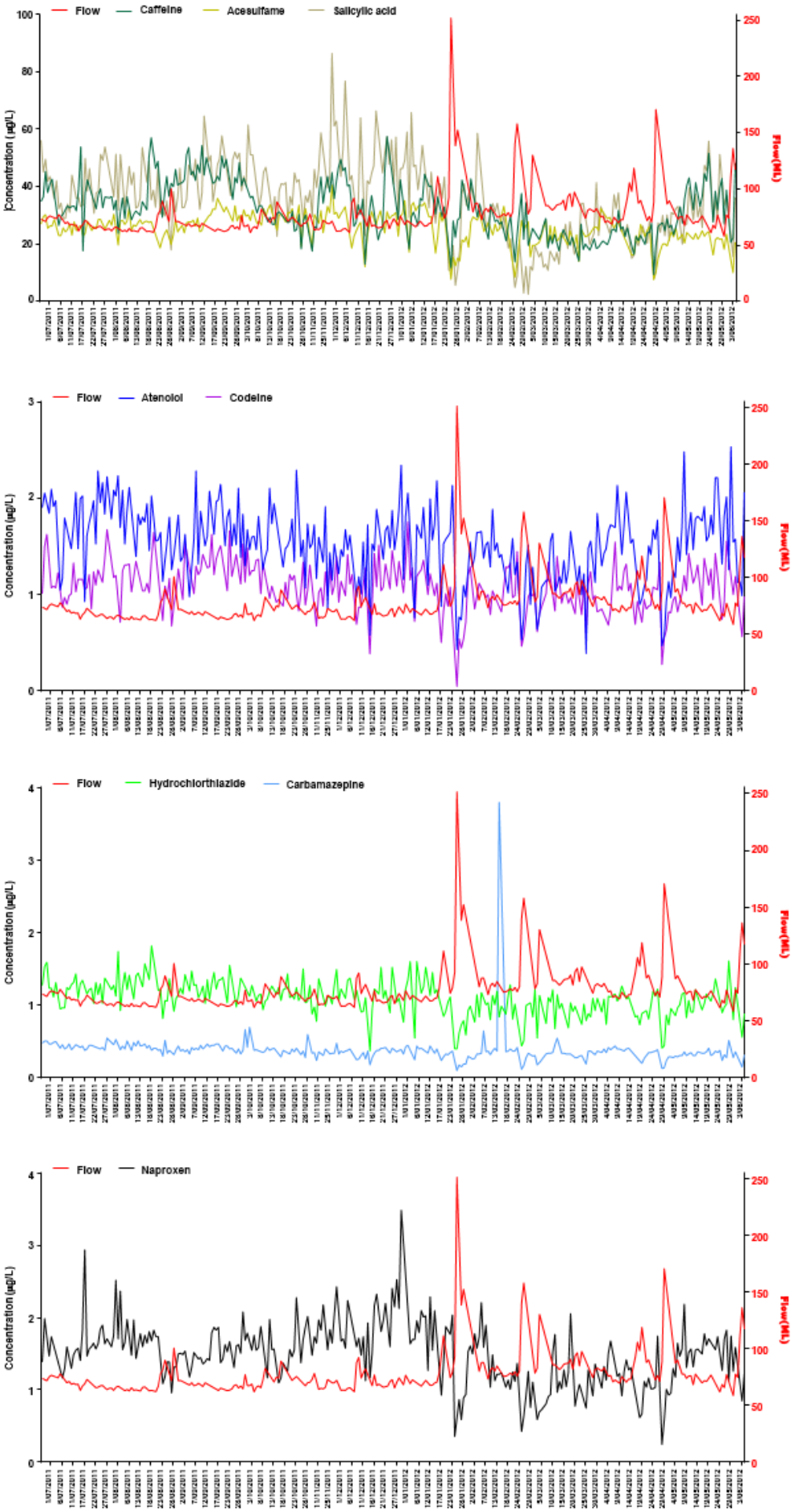


Figure S- 17 Daily concentrations (µg/L) of the high use chemicals for estimating the de facto population and the daily total wastewater volume (ML) over the monitoring period. Correlations (Spearman test: r= -0.51 to -0.62, p< 10-4) between concentrations of these chemicals and the flow rate are shown in Fig. S3B.

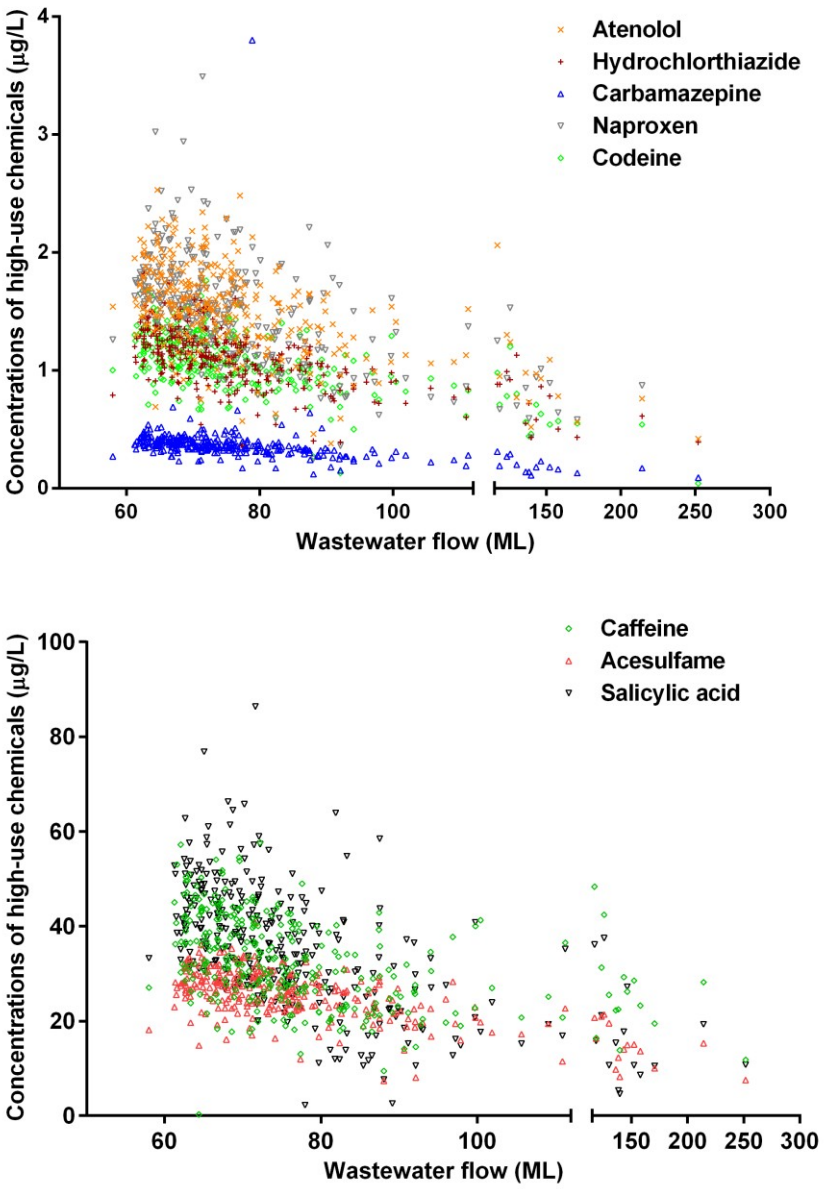


Figure S- 18 Concentrations (µg/L) of the high-use chemicals against the flow rate.

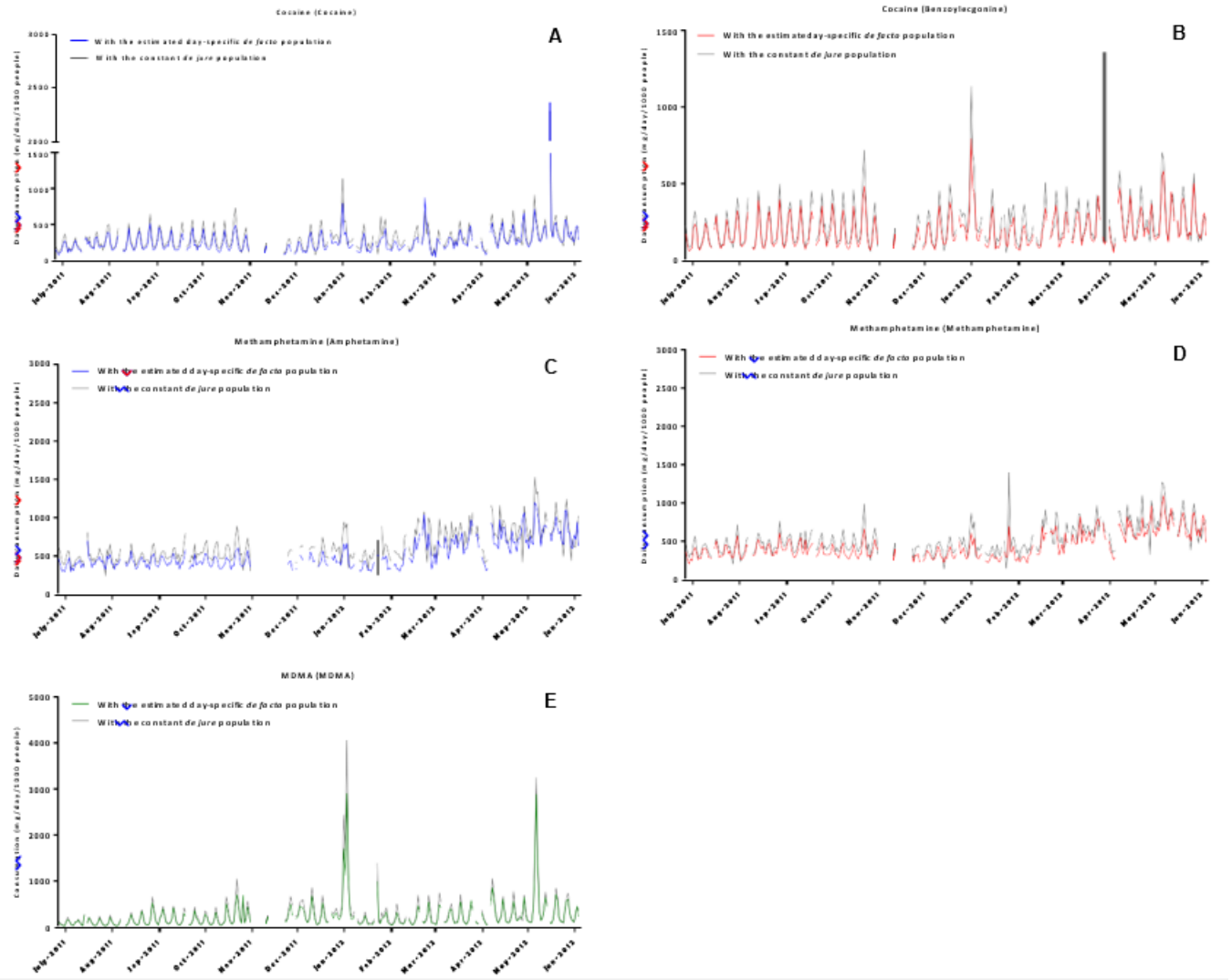
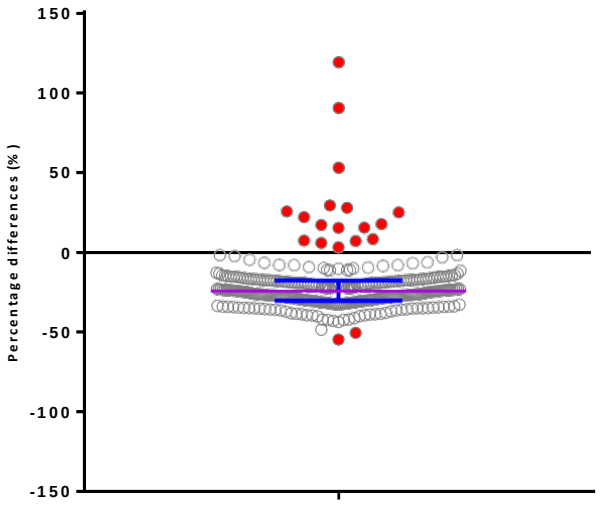


Figure S- 19 Comparison of drug consumption between a normalisation to the estimated day-specific *de facto* population (the coloured lines) and the *de jure* population (the grey line) for cocaine estimated from cocaine itself (A) and benzoylecgonine (B); for methamphetamine estimated from amphetamine (C) and methamphetamine itself (D); and for MDMA estimated from MDMA itself (E). Strong correlation (Spearman test) was observed for drug consumption (A–E: r= 0.86–0.98, $p<10^{-4}$) between a normalisation to the estimated day-specific *de facto* population and the *de jure* population.



Outliers		
Date	Day	%difference
28/07/2011	Thursday	25.2
2/08/2011	Tuesday	6.8
27/10/2011	Thursday	7.0
1/11/2011	Tuesday	7.9
28/11/2011	Monday	-50.4
14/12/2011	Wednesday	53.0
4/01/2012	Wednesday	17.4
24/01/2012	Tuesday	90.5
26/01/2012	Thursday	-54.6
24/02/2012	Friday	16.8
25/02/2012	Saturday	27.5
3/03/2012	Saturday	24.6
25/03/2012	Sunday	15.1
28/04/2012	Saturday	119.2
12/05/2012	Saturday	2.9
25/05/2012	Friday	21.7
30/05/2012	Wednesday	28.9
1/06/2012	Friday	14.9
2/06/2012	Saturday	5.6

Figure S- 20 Percentage differences in consumption with normalisation to an estimated *de facto* population and a *de jure* population. Blue line represents the inter-quartile range. Red circles represent outliers which were defined as above or below three times of the interquartile range of the dataset.

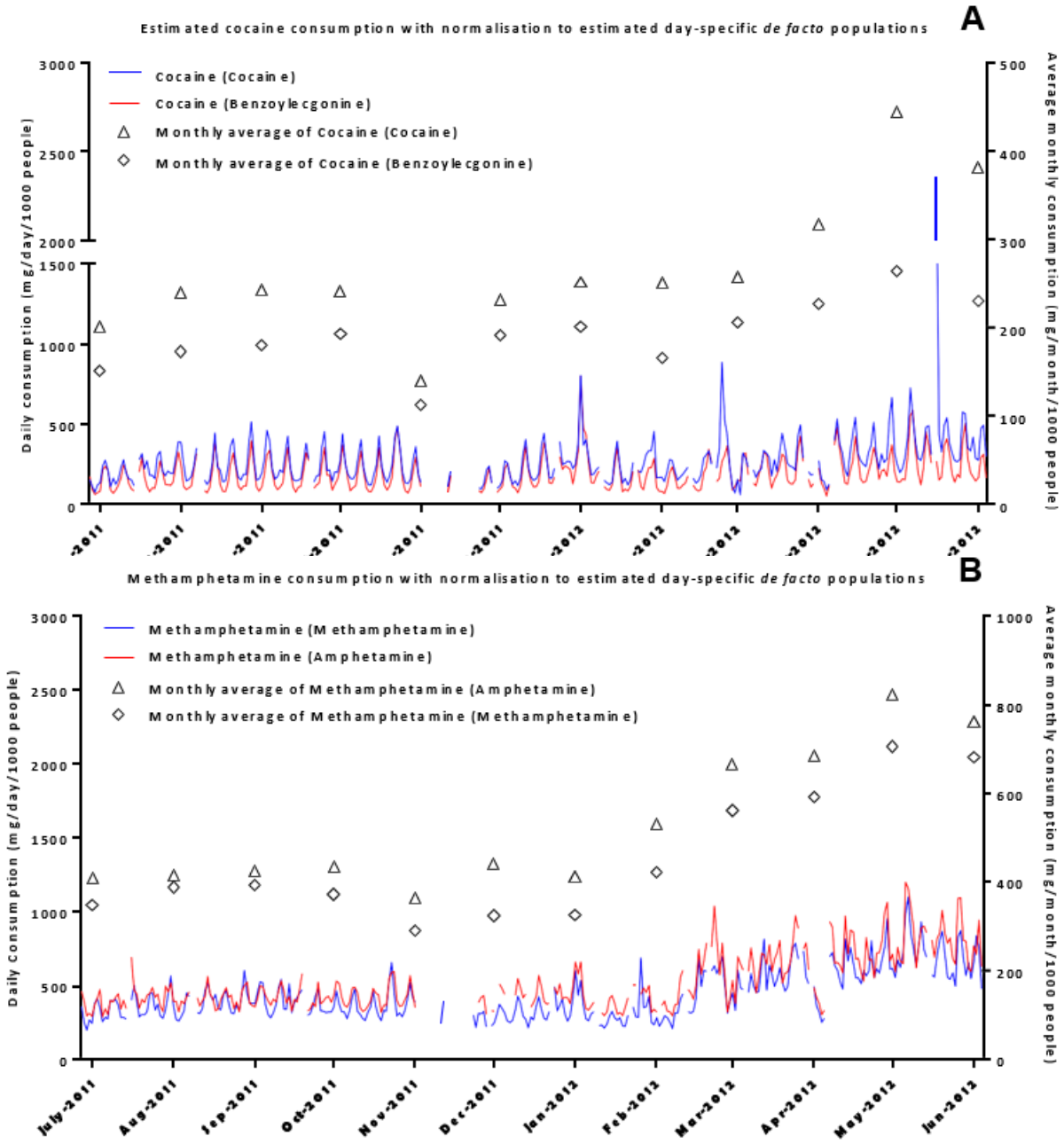


Figure S- 21 Estimated *de facto* population-normalised drug consumption for cocaine estimated from cocaine itself and benzoylecgonine (A) (Spearman test: r= 0.91, p<10-4) and for methamphetamine estimated from amphetamine and methamphetamine itself (B) (Spearman test: r= 0.90, p<10-4).

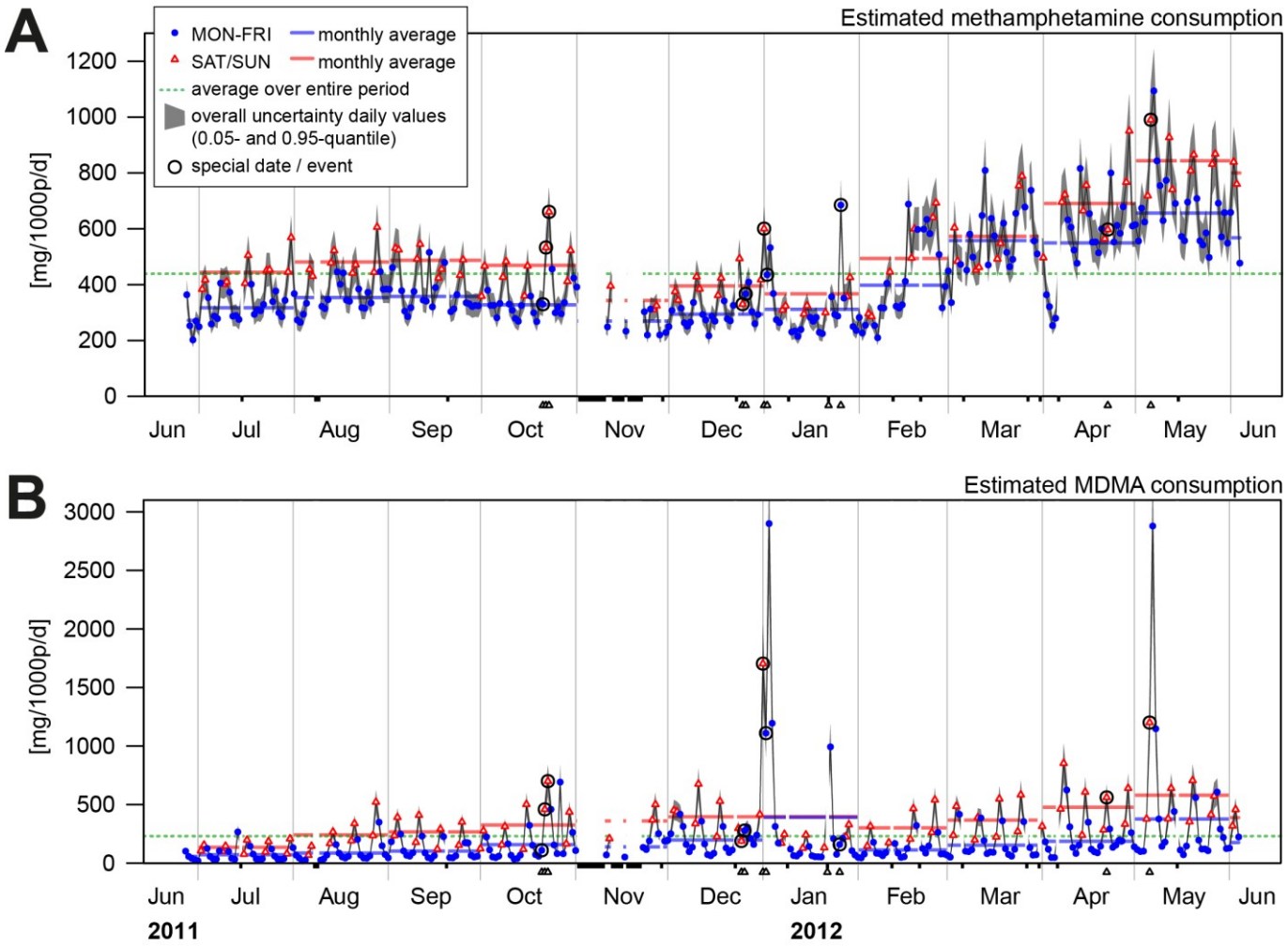


Figure S- 22 Estimated *de facto* population-normalised consumption for methamphetamine (A) and MDMA (B). Black bars along X-axis = missing date (see Table S- 20); triangles along X-axis = days with special dates/events. Grey area indicates Utot(de facto)= US+UC+UE+UP(de facto). Figure S- 15 shows a direct comparison of data between the weekends and weekdays for A (see Table S- 25 for the numerical data of the monthly average and the average over the entire monitoring period).

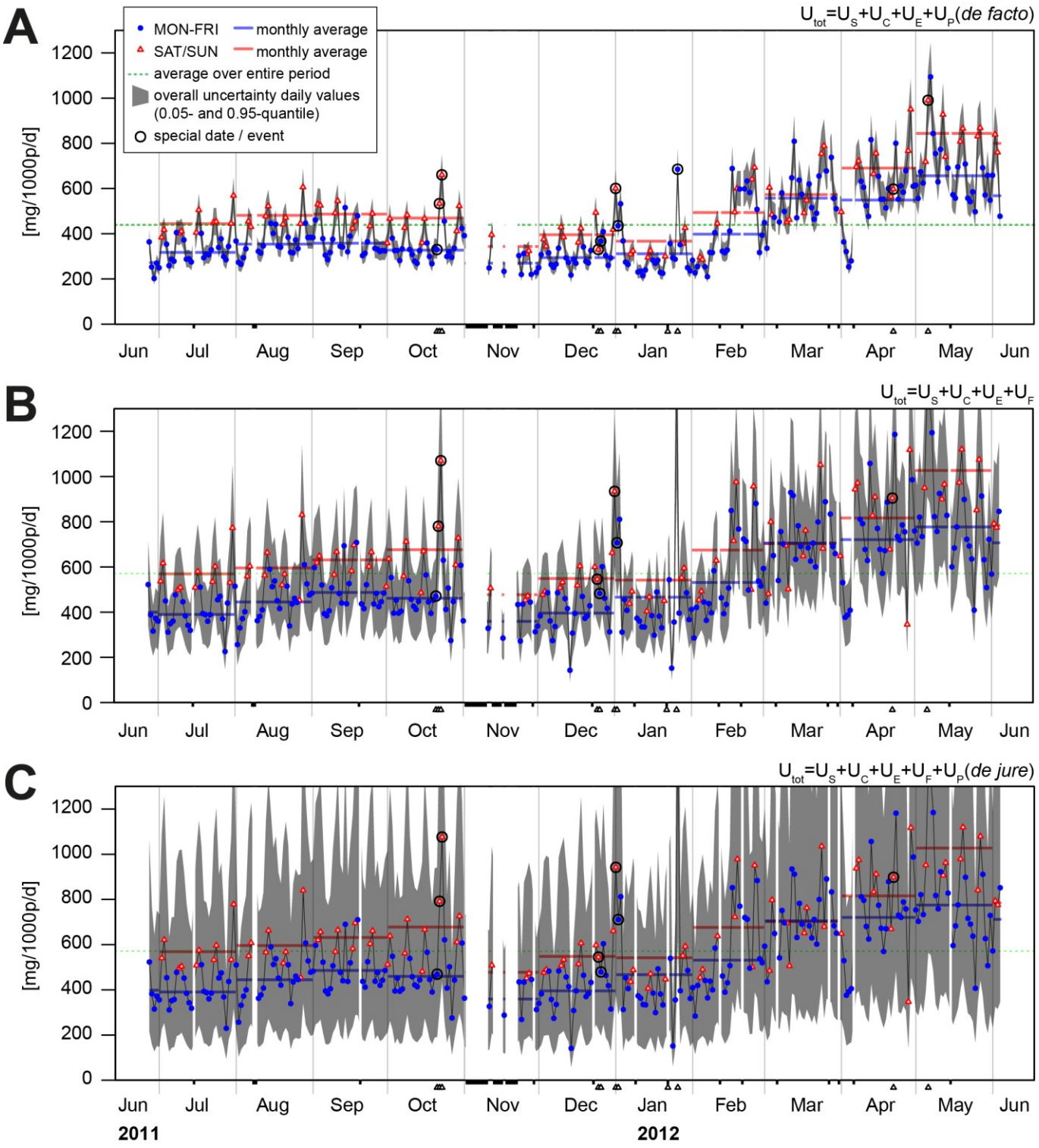


Figure S- 23 Estimated population-normalised consumption for methamphetmine considering different population estimates and uncertainty components: A. *de facto*-population normalised consumption with U_{tot}(*de facto*)= $U_S+U_C+U_E+U_P$(*de facto*); B. *de jure*-population normalised consumption with a typical Utot= $U_S+U_C+U_E+U_F$; C. *de jure*-population normalised consumption with U_{tot}(*de jure*)= $U_S+U_C+U_E+U_F+U_P$(*de jure*). Black bars along X-axis = missing date (see Table S- 20); triangles along X-axis = days with special dates/events.

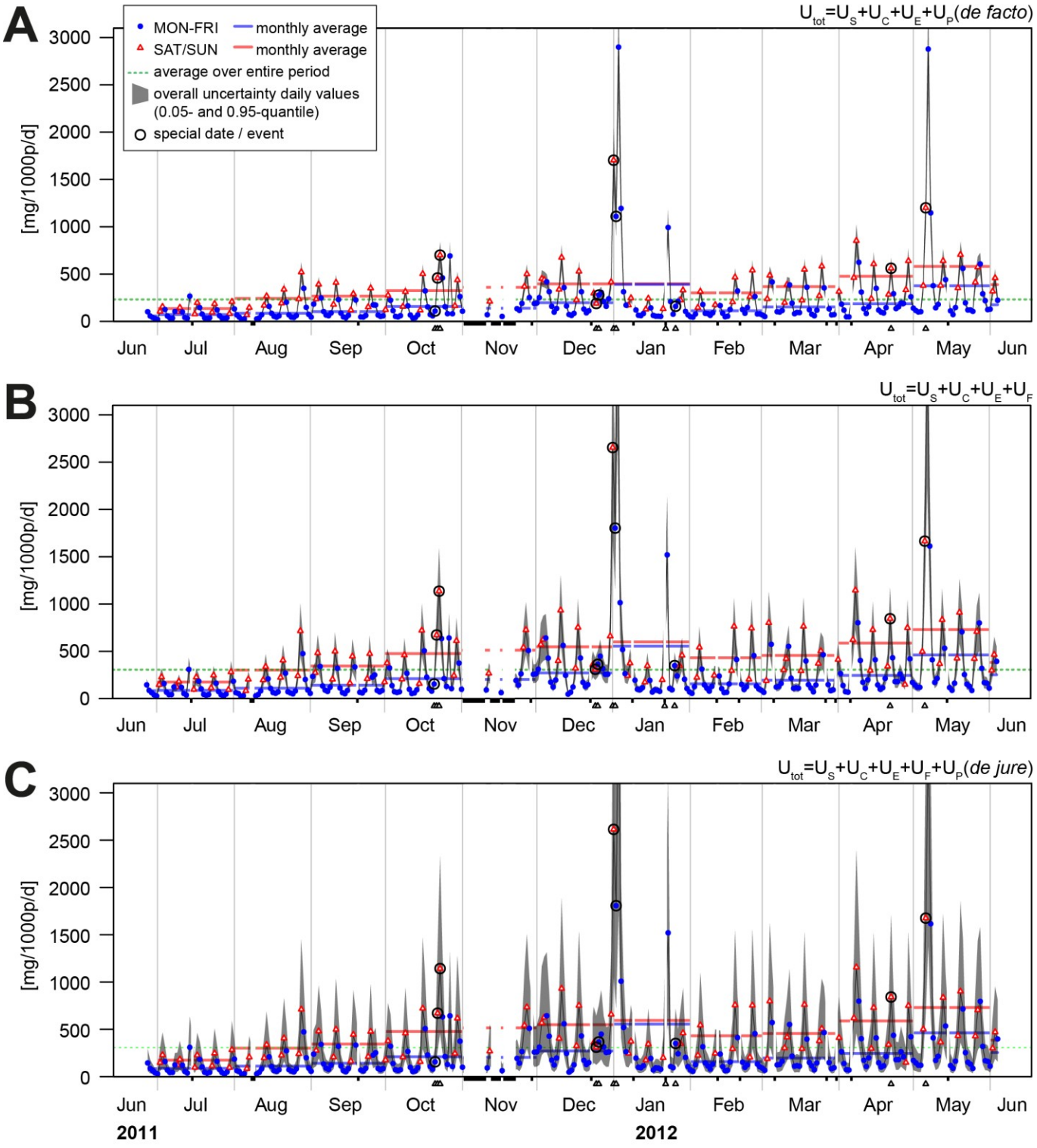


Figure S- 24 Estimated population-normalised consumption for MDMA considering different population estimates and uncertainty components: A. *de facto*-population normalised consumption with U_{tot}(*de facto*)= $U_S+U_C+U_E+U_P$(*de facto*); B. *de jure*-population normalised consumption with a typical $U_{tot}= U_S+U_C+U_E+U_F$; C. *de jure*-population normalised consumption with U_{tot}(*de jure*)= $U_S+U_C+U_E+U_F+U_P$(*de jure*). Black bars along X-axis = missing date (Table S- 20); triangles along X-axis = days with special dates/events.