Hospital Wastewater

Kristell Le Corre[1], Mohammad Katouli[2,4], Helen Stratton[3,4], Christoph Ort[1] and Jurg Keller[1]

August 2012

Urban Water Security Research Alliance
Technical Report No. 76

Queensland Government
National Research FLAGSHIPS Water for a Healthy Country CSIRO
Griffith UNIVERSITY
The University Of Queensland AUSTRALIA

Urban Water Security Research Alliance Technical Report ISSN 1836-5566 (Online)
Urban Water Security Research Alliance Technical Report ISSN 1836-5558 (Print)

The Urban Water Security Research Alliance (UWSRA) is a $50 million partnership over five years between the Queensland Government, CSIRO's Water for a Healthy Country Flagship, Griffith University and The University of Queensland. The Alliance has been formed to address South East Queensland's emerging urban water issues with a focus on water security and recycling. The program will bring new research capacity to South East Queensland tailored to tackling existing and anticipated future issues to inform the implementation of the Water Strategy.

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Enquiries should be addressed to:

The Urban Water Security Research Alliance
PO Box 15087
CITY EAST QLD 4002

Ph: 07-3247 3005
Email: Sharon.Wakem@qwc.qld.gov.au

Project Leader – Kristell Le Corre
The University of Queensland
ST LUCIA QLD 4072

Ph: 07-3346 3229
Email: k.lecorre@awmc.uq.edu.au

Authors:
1. The University of Queensland, Advanced Water Management Centre (AWMC), QLD.
2. Faculty of Science, Health and Education, University of the Sunshine Coast, QLD.
3. School of Biomolecular and Physical Sciences, Griffith University, QLD.
4. Smartwater Research Centre, QLD.

Le Corre, K., Katouli, M, Stratton, H., Ort, C and Keller, J. (2012). *Hospital Wastewater.* Urban Water Security Research Alliance Technical Report No. 76.

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Cover Photographs:

Top raw, from left to right:
* Sampling at Bundamba STP;
* Sampling pump connected to Bundamba Sewage treatment plant (STP) inflow meter.
* Flow proportional sampling set up in a sewer in front of Ipswich hospital.

Bottom raw, from left to right:
* Solid phase extraction of micropollutants in the Advanced Water Management Centre laboratories, The University of Queensland.
** Plate of antibiotic resistance pattern of a bacterium isolated from hospital wastewater.
** Testing the bacteria for their antibiotic resistance pattern in the laboratories of the Faculty of Science, Health and Education, University of the Sunshine Coast.

Photographers: *Kristell Le Corre (UQ), **Mohammad Katoutli (USC)

ACKNOWLEDGEMENTS

This research was undertaken as part of the South East Queensland Urban Water Security Research Alliance, a scientific collaboration between the Queensland Government, CSIRO, The University of Queensland and Griffith University.

Regarding the field study carried out at Caboolture, the authors would like to thanks David Fillmore, Rick Jones and Wayne Batchler from Moreton Bay Water (assistance in setting up proper sampling points and support during the campaign); Benjamin Tan and Mary Hodge from Queensland Health Forensic and Scientific Services (logistics and processing samples); John Doonan, Greg Jackson and Daniel Field from Queensland Health (making contacts and providing hospital data); Christa McArdell from Eawag and the Swiss National Science Foundation (Grant PBEZP2-122958 awarded to the first author).

Concerning the audit data evaluation, particular thanks go to Queensland Health and the Medication Services Queensland for providing hospital audit data and their support regarding understanding and processing of the hospital audit data; Maxine Robinson, Vanna Mabbott and Chris Raymond from the Drug Utilisation Sub-Committee (DUSC) of the Pharmaceutical Benefits Advisory Committee (PBAC) (Department of Health and Ageing, Australian Government) for providing statistics on Australian pharmaceutical consumptions and their support during the data processing.

The authors would also like to thank Queensland Urban Utilities for providing information on water consumptions of hospitals and flow data of the STPs investigated; for allowing access to the sewer in Ipswich for sampling.

Particular thanks go to Lend Lease and more specifically Sean O'Callaghan for his help in organising the sampling campaign and also Tim Bowman, Shane Holding, Nicolas Munroe and Richard Behan-Howell for their technical support (and enthusiasm) during the sampling. John Christiansen and Harry Baron from the West Moreton Hospital and Health Service at Ipswich hospital are also acknowledged for the information provided about the hospital.

The authors would also like to thank Jelena Radjenovic from the Advanced Water Management Centre (AWMC) at the University of Queensland for her immense help and support in the analysis of the pharmaceuticals in wastewater samples from Ipswich and Beatrice Keller for her help with regard to the analytical equipment at the AWMC. Jasmine Thompson, Aycan Gundogdu and Saiyuri Naicker at the University of the Sunshine Coast are also acknowledged for their excellent work with sampling, isolation and identification of bacteria and their antibiotic resistance pattern.

Finally, the members of the reference panel of the Hospital Wastewater project and more specifically Greg Jackson are acknowledged for their great support and contributions throughout the duration of this project.

FOREWORD

Water is fundamental to our quality of life, to economic growth and to the environment. With its booming economy and growing population, Australia's South East Queensland (SEQ) region faces increasing pressure on its water resources. These pressures are compounded by the impact of climate variability and accelerating climate change.

The Urban Water Security Research Alliance, through targeted, multidisciplinary research initiatives, has been formed to address the region's emerging urban water issues.

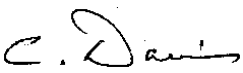
As the largest regionally focused urban water research program in Australia, the Alliance is focused on water security and recycling, but will align research where appropriate with other water research programs such as those of other SEQ water agencies, CSIRO's Water for a Healthy Country National Research Flagship, Water Quality Research Australia, eWater CRC and the Water Services Association of Australia (WSAA).

The Alliance is a partnership between the Queensland Government, CSIRO's Water for a Healthy Country National Research Flagship, The University of Queensland and Griffith University. It brings new research capacity to SEQ, tailored to tackling existing and anticipated future risks, assumptions and uncertainties facing water supply strategy. It is a $50 million partnership over five years.

Alliance research is examining fundamental issues necessary to deliver the region's water needs, including:

- ensuring the reliability and safety of recycled water systems.
- advising on infrastructure and technology for the recycling of wastewater and stormwater.
- building scientific knowledge into the management of health and safety risks in the water supply system.
- increasing community confidence in the future of water supply.

This report is part of a series summarising the output from the Urban Water Security Research Alliance. All reports and additional information about the Alliance can be found at http://www.urbanwateralliance.org.au/about.html.



Chris Davis
Chair, Urban Water Security Research Alliance

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EXECUTIVE SUMMARY

In 2007, the Urban Water Recycling Research Alliance (UWSRA) established a program to address South East Queensland's (SEQ's) emerging urban water issues with a focus on water security and recycling. As part of this program, the "*Purified Recycled Water*" project was developed to assess the health, safety and ecological risks of Purified Recycled Water (PRW) and, more specifically, to provide an assessment of the ability to control the quality of the water reaching an advanced water treatment plant (AWTP). This involved thorough investigations of how specific contaminants could be controlled at the source at locations such as hospitals, and the ability of wastewater treatment plants (WTPs) to remove microbial pathogens and targeted organic compounds.

Indeed, due to the high density of treated patients in a specific location, wastewater discharged from hospitals has been suspected to contribute significant loads of pharmaceutical residues (and other contaminants including detergents, solvent and pathogens such as antibiotic resistant bacteria) in municipal wastewater. But, since pharmaceuticals are also largely excreted by the general public at home, assessing the contribution of an individual hospital in a given catchment of a sewage treatment plant is essential in evaluating if treatment of specific contaminants (i.e. implementation of onsite treatment processes or separate collection of substances of potential concern) at the source would be an efficient and economic approach.

In this context, detailed experimental investigations were carried out at Caboolture Hospital and South Caboolture sewage treatment plant (STP) in 2008 as a sub-project of the Purified Recycled Water project. The results of this study, published by Ort *et al.* (2010a) (Chapter 1), indicated that this hospital was not a major contributor of pharmaceuticals in the influent of the STP to which it discharged its effluent. However, the conclusions of this study were based on a set of only 59 pharmaceutically active compounds for which an analytical method was available. Although Caboolture Public Hospital provides a large range of services and the average occupancy results in a density of 4.2 hospital beds per 1,000 inhabitants - the highest in SEQ for an individual hospital in the catchment of an advanced water treatment plant (AWTP) - these findings needed to be validated for a broader range of substances in catchments of AWTPs in the Western Corridor Recycled Water project.

For this purpose, a follow-up project funded by the UWSRA and exclusively focusing on hospital wastewater was launched in 2010. This two-year project, championed by Queensland Health, was undertaken by the Advance Water Management Centre (AWMC) in collaboration with Griffith University (GU) and the University of the Sunshine Coast (USC). It dealt directly with barrier 1, namely Source Control, of the seven-barrier process in place in SEQ for the production of PRW.

The *Hospital Wastewater* project addressed a large number of chemicals consumed in hospitals by evaluating audit data. A second sampling campaign was undertaken by the AWMC to confirm the audit data evaluation and the results obtained in the earlier research at Caboolture. In addition, the influence of hospitals on the transfer and survivability of antibiotic resistant and multi-resistant bacteria in municipal wastewater was investigated through a pilot study undertaken by GU and USC. Indeed, antibiotics are heavily used in hospitals and are often blamed for the transfer of antibiotic resistant and multi-antibiotic resistant bacteria to sewage, and ultimately surface waters.

Overall, the aim of the project was to provide a stronger basis for the evaluation of the importance of hospital wastewater, hence enhancing the understanding of pollutant fluxes originating from hospitals and helping regulators in the trade waste sector make informed decision on eventual needs for source control options.

Current approaches used to assess risks associated with the presence of pharmaceuticals in municipal wastewater are mainly based on experimental studies (Chapter 4). Recent studies on the detection of pharmaceuticals in various water sources show that the most studied - and eventually detected - compounds are typically the most consumed ones. Indeed, compounds to be analysed are often selected based on criteria such as usage, prescription numbers, sales and/or production amounts. Other parameters used by researchers include known occurrence of substances in the environment, drug class and availability of analytical methods. However, prioritisation strategies to select the compounds for investigation are rarely used. This means that in a majority of experimental studies, the compounds

analysed for may not necessarily be the most important ones in terms of toxicity or impact on the environment and human health.

A number of methodologies have recently been developed in the literature in order to prioritise research on pharmaceutical residues in the aquatic environment. Most of these methodologies use consumption data as a starting point to screen pharmaceuticals based on exposure assessment. To further refine those pharmaceuticals of potential concern, predictions of potential risks to the environment and/or human health are performed. Despite similarities in some of the methodologies, there is a need for a standardisation of strategies to generate prioritisation tools for various water sources that are transferable from one country to another. In the case of pharmaceuticals originating from hospitals, prioritisation strategies need to be more specific as they should not only focus on those compounds most consumed nationally or regionally, but also on consumptions in hospitals.

The initial phase of the hospital wastewater project therefore focused on the evaluation of audit data by developing a refined and extended consumption-based approach (Chapter 3). The evaluation of audit data deserves priority attention, as it covers large numbers of hospitals (107 in Queensland) and includes detailed information on mass consumption for all pharmaceuticals used in a hospital, usually on a yearly basis. This compares to sampling hospital effluent, which can only take place at selected locations, is restricted in time (a few days) and for which results are limited to compounds for which an analytical method exists.

Through this consumption-based approach, the contribution of six hospitals located in SEQ to the loads of 589 pharmaceuticals in municipal wastewater was predicted. The results of this study showed that for 63 to 84% of the pharmaceuticals investigated, individual contributions of hospitals to the influent of a STP were likely to be less than 15%. For these compounds, the selected hospitals were therefore not a major point source.

In addition, the possibility that hospital-specific substances are present at levels that may pose a risk for human health was also evaluated. Between 10 and 20% of the pharmaceuticals consumed in the selected hospitals were found to be exclusively used in these hospitals. For these hospital-specific substances, 57 distinct pharmaceuticals may cause concerns for human health as concentrations predicted in hospital effluents are less than 100-fold lower than effect thresholds. However, concentrations of pharmaceuticals in raw wastewater (from hospital or domestic sources) are expected to be significantly reduced after conventional wastewater treatment and advanced water treatment. Therefore, the results obtained for hospital-specific compounds indicate that these are unlikely to be present in STP effluents at levels representing a risk to humans. Nevertheless, 12 compounds were identified which are less than 100 times below a concentration “of no concern” in the influent of STPs. They warrant more detailed investigations including: environmental and human toxicity assessment; biodegradation assessment; and treatment or source control options.

As a conclusion, the results of this study suggested that the contribution of hospitals to the total load of pharmaceuticals in the influent of a STP and risks of human exposure to the pharmaceuticals exclusively administered in the investigated hospitals were limited. Decentralised wastewater treatment at the hospitals investigated would, therefore, not have a substantial impact on pharmaceutical loads entering STPs, and finally, the environment.

To confirm the outcomes of the Caboolture study and validate the consumption-based approach, the second phase of this project focused on an additional sampling campaign at Ipswich Hospital (296 beds) and its corresponding STP (Bundamba) serving a population of 75,000 people. The concentration of a set of pharmaceuticals previously measured by Ort *et al*. (2010a) at Caboolture Hospital was measured in both the hospital and municipal wastewater. Based on recommendations for sampling developed and published by Ort *et al*. (2010 b and c) as part of the PRW sub-project on hospital wastewater, a flow-proportional sampling system was installed in a sewer collecting effluent from Ipswich Hospital. A similar system was installed to collect influent to the Bundamba STP. Both systems were put in place to collect wastewater over 24-hour cycles over three consecutive days.

The results obtained at the Ipswich case study site confirmed that a hospital is unlikely to contribute significantly to the loads of pharmaceuticals in municipal wastewater, with contributions below 15% for a majority of the compounds investigated. Out of the 34 substances detected in hospital and municipal wastewater, only two substances resulted in maximum contributions above 15%. When

compared to predictions using audit data, the contributions measured at Ipswich were either of the same order of magnitude or below, therefore confirming that predictions using audit data reflect results obtained experimentally for analytically quantifiable compounds.

Overall, the consumption-based approach developed in the current project proved a unique opportunity to screen for pharmaceuticals used in hospitals and identifying priority pollutants in hospital wastewater (HWW) explicitly accounting for site-specific conditions. The contribution determined using such a tool were found to be in good agreement with contributions determined experimentally at two case study sites and can therefore be used to predict the contribution of a wide range of substances for which no analytical methods are available. The next step in the validation process of the consumption-based tool would be the development of analytical methods for hospital-specific substances identified through this approach.

In parallel to the work performed on the evaluation of hospitals' contributions to pharmaceutical contamination in municipal wastewater, the GU/USC team investigated the presence and prevalence of antibiotic resistant bacteria in hospital wastewater (hospital 1) and two independent STPs not receiving wastes from this hospital. The group also investigated transmission of antibiotic resistant bacteria from wastewater of another hospital (hospital 2) to its receiving STP and their survival through treatment processes.

The results of both studies showed that certain clonal groups of resistant bacteria were constantly present in HWW of both hospitals. These clones were highly resistant to a number of antibiotics. STPs that did not receive wastes from hospital 1 were also shown to contain antibiotic resistant strains but the number of antibiotics to which these strains were resistant was significantly lower than those found in wastewater from hospital 1. *E. coli* strains with or without the ability to produce the extended spectrum beta-lactam (ESBL) enzyme were isolated from wastewater from hospital 1 and STPs not connected to any major hospital or healthcare facilities. When tested for their resistance to common antibiotics, 9% of the *E. coli* strains isolated in municipal wastewater were resistant to imipenem and up to 78% of them were resistant to tetracycline. In contrast, the resistance of *E. coli* strains isolated from HWW against these two antibiotics was more than 90%. Overall, the resistance of HWW strains was significantly higher than that of STP strains for a majority of the antibiotics investigated.

When looking at the possible transmission of resistant strains from a hospital to municipal wastewater, the results of this study showed that common types of Gram-positive bacteria, especially methicillin resistant *Staphylococcus aureus* (MRSA), and Gram-negative strains isolated in hospital wastewater are able to survive in sewer networks and reach the inlet of STPs. However, Gram-negative strains tend to survive far better through sewage treatment processes than Gram-positive strains. In terms of resistance, these Gram-negative strains were found to be resistant to higher numbers of antibiotics (8.9 antibiotics on average) compared to Gram-positive bacteria (5.1 antibiotics on average).

Finally, this study indicated that antibiotic resistant strains are unlikely to lose their resistance once they are released into the wastewater and after their transition to a STP. However, the significance of this for public health is not clear and will require further work to characterise and quantify the input of multidrug resistant bacteria from hospitals compared with those originating from the general community or other wastewater related sources.

In conclusion, the outcomes of both experimental and predictive approaches suggest that the implementation of decentralised treatment systems for hospital wastewater as a strategy to reduce pharmaceutical residues in municipal wastewater would have little effect. This may be different in STP catchments with substantially higher numbers of hospital beds relative to the general population. Furthermore, the work on antibiotic resistance performed during this project showed that further attention should be given to the impact of hospital wastewater on the propagation of antibiotic resistant bacteria before further consideration is given to on-site treatment of hospital wastewater for source control.

PART A: CONTRIBUTION OF HOSPITALS TO PHARMACEUTICAL LOADS IN MUNICIPAL WASTEWATER

Authors

- Kristell Le Corre
- Christoph Ort
- Jurg Keller

The University of Queensland, Advanced Water Management Centre, Brisbane, QLD 4072, Australia.

1. EXPERIMENTAL APPROACH (I): DETERMINING THE FRACTION OF PHARMACEUTICAL RESIDUES IN WASTEWATER ORIGINATING FROM A HOSPITAL - CABOOLTURE CASE STUDY

This chapter presents the research undertaken in the UWSRA *Purified Recycled Water* project by Christoph Ort[a], Michael G. Lawrence[a], Julien Reungoat[a], Geoff Eaglesham[b], Steve Carte[b] and Jurg Keller[a] and published in Water Research, Volume 44, Pages 605-615, January 2010 (see Ort *et al.,* 2010a).

[a] The University of Queensland, Advanced Water Management Centre (AWMC), Brisbane, QLD 4072, Australia
[b] Queensland Health Forensic and Scientific Services, Organics Laboratory, QLD 4108, Australia

1.1. Abstract

Pharmaceutical residues in water are frequently analysed and discussed in connection with sewage treatment, ecotoxicity and, natural and drinking water quality. Among different localities hospitals are suspected, or implied, to be a major and highly variable source of pharmaceuticals that substantially contribute to the total wastewater load. In this study, the contribution of pharmaceuticals from a hospital to a sewage treatment plant (STP) serving around 45,000 inhabitants was evaluated. Approximately 200 hospital beds result in a hospital bed density of 4.4 beds per 1,000 inhabitants, which is a typical value for developed world countries. Prior to sampling, a sound systems analysis was performed, and a sophisticated continuous flow-proportional sampling regime was applied. Hence, overall experimental uncertainty was reduced to a minimum, and measurements provide clear evidence that, for 28 of 59 investigated substances, over 85% of the pharmaceutical residue loads do not originate from the hospital when applying a conservative error estimation. Only for 2 substances, trimethoprim (18%) and roxithromycin (56%), was the maximum observed contribution of the hospital >15%. On average, the contribution of the hospital for the compounds detected in both, hospital effluent and sewage treatment plant influent was small and fairly constant. Five compounds were only detected in hospital wastewater and 24 neither in the hospital wastewater nor in the total wastewater at the influent of the STP. For these compounds, no experimental contribution could be calculated. For the compounds where audit data for both the national consumption and the specific hospital under investigation were available, a prediction of the fraction of pharmaceuticals originating from the hospital was performed. Three quarters of the compounds, classified with the existing audit data, were in the same ''hospital contribution category'' as determined by measurements. For most of the other compounds, plausible reasons could be identified to explain the observed deviations.

1.2. Introduction

1.2.1. Brief Overview

Hospital wastewater (HWW) is normally discharged directly, without pre-treatment, to sewers. Despite mostly being only a small fraction of the total wastewater volume in the influent of a sewage treatment plant (STP), HWW has gained increasing scientific and public attention in the last decade. This is, in part due to the observation and expectation that HWW is a source for undesirable constituents, such as (multi-)antibiotic-resistant bacteria (Baquero *et al.*, 2008; Kümmerer, 2004a). In other publications, the emission from hospitals was estimated for antibiotics, anaesthetics, disinfectants, heavy metals, AOX (Adsorbable Organic Halogens), iodised X-ray contrast media and cytostatic agents (e.g. Kümmerer, 2001). The latter were also investigated in detail by Lenz *et al.* (2007). Furthermore, a number of toxicity assays were performed (Boillot *et al.*, 2008; Ferk *et al.*, 2009; Hartmann *et al.*, 1998). As a result, it has been suggested in some studies that pre-treatment of HWW prior to discharge into the sewers provides a reasonable solution (Gautam *et al.*, 2007; Lenz *et al.*, 2007; Pauwels and Verstraete, 2006). However, this view is not unanimously supported. The separate treatment of HWW to reduce the development of resistant bacteria was questioned (Kümmerer, 2009): the substantial amount of antibiotics used outside of hospitals (in Germany more than 75%) seems to be a plausible reason that resistant bacteria are also abundant in wastewater not receiving any HWW. Additionally, Boillot *et al.* (2008) found quantitatively far fewer microorganisms

in the effluents of hospitals than in urban wastewaters, which is consistent with other studies. With regard to pharmaceuticals, Lenz *et al.* (2007) report that: 1) for some pharmaceuticals merely a small fraction of the amounts administered in the hospital were actually found in its effluent (i.e. 0.1–0.2% for doxorubicin, 0.5– 4.5% for 5-fluorouracil and 27–34% for total platinum); and 2) a complete onsite wastewater treatment process is needed to significantly remove targeted pharmaceuticals. This includes full physical and biological treatment steps, not only advanced processes. Capturing all sources within a hospital (wards, laboratories) may be further complicated by the fact that different facilities discharge through different pipes to the common sewer. This particularly holds true for large existing hospital complexes.

Therefore, local circumstances need to be considered and the contribution of an individual hospital needs to be assessed in relation to the total load in a STP catchment. To our knowledge, only a few publications explicitly quantify pharmaceutical residues (subsequently referred to as 'pharmaceuticals') excreted within hospitals compared to the total pharmaceutical load in the corresponding STP influents (Feldmann *et al.*, 2008; Heberer and Feldmann, 2005; Thomas *et al.*, 2007). However, these studies are limited to a small number of pharmaceuticals, or make an assumption on the water flow instead of measuring the wastewater flow onsite to determine actual loads.

In view of the local situation in SEQ where it is proposed to recycle wastewater for indirect potable reuse, it is sensible to consider whether pre-treatment of HWW will provide a significant benefit. From two previous research papers relevant for the region of interest also dealing with pharmaceuticals the contribution of hospitals cannot be derived (Khan and Ongerth, 2004; Watkinson *et al.*, 2009).

Therefore, the goal of our study is to determine accurately the contribution of a hospital to the total pharmaceutical load found at the inlet of the corresponding STP by means of measurements. Additionally, this experimentally data obtained from a limited time period is then compared with readily available audit data. It shall be assessed whether the contribution of a hospital can be predicted reliably without any additional administrative effort, i.e. without extra surveys on the hospital wards for day-specific consumptions. If measurements matched with the prediction, the same kind (comprehensiveness and quality) of information can be used at other locations to make a prediction, a priori without laborious measurements.

The focus of this research is on dissolved pollutants which cannot be eliminated in conventional wastewater treatment. Pollutants showing poor to moderate biological removal need to be transformed by chemical reactions (e.g. oxidation) or separated by physical processes (e.g. adsorption onto activated carbon).

1.2.2. Systems Analysis

The prediction and experimental quantification of pharmaceutical mass fluxes in the wastewater of a specific STP catchment are laborious. A sound understanding of the whole system is required prior to setting up a predictive model, and performing a confirmative sampling campaign. This particularly holds true when attempting to attribute different fractions to a multitude of individual sources, for example if there are several hospitals and multiple smaller healthcare facilities in a catchment. Due to the lack of generally accessible consumption data at sufficiently high spatial and temporal resolution, models often provide only a prediction of an average load. Additionally, the latter is prone to uncertainty due to varying transformations of pharmaceuticals during human metabolism.

While it would be ideal to have a list of all health care facilities with size, services provided and precise pharmaceutical consumption, just obtaining generally available consumption data is a tedious task in itself. The ''institutional resolution'' is often not sufficient without additional administrative effort, i.e. temporary surveys of the wards in the hospital(s) under investigation (Feldmann *et al.*, 2008; Kümmerer, 2001). Furthermore, the (average) household pharmaceutical consumption needs to be estimated from national or state-wide sales and/or prescription data if regional data is not available.

Moreover, collecting representative samples requires a thorough knowledge of the sewer layout and awareness of potentially highly variable concentrations and loads in the course of a day. Clearly, accurate chemical analysis of a non-representative sample is not adequate to characterise a real full-scale system.

1.2.3. Sampling Issues

Accurately quantifying pharmaceutical loads in hospital effluents or sewers close to any source (sub-catchments, households or industry) is a demanding undertaking. It requires a substantial experimental effort and is still prone to uncertainties. The latter are extremely hard to quantify if sampling is carried out with conventional (unsophisticated) devices, i.e. auto-samplers operated in a discrete sampling mode with (too) long time intervals, or grab samples. Rarely are fluctuations of concentrations and loads assessed in separate experiments at high temporal resolution prior to the ''real'' measuring campaigns.

These pre-experiments are very expensive and may not provide the data to answer the actual research question. However, if the applied sampling protocol does not result in the collection of a representative sample, then the care taken in the following processes of transport, storage, preparation and chemical analyses with a sophisticated method cannot make up for this deficiency (de Gruijter *et al.*, 2006). Subsequent (even sophisticated) statistical analyses of non-representative samples are unreliable and the resulting conclusions will therefore be of limited value. In some cases, the large variation observed in previous studies may not be ''true natural variation'' but instead, may simply be an artefact caused by inadequate sampling (Ort *et al.*, 2010).

Therefore, strong emphasis has been put on obtaining representative samples for this study. In Ort and Gujer (2006), a method was presented to estimate the required sampling frequency in order to not exceed a certain sampling error. In gravity sewers, this results in fairly short time intervals if the substance of interest is contained in a small number of ''wastewater pulses'' per day (e.g. toilet flushes containing a specific excreted pharmaceutically active compound).

Sampling frequencies that are too low result in large sampling uncertainties, especially in the case of only a few patients per day (Weissbrodt *et al.*, 2009). The often claimed problem of ''limited storage capacity in an auto-sampler'' can be easily solved by replacing the glass bottles more than once per day. This may be more laborious, but it is a much better solution than using a time-proportional sampling mode, which does not take samples weighted according to the flow in the sewer. In contrast, physical boundary conditions such as deep sewers resulting in long dead times for purging the sampling hose or limited access to pressurised sewers are more difficult to overcome.

1.3. Material and Methods

1.3.1. Sewage Treatment Plant and Catchment Characteristics

A total of approximately 45,000 inhabitants in two geographically separated sub-catchments, Morayfield and Caboolture, are connected to the South Caboolture STP (subsequently only referred to as STP) which is operated with two sequencing batch reactors (SBRs). It treats a daily dry weather flow of approximately 10,000m^3. During long dry periods with high level water restrictions, this value can drop to 7500m^3 per day.

Morayfield is drained by gravity sewers and contributes two thirds of the total wastewater. It is only pumped once, at the STP itself. Caboolture makes up for one third of the total influent and is a largely pressurised sewer system with numerous pumping stations. At specific times of the day, the flow is diverted at the influent of the STP and stored in two large buffer tanks (800m^3 each) before being pumped to the SBRs. This combination of sewers and the complex influent layout of the STP results in very high hydraulic fluctuations (Figure 1). Hours with almost zero flow contrast with hours around 250–300 L/s and in between, the flow varies rapidly and significantly. During wet weather the relative flow variations are less significant due to higher base flow.

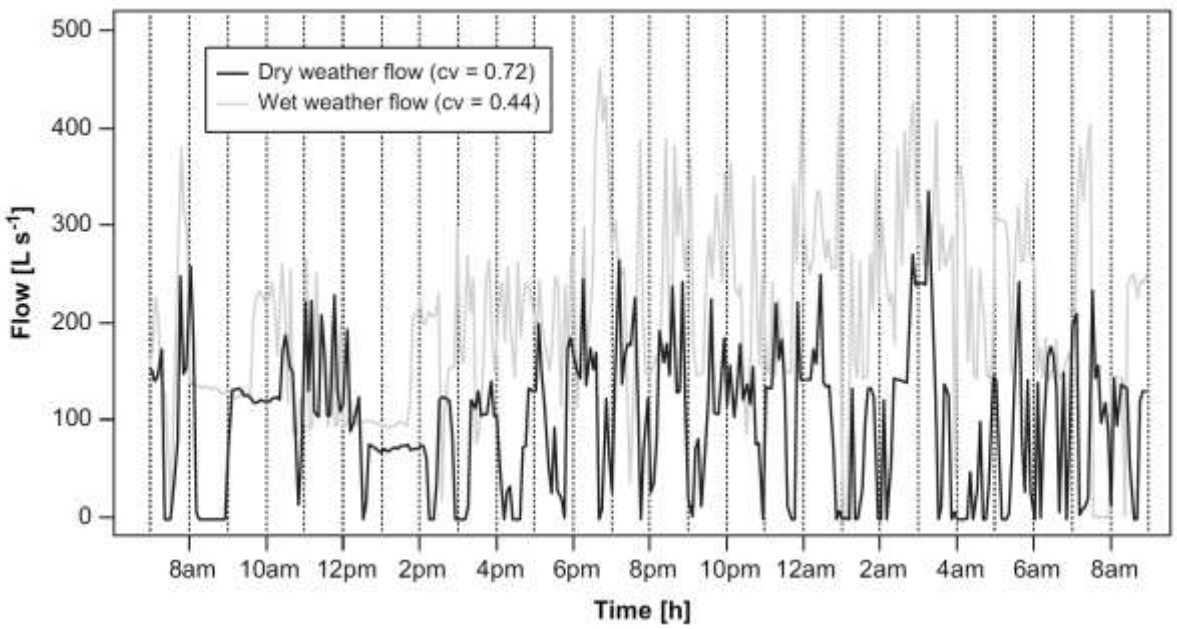


Figure 1. Two examples for typical flow patterns at the influent of the sewage treatment plant; cv = coefficient of variation (standard deviation/mean).

1.3.2. Hospital Characteristics

Caboolture Public Hospital has 190 beds and offers all services of a modern regional hospital (listed in Table SI 1, see supporting information A). A small private hospital providing mainly day surgery (only around 10 beds) and a small dental surgery also drain into the same sewer. The wastewater from the private hospital cannot be accessed separately. Other small health care facilities within this sewer catchment make consultations to out-patients, and therefore, the wastewater from these facilities are not expected to significantly add to the pharmaceutical load of the STP. The hospital bed density for the whole STP catchment is 4.4 beds per 1,000 inhabitants. All HWW is collected in a sewage pumping station (SPS CT-51, subsequently referred to as SPS) before being pumped to the primary rising main. There is no residential wastewater contributing to this SPS and the hydraulic residence time in the main sewer to the STP is approximately 30 min to 1 h (hydraulic calculations provided by the Regional Council for the decisive time in the morning when samples at the SPS and the STP needed to be coordinated). The average daily volume during dry periods pumped at the SPS is approximately 75m^3 which is 1% of the total wastewater volume discharged to the STP. The occupancy of hospital beds in Caboolture during the sampling period was close to 100% which is representative for the year to date average.

Unfortunately no comprehensive database exists with regard to other health care facilities in the catchment of the STP. Hence, an internet search was performed. Four aged care facilities with a total capacity of 443 beds were found (297 high care and 146 low care) with an unknown occupancy rate. Furthermore, a total of 14 addresses for doctors plus 12 dentists were found. If mass fluxes at the influent of the STP were significantly higher than expected from average national consumption and hospital usage, further investigations of these facilities would be warranted.

1.3.3. Sampling

Continuous flow-proportional sampling modes were applied in this study to minimise sampling error. Continuously diverting a small flow-proportional side stream is conceptually the best solution to obtain representative samples for dissolved compounds. However, low velocities in the side stream prevent proper sampling of solids and long-term operation may lead to biofilm growth. Due to the limited time of sampling biofilm growth is not considered problematic in this instance. Sampling over

consecutive days was preferred to the alternative option of collecting samples on single days distributed over a longer period. This drastically reduces the effect of unknown system behaviour: missing a ''decisive'' HWW packet at the STP is then limited to the first hour of the first day and the last hour of the last day. All other water packets are captured, although they might be attributed to the STP sample a day later. However, this would merely lead to higher variability of the hospital's contribution and not to a non-quantifiable effect.

1.3.3.1. Sampling Protocol for Caboolture Hospital (SPS CT-51)

The HWW is not easily accessible before it enters the SPS. Furthermore it would have been very difficult to set up an accurate flow meter to measure flow in a small open channel with intermittent, partially very low flows and to use this data to control the speed of the sampling pump. Instead, plumbers from the Regional Council fitted a tap in the rising main of the SPS (Figure 2).

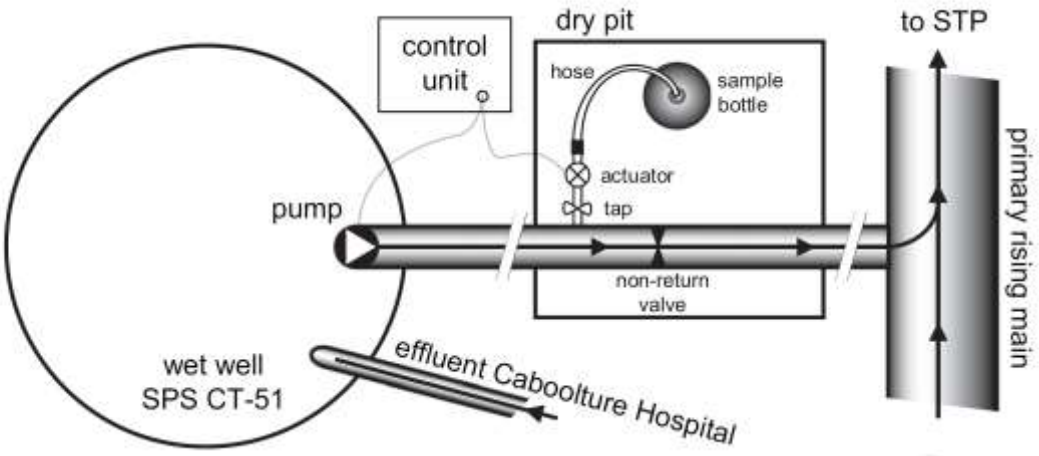


Figure 2. Schematic drawing of the sampling point at the sewage pumping station (SPS) CT-51 (not to scale): All hospital wastewater is discharged to the wet well of the SPS and intermittently pumped to the primary rising main leading to the sewage treatment plant (STP). Upstream of the non-return valve a stand pipe with a tap and an actuator was fitted. This allows for taking flow-proportional samples during individual pump cycles.

The tap is upstream of the non-return valve before the HWW enters the primary rising main leading to the STP. Electricians from the Regional Council installed an actuator after the tap which only opens when the pump of the SPS empties the wet well. Water runs without a sampling pump due to the pressure in the rising main. Under normal operating conditions, there are about 24 pumping cycles per day, triggered automatically based on to the water level in the SPS. While it was found that the flow during one cycle is fairly constant, it can vary significantly among cycles due to variable hydraulic conditions in the primary rising main. Therefore, a manual operating mode was adopted, disabling the auto level control. This allowed for using the full storage capacity of the wet well. Starting at 7 AM it was emptied again at 12 PM, 6 PM and 7 AM the following day which required personnel to be present three times per day (and operating in a confined space). The pump operates at about 2500 L/min and the sampling side stream was adjusted with the tap to approximately 1 L/min, resulting in a sampling volume of about 10 L per pump cycle. In comparison, the dead volume of the tap installation including hose was 0.5 L (ca. 5% of the sampling volume).

The three samples were collected in separate glass bottles, and analysed separately. The concentrations of the individual samples were multiplied with the flow for the corresponding pump cycle, and summed to obtain a 24-h load. Rough diurnal variations could also be determined with this sampling procedure, but they are not relevant for the system and time scales under investigation, and hence they are not further discussed in this paper.

1.3.3.2. Sampling Protocol at the Sewage Treatment Plant

To sample for the same ‘‘water packets’’ as at the SPS, sampling started at 7:45 AM in the influent of the STP. The storage tanks start filling at 8 AM and are emptied completely during night time, and in the early morning hours. This guarantees that wastewater is not stored and dragged on over different 24-h sampling periods. Flow rates in the influent are routinely measured at high temporal resolution. A wire connected to an analogue digital converter provides a 4–20mA signal from the PLC (programmable logic controller) linear to the flow in the sewer to control the speed of the sampling pump. The peristaltic pump (Watson Marlow 520UN, programmable interface, water proof casing, equipped with a 520R2 pump head and 3.2mm tube bore) was tested in the lab to ensure its linear behaviour over the full speed range under similar physical boundary conditions (suction height approximately 2 m, pressure height negligible). The pump speed was set to 0 rpm (revolutions per minute) for 0 L s^{-1} in the sewer (pumping 0 mL min^{-1}) and to 34 rpm for 1,000 L s^{-1} (pumping 69.4 mL min^{-1}). The finest increment of the pump is 0.1 rpm equivalent to 2.9 L s^{-1} wastewater flow in the influent of the STP. With this setup approximately 15 L of wastewater were collected in a 20 L glass bottle which was located in a refrigerated container. Two field blanks were collected: to this end 0.5 L of MilliQ water was used to rinse the sampling tube and subsequently 0.5 L MilliQ water was pumped through the tube to be analysed in the laboratory. No substances were detected above the limit of quantification.

1.3.4. Chemical Analyses

After collection, the continuously refrigerated samples were transported to the laboratory where they were filtered the same day and preserved before analysis. All samples were analysed for 59 substances by Queensland Health Forensic and Scientific Services (QHFSS). A detailed description of the method consisting of solid phase extraction followed by concentration prior to quantification by LC–MS/MS (liquid chromatography coupled with tandem mass spectrometry) is given in the supplementary information SI 2, accompanied with an alphabetical list of all compounds (see Table SI 3-Table SI 4).

As the method does not allow for correction of absolute analytical extraction recoveries in raw wastewater samples, we report relative loads. In order to compare hospital effluent samples with samples from the influent of the STP, it is necessary to assume that matrix effects between these sample types are similar. Any systematic error in recovery is therefore cancelled out when calculating ratios of loads, i.e. contribution of HWW to the total influent of the STP.

1.3.5. Uncertainty Assessment

Flows in completely filled pressurised pipes can be measured more accurately than flows in open water channels (gravity flow). For this study, a maximum error of ±10% was assumed, which equals to ± 6% ($=10/3^{0.5}$) as single standard deviation of a normal distribution. For chemical analysis, a random uncertainty (reproducibility) of ± 20% for all compounds was chosen (see Table SI 2 -Table SI 4). The two errors are independent, and Gaussian error propagation results in an overall uncertainty estimate for calculated loads of ± 21% ($= [6^2 + 20^2]^{0.5}$). The flow-proportional continuous sampling procedure covers all fluctuations in the wastewater over time. Since it is a reasonable assumption that dissolved compounds are completely mixed over the whole pipe cross section in the influent works, no additional errors need to be taken into account due to sampling.

1.3.6. Pharmaceutical Audit Data

1.3.6.1. National Consumption

An extract from the DUSC database (Drug Utilisation Sub- Committee) for the year 2008 is listed for the compounds investigated in this study (see supporting information A3, Supporting information A3: Table SI 5). It comprises the sum of subsidised drugs (subsidised under the Pharmaceutical Benefits Scheme (PBS) and the Repatriation Pharmaceutical Benefits Scheme (RPBS) and processed by Medicare Australia) and non-subsidised drugs (under PBS co-payment and private prescriptions). The amounts of non-subsidised drugs were estimated from continuous data on all prescriptions dispensed from a validated sample of 370 community based pharmacies. The available data do not include drugs

dispensed to public hospital in-patients, pharmacy over-the-counter drugs (i.e. non-prescription) and drugs supplied by supermarkets.

1.3.6.2. Amounts Administered to In-Patients in Caboolture Public Hospital

No specific survey was carried out during the sampling period on the wards. Routinely stored audit data for a current 12-month period (2007–2008) was made available by the pharmacy of the Caboolture Public Hospital. For each pharmaceutical, a specific database query was performed to derive the amounts exclusively used for hospitalised in-patients; pharmaceuticals given to out-patients (in consultations and pharmacy) were not considered, since they will be taken and excreted at home. The total annual hospital load was determined after summing the contributions of all medications containing the pharmaceutically active compound of interest. It has to be noted that the amounts derived from this database are amounts supplied by the pharmacy to the individual wards and not the amounts effectively administered. However, it is generally not the hospital's policy to discard drugs to the (solid or liquid) waste system, both from a financial and environmental point of view. Nevertheless, some unused drugs for in-patients may be collected on the wards and returned to the pharmacy for reuse or proper disposal. Hence, these drugs do not contribute to the load in the HWW. However, in discussion with relevant hospital staff these amounts are considered to be very limited and are not assessed within this study.

1.4. Results and Discussion

1.4.1. Evaluation of Wastewater Volumes

The four consecutive weekdays, mid-February 2009 when sampling took place, were during a wet period, with flows 1.5–2 times higher than normal dry weather flow (i.e. surface runoff in catchments and infiltration to sewage pumping stations). In Table 1, the flows at the two sampling locations over the corresponding 24-h periods are summarised. During the sampling period, the hospital contributed less than 1% of the total wastewater flow to the STP.

Table 1. Wastewater volumes over 24 h at the SPS CT-51 (hospital wastewater) and the influent to the STP.

		Influent STP [m3]	Hospital Wastewater (Flow at SPS)	
		7:45 AM – 7:45 AM of the following day	7 AM–7 AM of the following day [m3]	Fraction of Influent STP [%]
Day 1	16/2/09	14,064	109	0.8
Day 2	17/2/09	16,921	129	0.8
Day 3	18/2/09	19,059	138	0.7
Day 4	19/2/09	14,347	127	0.9

1.4.2. Evaluation of Relative Pharmaceutical Loads

To obtain relative pharmaceutical loads, measured concentrations were multiplied with the corresponding 24-h flow at each sampling location and normalised by the highest STP influent load. Four examples representing four different groups of pharmaceuticals are charted in Figure 3. Absolute concentration values are not reported because they are difficult to compare among different studies; they highly depend on the sewer system (separate or combined) and on the hydraulic conditions (dry or wet weather flow). The key figures chosen for statistical evaluation are presented in Table 2, and discussed subsequently in detail for one example (atenolol, a beta-blocker, see also Figure 3A).

The numbers in black circles (❶) refer to the corresponding column in Table 2:

❶ Concentration values for atenolol in the influent of the STP were, on average, 10 times higher than the limit of quantification (LOQ).

❷ The concentrations in the hospital effluent were on average 2 times higher than in the STP influent.

❸ The STP influent loads show only little day-to-day variation (cv ¼ 0.06, cv ¼ coefficient of variation ¼ standard deviation/mean).

❹ Day-to-day variation is smaller than the estimated overall uncertainty.

❺ The loads in the hospital effluent varied more (cv ¼ 0.27).

❻ On average the hospital contributed only 1.8% to the total atenolol load in the influent of the STP. For a conservative error estimation, a maximum contribution of the hospital was calculated by dividing the upper uncertainty value of the hospital effluent by the lower uncertainty value of the STP influent for each day (see Figure 3). Over all four days, the highest maximum contribution for atenolol was 3.5%.

❼ Over all four days, the smallest minimum contribution for atenolol was 0.9% (analogue procedure as in ❻).

❽ The prediction for an average contribution of the hospital based on audit data is 0.6% (see more details in Section 1.4.4).

❾ Classification of all substances according to maximum observed contribution from the hospital (❻).

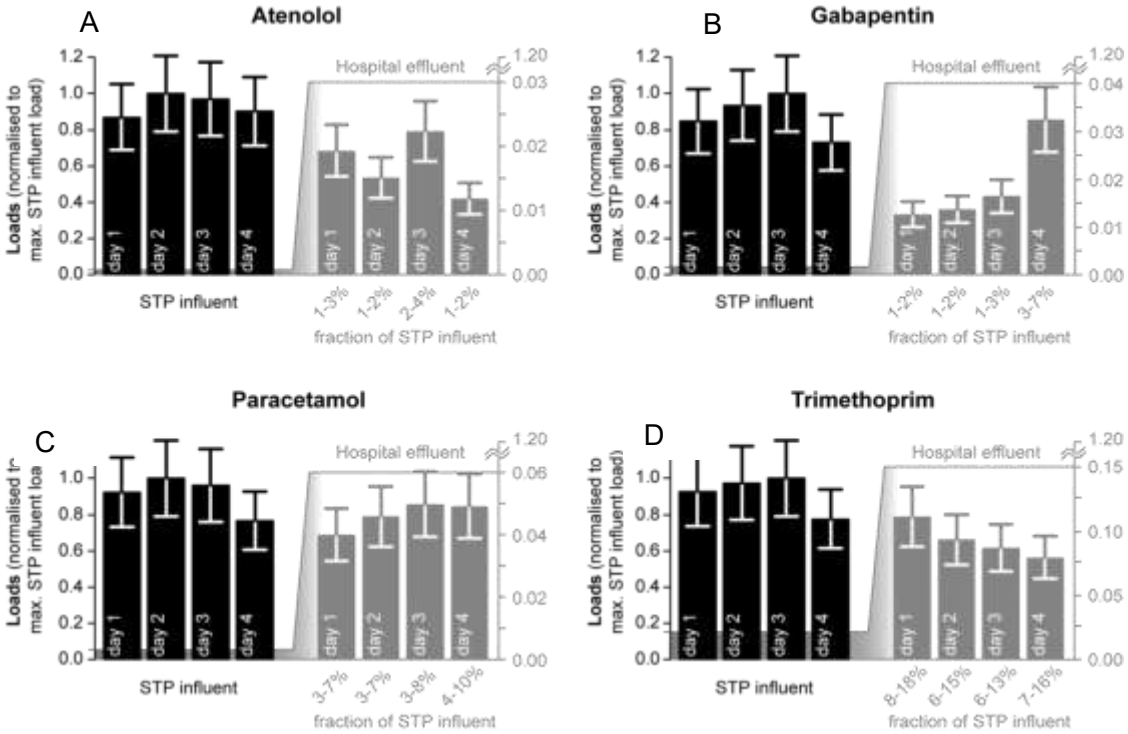


Figure 3. Measured, relative pharmaceutical loads over 24-h periods in the influent of the STP and effluent of the hospital for four consecutive weekdays. Error bars include uncertainty of flow measurements (±6%) and chemical analysis (±20%), resulting in an overall uncertainty of ±21% (single standard deviation). Note the different scales for the y-axis of STP influent and hospital effluent.

The consistent results for atenolol are reflected across most of the 30 detected substances. Representatives of other pharmaceutical groups show also fairly constant loads over the four-day period: gabapentin (an anticonvulsant), paracetamol (an analgesic) and trimethoprim (an antibiotic, see Figure 3B-D).

Of the 59 substances, five were detected only in the HWW but not in the influent of the STP and 24 substances were not detected above the LOQ in any of the samples. The 30 substances detected at both locations were classified for the hospital's contribution to the total influent of the STP. To this end, the maximum observed contribution including uncertainty as a conservative estimate was used (see description before in ❻). The hospital's contribution for 17 substances was at all times ''smaller than 5%'', 11 additional substances fall in the category ''smaller than 15%'' and only two substances were ''above 15%'' (trimethoprim and roxithromycin with a worst case estimate of 18% and 56% respectively). For most substances quantified in both STP influent and hospital effluent, the variations of the loads in the HWW were on average 2.4 times higher than in the influent to the STP. The small number of hospital patients compared to the potentially large number of individuals taking these pharmaceuticals at home is a valid explanation for this observed difference in variation.

Four out of the five substances only detected in the HWW were just above the LOQ. With the 100 fold dilution in the influent of the STP, the LOQ would have to be at least three orders of magnitude lower to reliably quantify the hospital's (high) contribution. When assuming that the concentrations in the influent of the STP were equivalent to the corresponding LOQ, only a one-sided estimation with regard to the hospital's contributions from >5% up to >50% can be made. However, in some cases this deviates from the prediction based on audit data (see section 1.1.1).

Table 2. Classification of substances according to the contribution of the hospital to the total load in the influent of the STP (see section 1.1.1 for more explanations of key figures marked with black circles). LOQs for all compounds are between 0.1 and 2 µg L^{-1}.

Classification ❺	Substance	Therapeutic Group	$\frac{\overline{C}_{STP}}{LOQ}$ ❶	$\frac{\overline{C}_{hospital}}{\overline{C}_{STP}}$ ❷	Coefficient of Variation for Loads		Concentration of Hospital Wastewater [% of total STP influent]			
							Measured			Average Predicted with Audit Data[d] ❽
					Influent STP ❸	Hospital ❹	Min ❼	Mean ❺	Max ❻	
Max ≤ 5%	Atenolol	BB	10.7	2.0	0.06	0.27	0.9	1.8	3.5	0.6
	Atorvastatin	HL	<LOQ	–	–	0.47	–	3c	–	0.9
	Caffeine	–	296.4	3.0	0.07	0.17	1.4	2.6	4.4	–
	Carba-mazepine	AC	6.3	0.6	0.17	0.65	0.0	0.4	1.3	1.9
	Cephalexin[a]	AB	33.8	0.9	0.21	1.33	0.0	0.4	1.2	5.9
	Citalopram	AD	<LOQ	–	–	0.19	–	4c	–	1.6
	Codeine	AG	1.6	1.8	0.08	0.47	0.5	1.5	3.7	6.6
	DEET	IR	24.2	0.2	0.22	0.18	0.1	0.2	0.3	–
	Diclofenac	AI	<LOQ	–	–	0.53	–	1c	–	1.8
	Hydrochloro-thiazide	DI	8.7	2.7	0.24	0.30	1.1	2.0	3.7	0.5
	Iopromide[b]	XC	1.3	3.9	0.20	–	1.4	2.1	3.2	–
	Naproxen	AI	1.9	3.0	0.11	0.34	0.8	2.3	4.4	0.3
	Oxazepam	AL	5.5	1.2	0.08	0.41	0.4	1.2	2.8	1.5
	Oxycodone	AG	<LOQ	–	–	0.33	–	3[c]	–	5.0
	Sulphametho-xazole	AB	6.7	1.1	0.05	0.65	0.2	0.8	2.2	6.7
	Temazepam	SE	2.3	1.9	0.06	0.23	0.7	1.6	3.1	4.3
	Venlafaxine	AD	11.2	2.6	0.16	0.27	0.9	2.0	5.0	2
5% <max <15%	Erythromycin	AB	7.7	2.8	0.41	0.28	0.8	2.6	5.5	4.3
	Erythromycin	AB	7.7	2.8	0.41	0.28	0.8	2.6	5.5	4.3
	Gabapentin	AC	56.5	3.2	0.13	0.49	1.0	2.3	6.8	4.6
	Gemfibrozil	HL	3.9	5.3	0.26	0.85	0.7	4.1	10.0	0.4
	Ibuprofen	AI	70.6	6.3	0.08	0.18	2.7	4.6	8.5	49
	Metoprolol	BB	3.5	4.6	0.19	0.29	2.0	4.1	7.0	2.3
	Paracetamol	AG	1293.6	6.8	0.11	0.10	2.8	5.1	9.8	10
	Ranitidine	HB	3.2	6.2	0.12	0.38	1.3	4.9	11.0	5.7
	Salicylic acid	m	60.1	4.9	0.26	0.18	1.8	4.9	10.8	11
	Tramadol	AG	11.0	3.4	0.18	0.26	1.2	2.5	6.0	6.7
	Triclosan	BI	<LOQ	–	–	0.25	–	6[c]	–	–
Max >15%	Roxithromycin	AB	1.4	28.4	0.22	0.20	11.7	25.7	56.0	19
	Trimethoprim	AB	3.4	13.3	0.11	0.15	5.7	10.1	18.3	14
All values at STP <LOQ	Ciprofloxacin	AB	<LOQ	–	–	0.24	–	–	≥50[h]	10
	Desmethyl Citalopram	m	<LOQ	–	–	0.27	–	–	≥5[h]	–
	Indomethacin	AI	<LOQ	–	–	0.44	–	–	≥15[h]	10
	Lincomycin[g]	AB	<LOQ	–	–	0.53	–	–	≥50[h]	–
	Sertraline	AD	<LOQ	–	–	0.22	–	–	≥5[h]	1.2
All values < LOQ[e]	Acetylsalicylic acid (11%),[e] Chloramphenicol (0.1%) Chlortetracycline, Cyclophosphamide (1.6%), Dapsone, Desmethyl Diazepam (11%), Diazepam (11%), Doxylamine, Enrofloxacin, Fluoxetine (0.8%), Fluvastatin, Ifosfamide, Norfloxacin (3.2%), Oxytetracycline, Phenytoin (4.2%), Praziquantel, Propranolol (0.8%), Simvastatin (0.5%), Sulphasalazine (0.7%), Sulphadiazine, Sulphathiazole, Tetracycline, Tylosin, Warfarin (1.9%)									

– Not available.

a Only detected twice in hospital (influent STP four times).

b Only detected once in hospital (influent STP twice).

c Calculated with average loads measured in the influent of the same STP (three non-consecutive days in 2008 during very low dry weather flows).

d Audit data for hospital 2007–2008, audit data for national consumption 2008 (DUSC database).

e Numbers in brackets are the fraction of the hospital based on audit data (same as for column for the other compounds).

f AB= antibiotic, AC = anticonvulsant, AD = antidepressant, AG =analgesic, AI = anti-inflammatory, AL = anxiolytic, BB = beta-blocker, BI = biocide, DI = diuretic, HB = histamine blocker, HL = hypolipidemic agent, IR = insect repellent, m= metabolite, SE = sedative, XC = X-ray contrast media.

g More than 97% of national consumption used in agriculture (Watkinson *et al.*, 2009).

h When assuming C_{STP} = LOQ.

1.4.3. Comparison with Audit Data

If the consumption of pharmaceuticals in a STP catchment can be estimated from existing national sales or prescription data, and audit data for the hospital are available, the contribution of the hospital can be calculated with the following equation **1**:

$$\text{Contribution (hospital)} = \frac{\text{Cons}_{\text{Cab.Hosp}} \cdot \text{excretion ratio}}{\text{Cons}_{\text{Cab.Pop}} \cdot \text{excretion ratio} + \text{Cons}_{\text{Cab.Hosp}} \cdot \text{excretion ratio}} \quad (1)$$

$$\cong \frac{\text{measured load (hospital)} \cdot \text{recovery} \cdot \text{accuracy}}{\text{measured load (STP catchment)} \cdot \text{recovery} \cdot \text{accuracy}}$$

$$\text{with } \text{Cons}_{\text{Cab.Pop}} = \frac{\text{Cons}_{\text{AUS}}}{20,000,000} \times 45,000$$

where Cons is the consumption, Cab stands for Caboolture, Pop. for population, AUS for Australia and Hosp. for hospital. It becomes evident that the transformation due to human metabolism (excretion ratio) cancels out of the equation when assumed to be similar for patients in the hospital and for people at home. The consumption of pharmaceuticals in the STP catchment is estimated by calculating an average per capita consumption from the national consumption data multiplied with the number of inhabitants in the catchment. The consumption of in-patients in the hospital is added to the domestic consumption to obtain an estimate for the total STP influent load (see also Table SI 5).

The prediction for 27 compounds where both national and hospital audit data were available, in some cases, deviated significantly from the experimentally determined values. However, only eight substances would have been classified differently based on audit data when applying strict boundaries for the classification, which does not change the overall picture substantially.

Possible reasons for three examples are briefly discussed: 1) The overestimation in the case of ibuprofen may be reasonably explained by the fact that the national consumption is likely to be substantially underestimated because ibuprofen can also be obtained over the counter and in supermarkets without prescription. 2) A patient who regularly takes histamine blockers (at home) is likely to take them with him if he is being hospitalised (for any treatment not related or interfering with histamine blockers). This is one of the cases where patients may bring their own medication to the hospital and is also assumed to be valid for beta-blockers and diuretics. 3) In some countries, trimethoprim is often applied together with sulphamethoxazole (combination item) and hence would be expected in a similar ratio. In Australia, the consumption pattern is different: 70% of trimethoprim is sold as single item (general public) and in the hospital under investigation even 90% is administered as an individual compound.

In other cases, the explanation may be sought in a higher or lower than average number of patients being treated during the sampling period in the hospital. However, if the number of treated patients shall be estimated from measurements, the excretion ratio and absolute recoveries for chemical analyses need to be taken in to account (equation **1**). This makes it difficult to compare measured influent loads from a STP with audit data from an individual health care facility to reliably calculate the health care facility's contribution to the total influent of the STP.

1.4.4. Comparison with Other Studies

The Caboolture catchment, with 4.4 beds per 1,000 inhabitants, is comparable with two other studies (3.6 and 4.4 beds per 1,000 inhabitants, see Table 3). Without audit data for the hospitals and general public, the load estimations based on measured concentrations and an estimate for wastewater based on average water consumption in the study by Thomas *et al.* (2007) make a direct comparison difficult. However, higher contributions were also found for paracetamol and trimethoprim. In the study by Weissbrodt *et al.* (2009) the loads at the influent of the STP were not measured. The percentage determined in this study is the amounts measured in the sewer divided by the amounts administered on the corresponding days. The compounds investigated in the Swiss study are iodinated

X-ray contrast media and cytostatics, both compounds almost exclusively administered in hospitals. Only 50% of the X-ray contrast media and a maximum of 7.5% of the cytostatics were quantified in the hospital's effluent, implying that the remaining part is most likely ''carried home'' by patients and excreted in household toilets. In the studies by Heberer and Feldmann (2005) and Feldmann *et al.* (2008) the hospital bed density is significantly higher (12.1 beds per 1,000 inhabitants) with a sub-catchment bed density of 24. Pharmaceutical loads were measured in the influent of the STP and in selected hospital effluents. With day-specific hospital consumption data, the contribution of the other hospitals was estimated, resulting in a total hospital contribution of 15% (carbamazepine), 10% (diclofenac) and 50% (metamizole, not measured in our study). Although the results seem to be in good agreement with our study, the limited number of compounds, the various approaches used, and the different catchment characteristics preclude a comprehensive comparison.

Table 3. Comparison with other hospital wastewater studies.

Number of Hospitals	Number of Beds per 1,000 Inhabitants	Investigated Substances (% in Influent of the Corresponding STP Originating from Hospitals)	Location of Study
2	4.4	Diclofenac (1.4)[a] Ibuprofen (0.7)[a] Metoprolol (1.5)[a] Paracetamol (12)[a] Tetracycline (0.5)[a] Trimethoprim (14)[a]	Oslo, Norway (Thomas *et al.*, 2007)
1	3.6	5 X-ray contrast media (50)[b] Cytostatics (max 7.5)[b]	Winterthur, Switzerland (Weissbrodt *et al.*, 2009)
More than 5	12.1	Carbamazepine (15)[c] Diclofenac (10)[c]	Berlin, Germany (Heberer and Feldmann, 2005)
More than 5	12.1	Metamizol (50)[c]	Berlin, Germany (Feldmann *et al.*, 2008)

a Concentrations measured over 12 weeks, loads estimated with water consumption, sum of the two major hospitals (an unknown number of other smaller hospitals/health care facilities are located in the catchment).
b Influent STP was not measured in this study, percentage refers to loads of pharmaceuticals quantified in the hospital's effluent compared to day-specific administered amounts.
c Only measured in the effluent of one hospital and then extrapolated for the whole catchment based on audit data of the other hospitals.

1.4.5. Hospital Wastewater Treatment and Catchments in SEQ

Over 800 pharmaceuticals, disinfectants and other substances are recorded in the DUSC and the hospital databases. Whilst the 59 substances analysed for in this study presents one of the more comprehensive studies of the relative contribution of a hospital to total load in wastewater, we do not claim that these results can be extrapolated for each of these 800 substances, at all hospitals, or medical research activities in general. As is often the case, the selection of these 59 substances was based upon the availability of a validated analytical method. Despite this ''limitation'', even if there were substances that originate almost exclusively from hospital wastewater, or if measures were taken to prevent pharmaceutical residues entering hospital wastewater (source control, separate collection of urine and faeces (Heinzmann *et al.*, 2008)) or if hospital wastewater was treated on site, over 85% of the total load for the majority of the pharmaceuticals investigated in this study would still reach to the STP because they are excreted by the public at home in their households. Even for very specific compounds, almost exclusively administered in hospitals, the trends in many health care systems are moving towards shorter hospitalisations or even treatment of out-patients (particularly diagnostics). Two examples are the iodinated X-ray media and cytostatics: although administered in high amounts in hospitals, they cannot be recovered to 100% and hence solely attributed to hospital effluent (Weissbrodt *et al.*, 2009).

The three catchments of main interest within SEQ, the ones with advanced water treatment plants (AWTPs) for providing PRW to the region (for planned indirect potable reuse), have approximately 8 hospital beds per 1,000 inhabitants (Luggage Point, eleven hospitals), 0.4 (Gibson Island, one hospital) and 1.7 (Bundamba, five hospitals). While the hospital in Caboolture (this study) contributes 4.2 beds

per 1,000 inhabitants (total in the catchment 4.4), the biggest individual hospital in the catchment of Luggage Point accounts for only 1.5 beds per 1,000 inhabitants. A desktop exercise analysing audit data from the sum of all hospitals in these catchments is proposed to evaluate if further steps are required. This includes the planning of future sampling campaigns and the potential benefit of treating some hospitals' wastewater at the source.

1.5. Conclusions

- *Measurements:* For several widely applied pharmaceuticals, an individual hospital seems to be a small additional point source in the catchment of a sewage treatment plant. In this study, a hospital with 4.4 hospital beds per 1,000 inhabitants contributed less than 15% to the total load in the influent of the sewage treatment plant for 28 substances, detected in both hospital effluent and STP influent, which is in good agreement with estimates from other studies. Considering a conservative worst case uncertainty estimation, the hospital contribution only exceeded 15% for two substances, roxithromycin (max. 56%) and trimethoprim (max. 18%).
- *Audit data:* The contribution of the hospital calculated with audit data and the chosen classification reveals good agreement with actual measurements for three quarters of the substances. National audit data to calculate the consumption by the general public in a catchment and hospital data for in-patients appear to be good predictors. This approach can be used with some confidence for substances where no analytical method exists to experimentally determine concentrations and loads or where the LOQ is not low enough. This needs to be tested for other countries (dependent upon the comprehensiveness and quality of national and hospital audit data).
- *Sampling in general:* Sampling campaigns in hospital wastewater are prone to high uncertainty due to a highly dynamic system (flow and concentrations). All effort should be undertaken to understand the system (behaviour) prior to setting up a sound sampling protocol to ensure that representative samples can be obtained.
- *Other catchments in SEQ:* The preliminary analysis based on hospital bed densities suggests focusing on the catchment of the STP at Luggage Point (approximately 8 hospital beds per 1,000 inhabitants). However, it has to be noted that this hospital bed density consists of 3 major public hospitals and a series of private hospitals. Since measurements will be very expensive to assess all hospitals' contributions. A detailed desktop analysis of all audit data is planned to identify if there are major sources and if measurements at selected locations may be appropriate.
- *Hospital wastewater treatment:* If, for whatever motivation, hospital wastewater shall be treated separately on site, it must be noted that for many substances no major overall reduction can be achieved since many pharmaceuticals are taken on a regular basis at home. With the current trend to shorter hospitalisations and treatments (diagnostics) of outpatients, this also holds true for many compounds.

2. PREDICTIVE APPROACH: CONSUMPTION-BASED APPROACH FOR ASSESSING THE CONTRIBUTION OF HOSPITALS TOWARDS THE LOAD OF PHARMACEUTICAL RESIDUES IN MUNICIPAL WASTEWATER

This chapter presents the research undertaken in the UWSRA *Hospital Wastewater* project by Kristell S. Le Corre[a], Christoph Ort [a,b], Diana Kateley[c], Belinda Allen[c], Beate I. Escher[d] and Jurg Keller[a] and published in Environment International, Volume 45, Pages 99-111, September 2012 (see Le Corre *et al.*, 2012).

[a] The University of Queensland, Advanced Water Management Centre (AWMC), Brisbane, QLD 4072, Australia.
[b] Swiss Federal Institute of Aquatic Science and Technology (Eawag), CH-8600 Duebendorf, Switzerland.
[c] Medication Services Queensland, Clinical and Statewide Services Division, Queensland Health, Herston, QLD 4029, Australia.
[d] The University of Queensland, National Research Centre for Environmental Toxicology (Entox), Brisbane, QLD 4108, Australia.

2.1. Abstract

Hospitals are considered as major sources of pharmaceutical residues discharged to municipal wastewater, but recent experimental studies (see Chapter 1) showed that the contribution of hospitals to the loads of selected, quantifiable pharmaceuticals in sewage treatment plant (STP) influents was limited. However such conclusions are made based on the experimental analysis of pharmaceuticals in hospital wastewater which is hindered by a number of factors such as access to suitable sampling sites, difficulties in obtaining representative samples and availability of analytical methods. Therefore, this study explores a refined and extended consumption-based approach to predict the contribution of six selected Australian hospitals to the loads of 589 pharmaceuticals in municipal wastewater. In addition, the possibility that hospital-specific substances are present at levels that may pose a risk for human health was evaluated.

For 63 to 84% of the pharmaceuticals investigated, the selected hospitals are not a major point source, with individual contributions likely to be less than 15%, which is in line with previous experimental studies. In contrast, between 10 and 20% of the pharmaceuticals consumed in the selected hospitals are exclusively used in these hospitals. For these hospital-specific substances, 57 distinct pharmaceuticals may cause concerns for human health as concentrations predicted in hospital effluents are less than 100-fold lower than effect thresholds. However, when concentrations were predicted in the influent of the corresponding STP, only 12 compounds (including the antineoplastic vincristine, the antibiotics tazobactam and piperacillin) remain in concentration close to effect thresholds, but further decrease is expected after removal in STP, dilution in the receiving stream and drinking water treatment.

The results of this study suggest that risks of human exposure to the pharmaceuticals exclusively administered in the investigated hospitals are limited and decentralised wastewater treatment at these sites would not have a substantial impact on pharmaceutical loads entering STPs, and finally the environment.

Overall, our approach demonstrates a unique opportunity to screen for pharmaceuticals used in hospitals and identifying priority pollutants in hospital wastewater explicitly accounting for site-specific conditions. Being based on consumption and loads discharged by hospitals into municipal wastewater, it is not limited by: 1) the big effort to obtain representative samples from sewers; 2) the availability of sensitive chemical analysis; or 3) a pre-selection of consumption data (e.g. consumption volume).

2.2. Introduction

The worldwide consumption of pharmaceuticals has increased significantly since the 1950s as a direct result of a combination of factors including population growth, the fast development of medical science, ageing of the population, and practitioners' prescription habits (OECD, 2009). In Australia, this trend is well illustrated by the net increase in the number of prescriptions over 55 years from 0.4 prescriptions per person in 1948 up to 8.3 in 2003 as reported by Costanzo and Watkinson (2007).

However, more recently, pharmaceuticals have raised scientific and public concerns regarding their potential impact on the environment and human health. In fact, with the development of sensitive analytical techniques which make the detection of more and more active pharmaceutical ingredients (API) possible, it is now well established that pharmaceuticals (and their metabolites) are present in the environment (Kümmerer, 2004b) with wastewater being the primary entry route. Sources include households (Sanderson *et al.*, 2004), agriculture and pharmaceutical industries (Kümmerer, 2004b) and hospitals are often pointed out as major contributors to pharmaceutical residues in influents of municipal STPs (Ternes *et al.*, 2006; Hawkshead, 2008). As a result, the collection of hospital wastewater together with domestic wastewater has been criticised and a dedicated (pre-)treatment of hospital wastewater has been recommended (Verlicchi *et al.*, 2010; Gupta *et al.*, 2009; Pauwels and Verstraete, 2006).

However, other recent studies showed that the impact of hospitals on the loads of APIs in municipal wastewater was limited. For instance, Thomas *et al.* (2007) showed that the contribution of two Norwegian hospitals to the loads of selected APIs was typically below 2%, except for paracetamol (12%). In their study of mass flows of iodinated contrast media (ICM) and cytostatic agents in the effluents of a Swiss hospital, Weissbrodt *et al.* (2009) showed that only a part of these compounds (49% for ICM and 5.5% for cytostatics) were expected to be excreted within the hospital. Ort *et al.* (2010a) concluded that the contribution of an individual hospital to the total load of 59 APIs in the influent of the corresponding STP was unlikely to exceed 15% (see Chapter 1 of this report). This questions the efficacy of a dedicated treatment of hospital wastewater as a means of limiting pharmaceuticals residues in municipal wastewater and reducing their release to receiving water bodies. But, are experimentally measurable pharmaceuticals the most relevant ones to focus our attention on?

According to Runnalls *et al.* (2010), approximately 150 pharmaceutical compounds have been detected in sources including wastewater, surface water, groundwater and more recently drinking water. However, the vast number and diversity of APIs available worldwide make it difficult to assess if these compounds, analysed in the aquatic environment, are the ones likely to present the highest risks for the environment and human health. For example, around 3,000 drugs were available in the United States in 2010 (Bruce *et al.*, 2010). Similarly, about 4,900 active ingredients are currently authorised by the therapeutic good administration for use in Australia (TGA, 2011). This means that more than 95% of the pharmaceuticals available in these countries have never been investigated in water and wastewater sources although some of them could present a higher risk than those currently analysed for. For instance, Escher *et al.* (2011) found that some moderately used pharmaceuticals such as amiodarone, clotrimazole and ritonavir have rarely been investigated although they could pose an environmental risk because they are expected to be highly toxic to aquatic organisms.

Furthermore, complex and inaccessible sewer systems around hospital premises with high flow and concentration variations will always challenge sampling (Ort *et al.*, 2010b) or even make the collection of representative samples impossible. Additionally, measurements at one site, irrespective of their accuracy, can never be meaningfully transferred to any other location without profound knowledge on the system under investigation (hospital audit data and consumption by the general population).

This highlights the need for prioritisation methodologies to identify and quantify pharmaceuticals of concern and to determine if hospital-specific pharmaceuticals should receive priority attention when compared to pharmaceuticals used by the general population. Ultimately, such a methodology should serve as a tool to determine whether frequently detected compounds are the most important ones not only in terms of potential exposure but also in terms of toxicity because risk is determined by exposure and effect. Such a tool is of particular interest in countries where recycling of wastewater has become common practise and where, ultimately, indirect potable reuse is planned. Under this perspective, identifying and controlling the impact of source waters on water quality and more specifically on human health is a priority (NRMMC, 2008; Busetti and Heitz, 2011).

2.3. Material and Method

The project investigated a novel consumption-based approach to predict the contribution of a selection of public hospitals located in SEQ (Australia) to the loads of 589 pharmaceuticals in municipal wastewater. The potential presence of hospital-specific substances at levels that may present a risk for human health has been evaluated by comparing estimated concentrations in hospital effluents and STP influents with effect threshold concentrations calculated based on the methodology used in the Australian Water Recycling Guidelines for the Augmentation of Drinking Water Supplies (NRMMC, 2008). This approach is highly conservative because concentrations in wastewater prior to any treatment are compared to stringent guideline values determined for recycled water intended for (in)direct potable water supply.

2.3.1. Pharmaceutical Database

2.3.1.1. Hospital Audit Data

Pharmaceutical consumption audit data from 107 public hospitals in Queensland were collected by Medication Services Queensland (MSQ) (Queensland Health, Clinical and Statewide Services Division, Queensland Government) for the year 2008.

The database lists medicines dispensed annually to in-patients in public hospitals by generic name, strength, brand, form (i.e. tablets, injections, ointments etc.) and quantity.

In 2008, the database comprised 70,319 entries. As the audit data is a list of all items used by each hospital, these include prescription drugs but also non-prescription drugs and other general items such as dispensers, nozzles and needles.

2.3.1.2. National Consumption

Consumption data of pharmaceuticals by the general population in Australia were compiled by the Drug Utilisation Sub-Committee (DUSC) of the Pharmaceutical Benefits Advisory Committee (PBAC) (Department of Health and Ageing, Australian Government) for the year 2008. Consumption data are based on the date of supply or dispensing of prescriptions.

The information provided in the database includes:

- The number of prescriptions submitted for payment of a subsidy under the Pharmaceutical Benefits and Repatriation Pharmaceutical Benefits Schemes (PBS/RPBS) supplied by Medicare Australia.
- Estimates of non-subsidised medicines (under co-payment and private prescriptions) calculated from continuous data on all prescriptions dispensed from a validated sample of community based pharmacies.

Data on prescription medicines dispensed to in-patients in public hospitals, non-prescription medicines (i.e. available over the counter – OTC drugs) and compounds sold in supermarkets are not taken into account.

In 2008, the database comprised 928 entries. These correspond to single and combined APIs and the annually sold mass.

2.3.2. Evaluation of the Audit Data

2.3.2.1. Hospital and Catchment Characteristics

Due to the large number of hospitals (107) listed for Queensland, the current evaluation has focused on six major hospitals (see Table 4) for characteristics of all catchments and hospitals. The hospitals selected are public hospitals located in three distinct catchments with populations ranging from 45,000 inhabitants in the catchment where the Caboolture Hospital (CAB) is located, up to 572,000 inhabitants in the catchment including the hospitals The Prince Charles (PC), Princess Alexandra (PA)

and Royal Brisbane and Women's (RBWH). The set of hospitals investigated varies in size and diversity of health services provided. Queen Elisabeth II Jubilee (QEII) is the smallest of the six hospitals with a total number of 132 beds. It provides a wide range of services including general medicine, orthopaedics, urology, gynaecology, general surgery, aged care and rehabilitation. RBWH is the largest hospital with 882 beds. RBWH is a general and teaching hospital and is the largest tertiary hospital in Queensland. It offers a comprehensive set of medical services such as general medicine, surgery, orthopaedics, psychiatry, oncology, trauma, comprehensive women's and newborn services (obstetric, gynaecological and neonatal intensive care). The volumes of water consumed at these two hospitals in 2008 were 95 m^3 d^{-1} and 627 m^3 d^{-1} respectively. QEII discharges its effluent to the Oxley Creek STP which in 2008 treated on average 55,336 m^3 d^{-1}. RBWH discharges its effluent to the Luggage Point STP with an average of 148,622 m^3 of wastewater treated per day in 2008 (Table 4).

Table 4. Characteristics of the investigated hospitals and catchments.

ID Key	Hospital Name	Number of Beds*	Population in Catchment Area	Number of Beds per 1,000 Inhabitants	Hospital Water Consumption* (m^3 d^{-1})	STP	Average Raw Influent Flow Rate to STP* (m^3 d^{-1})	Proportion of Influent Wastewater Flow Allocated to Hospitals (%)
QEII	Queen Elizabeth II Jubilee	132	280,000	0.5	95	Oxley Creek	55,300	0.2
CAB	Caboolture	190	45,000	4.2	126**	Caboolture	16,100**	0.8
IPS	Ipswich	296	75,000	3.9	176***	Bundamba	15,000	1.2
PC	The Prince Charles	533	572,000	0.9	541***	Luggage Point	148,600	0.4
PA	Princess Alexandra	754	572,000	1.3	773***	Luggage Point	148,600	0.5
RBWH	The Royal Brisbane and Women's Hospital	882	572,000	1.5	627	Luggage Point	148,600	0.4

**In 2008; ** Ort et al. (2010); ***Water consumption data only available for the year 2009.*

2.3.2.2. List of Compounds to Evaluate

The extraction of data for the year 2008 and the six hospitals investigated resulted in a reduced dataset containing 11,187 entries. Non-pharmaceutical items and duplicates were then removed from the list. The remaining entries were further screened to exclude naturally occurring substances such as hormones, sugars and enzyme as well as gaseous substances. Compounds available over the counter in Australia (TGA, 2011) were also excluded from the list since information on consumption for these substances are not available in the national consumption database.

These screening steps resulted in a list of 589 individual APIs to be evaluated. They cover a wide range of drug classes from common antibiotics and anti-inflammatories to more specific compounds such as antineoplastics (Supporting information B1 - Table SI 6).

2.3.2.3. Prediction of the Contribution of a Hospital to the Load of Pharmaceutical Residues in Municipal Wastewater

In the present study, annual consumption audit data collected from public hospitals in Queensland were compared with the consumption by the general population to allow the prediction of the contribution of a hospital for all APIs in the corresponding catchments of the STPs.

The consumption of a pharmaceutical in a catchment of a STP was estimated by calculating an average per capita consumption from the national consumption data multiplied with the number of inhabitants in the catchment. The consumption of in-patients in the hospital was added to the domestic consumption to obtain an estimate for the total STP influent load according to equation 2:

$$Contribution_{Hospital_{i,j}} = \left[\frac{Consumption_{Hospital_{i,j}} . X_r}{\left(Consumption_{Catchment_j} . Xr \right) + \left(Consumption_{Hospital_{i,j}} . Xr \right)} \right] \quad (2)$$

where:

$Consumption_{Hospital_{i,j}}$ is the consumption of the hospital i in catchment j for an API [g y^{-1}];

$Consumption_{Catchment_j}$ is the consumption of the same API by the general population in the catchment j where hospital i is located [g y^{-1}] (see equation (3);

X_r is the excretion rate for a given API [-]. *Note: this parameter cancels out of equation* (2 *when calculating contributions of hospitals*.

$$Consumption_{Catchment_j} = \left[\frac{Consumption_{Australia}}{Population_{Australia}} \right] . Population_{Catchment_j} \quad (3)$$

where:

$Consumption_{Australia}$ is the consumption data for a single API provided in the national consumption database [g y^{-1}];

$Population_{Australia}$ is the number of inhabitants in Australia, here rounded to 20,000,000;

$Population_{Catchment_j}$ is the number of inhabitants in the catchment j.

A $contribution_{Hospital_{i,j}}$ equal to 100% implies that this API was solely used at hospital i. In contrast, 0% $contribution_{Hospital_{i,j}}$ indicates that a certain amount of this API was consumed by the general population but not in any of the hospital wards.

2.3.2.4. Prediction of the Concentration of Pharmaceutical Residues in Hospital and Municipal Wastewater

Concentrations of APIs expected in hospital wastewater were estimated based on site-specific water consumption data provided by the hospitals and the total amount of a given API consumed per day at this hospital (equation 4). It was assumed that no metabolism occurred (i.e. the total amount of a given substance was excreted unchanged) resulting in a conservative concentration estimation:

$$Concentration_{eff.Hospital_i} = \frac{Consumption_{Hospital_i} \div 365}{Water\ consumption_{Hospital_i}} . 10^6 \quad (4)$$

where:

$Concentration_{eff.Hospitali}$ is the concentration of an API in the effluent of hospital i [µg L^{-1}];

$Water\ consumption_{Hospitali}$ is the average volume of water used daily at hospital i [L d^{-1}];

365 is the conversion factor between year and day;

10^6 is the conversion factor between [g L^{-1}] and [µg L^{-1}].

API concentrations in the influent of the STP (equation 5)to which the hospital discharges its effluent were based on the total amount of a given substance consumed per day - assuming that no metabolism occurred - in the catchment of the hospital and daily raw influent flow rates entering the STP provided by Queensland Urban Utilities:

$$Concentration_{inf.STP_j} = \frac{\left(Consumption_{Catchment_j} + \sum_i Consumption_{Hospital_{i,j}} \right) \div 365}{F_{STP_j}} .10^6 \quad (5)$$

where:

$Concentration_{inf.STPj}$ is the concentration of an API in the influent of the STP in catchment j to which hospital i discharges [µg L^{-1}];

$Consumption_{Catchmentj}$ is the consumption of the same API by the general population in the catchment j [g y^{-1}];

$Consumption_{Hospitali,j}$ is the consumption of the hospital i in catchment j for an API [g y^{-1}]*;

F_{STPj} is the wastewater volume at STP j [L d^{-1}].

**Hospitals PA, PC and RBWH discharge their effluent to the same STP, in that case the concentration estimation for an API in the influent of the STP takes into account the consumption data for that API at the three hospitals.*

2.3.2.5. Comparison of Predicted Concentrations with Risk of Exposure

Estimated concentrations in both hospital wastewater and influent of the corresponding STP were compared to effect threshold (ET) values, which were calculated based on the method used in the "Australian Guidelines for Water Recycling: Managing Health and Environmental Risks (Phase 2): Augmentation of Drinking Water Supplies" (NRMMC, 2008).

ET values for the entire list of compounds were determined based on acceptable daily intakes (ADI, µg kg_{BW}^{-1} d^{-1}). When no ADI was available, a substitute ADI (S-ADI, µg kg_{BW}^{-1} d^{-1}), was estimated according to equation 6 using the Lowest Daily Therapeutic Doses (LDTD, µg d^{-1}) available in the Australian Medical Information Management System (MIMS Australia, 2011).

$$S\text{-}ADI = \frac{LDTD}{SF \,.\, BW} \quad (6)$$

where:

BW is the assumed body weight for an adult (70 kg);

SF is a safety factor of 1,000 applied to all pharmaceuticals investigated except cytotoxic drugs for which a safety factor of 10,000 was applied to account for higher toxicity levels associated with these substances.

ET values (µg L^{-1}) were then calculated according to equation 7.

$$ET = \frac{ADI(or \quad S\text{-}ADI) \,.\, BW}{V} \quad (7)$$

where:

V is the assumed daily volume of water consumed by an adult (2 L d^{-1}).

A margin of exposure (MOE) was then determined according to equation 8 to compare concentrations expected in hospital effluents and in influents of STPs with ETs:

$$MOE = \frac{ET}{Concentration_{eff.Hospital_i\ OR\ inf.STP_j}} \quad (8)$$

A MOE > 100 implies that the pharmaceutical concentration predicted in either wastewater type (hospital effluent or STP influent) is more than 100-fold below a "concentration of no concern". This means that such a compound is unlikely to present a risk of reaching drinking water sources at elevated concentrations and to affect human health.

2.3.3. Conservative Assumptions of the Method

To avoid false negative results, i.e. missing a compound that actually might be of concern, a series of conservative assumptions were made on purpose and are outlined subsequently.

2.3.3.1. Conservative Assumptions Related to the Prediction of Contributions by Hospitals

- The hospital audit database collected by MSQ excludes dispensing of APIs to out-patients and prescriptions to patients in day-admission clinics. However, there may be a few instances where the patient classification (in-, out- or day-patient) may have been recorded incorrectly. If day-admitted patients have been mistakenly classified as 'in-patients' in the dispensing process, the consumption data will have been included in the database. In this case the amount of APIs dispensed and excreted in the hospital would be overestimated.
- If out-patients are not accounted for in the consumption by the general population, the amount of APIs will lead to an underestimation of the consumption in the general population and consequently hospital contributions would also be overestimated in these cases.

2.3.3.2. Conservative Assumption Related to the Predictions of Wastewater Concentrations

- Pharmaceuticals used at the hospital and by the general population were assumed to be excreted unchanged (i.e. no metabolism), therefore, concentrations of pharmaceuticals predicted in raw wastewater (from hospital or domestic sources) are conservative estimates. This partially also accounts for unknown non-compliance and improper disposal.

2.3.3.3. Conservative Assumptions Related to the Evaluation of Risk

- Concentrations predicted in hospital and raw municipal wastewaters are compared to effect thresholds derived from guidelines for water recycling and augmentation of drinking water supply. Concentrations of pharmaceuticals in raw wastewater are likely to be significantly lower after conventional wastewater treatment, advanced water treatment, dilution in the receiving water bodies and drinking water treatment. Consequently, MOEs of compounds will then increase significantly.
- Any MOE value above 1 indicates that predicted concentrations are below the calculated effect thresholds. From a precautionary principle point of view, a conservative safety factor of 100 was added. This implies that MOE values below 100 (instead of 1) flag compounds potentially causing concerns for human health.

2.3.4. Uncertainty Assessment for Predicted Concentrations

2.3.4.1. Metabolism

One of the main simplifications is the assumption that no metabolism occurs, which has no impact on the estimation of a particular hospital's contribution to the total load of an API in municipal wastewater. This is reasonable as there is no evidence that the excretion rate was different between hospital patients and people at home and hence it cancels out in equation 2. In contrast, this does not hold true for the estimation of concentrations in hospital effluents and municipal wastewater. If metabolism was accounted for, predicted concentrations would be lower and consequently MOEs higher. Moreover, excluding excretion rates at this screening level partly counterbalances parameters which are not considered in the database and calculations of the present study (e.g. non-compliance and improper disposal of unused medicine). If excretion rates were considered in the calculations, uncertainties thereof could lead to higher or lower concentrations (two-sided). Avoiding the discussion on levels of excretion rates for individual APIs leads to a one-sided assessment: concentrations can only be overestimated which is adequate for a rapid screening and prevents false negative results.

2.3.4.2. Spatial and Temporal Variations

According to equations 4 and 5, uncertainty of predicted concentrations is mainly impacted by uncertainties of API consumption and flow data or their spatio-temporal variability.

Amounts of APIs used in hospitals are carefully checked, site-specific data. Uncertainty due to location must therefore not be considered. Year-to-year variability at any location was assessed by comparing 2008 with 2009 consumption at all hospitals for hospital-specific APIs (data not shown). This comparison resulted in differences between 22 and 44%. For temporal variability of all APIs in hospitals, we conservatively assume an uncertainty of 50% (rsd, relative standard deviation). The consumption by the general population could vary regionally and hence it might differ from national average. For this unknown variation we assume 50% uncertainty (rsd). This is considered a generous assumption in view of the relatively large catchments investigated in this study since large catchments are expected to differ less from the national average than small ones.

Flow data for hospitals were compiled from yearly records of water consumption and for STPs from or daily inflow rates. To account for seasonal or day-to-day variability of dry weather wastewater volumes and flow measurement, errors a total uncertainty of 50% (rsd) was assumed.

It is a realistic assumption that uncertainties for both pharmaceutical loads (hospital and general population) and flow data are mutually independent. Applying error propagation rules to equation 4 results in an uncertainty of predicted concentrations of 70% (*i.e.* $\sqrt{0.5^2 + 0.5^2}$). For equation 5, a maximum uncertainty of 70% results (the uncertainty of the numerator is ≤50%). We are mainly interested how the results are impacted if predicted concentrations increase. Therefore, only the case where concentrations were 70% higher was assessed for compounds used exclusively in hospitals (i.e. 97-100% contribution).

Compounds for which MOEs would fall below 100 if predicted concentrations were 70% higher are not discussed in detail but considered in a dedicated section (see 2.4.4) and clearly illustrated in Figure 6 and Figure 7 (i.e. as grey bars) and Table 6 and Table 7 (i.e. values in square brackets).

2.4. Results and Discussion

2.4.1. Hospital Contributions

For this study, the contributions of six hospitals to the loads of 589 APIs in the influent of the STP to which they discharge their effluents were evaluated. As expected, the larger the number of hospital beds and diversity of wards, the larger the number of compounds used. In 2008, out of 589 compounds investigated, the number of compounds returning a contribution value was 487 at QEII and CAB, 502 at IPS, 524 at PC, 541 at PA and 548 at RBWH (Supporting information B2).

Figure 4 shows the distribution of the contributions for the six hospitals investigated. The distribution patterns obtained were similar at each of the six hospitals. For 63 to 84% of the compounds, the contributions of an individual hospital are likely to be less than 15% (Supporting information B - Table SI 10 and TableSI 11). The percentage of compounds belonging to the contribution class [0-15%) decreases with the increasing size of the hospital, in terms of number of beds. These results suggest that for a large amount of the compounds investigated, hospitals are not a major point source of pharmaceutical residues in municipal wastewater. For these compounds, at least 85% of the loads originate from households and would reach the corresponding STP even if hospital effluents were treated separately.

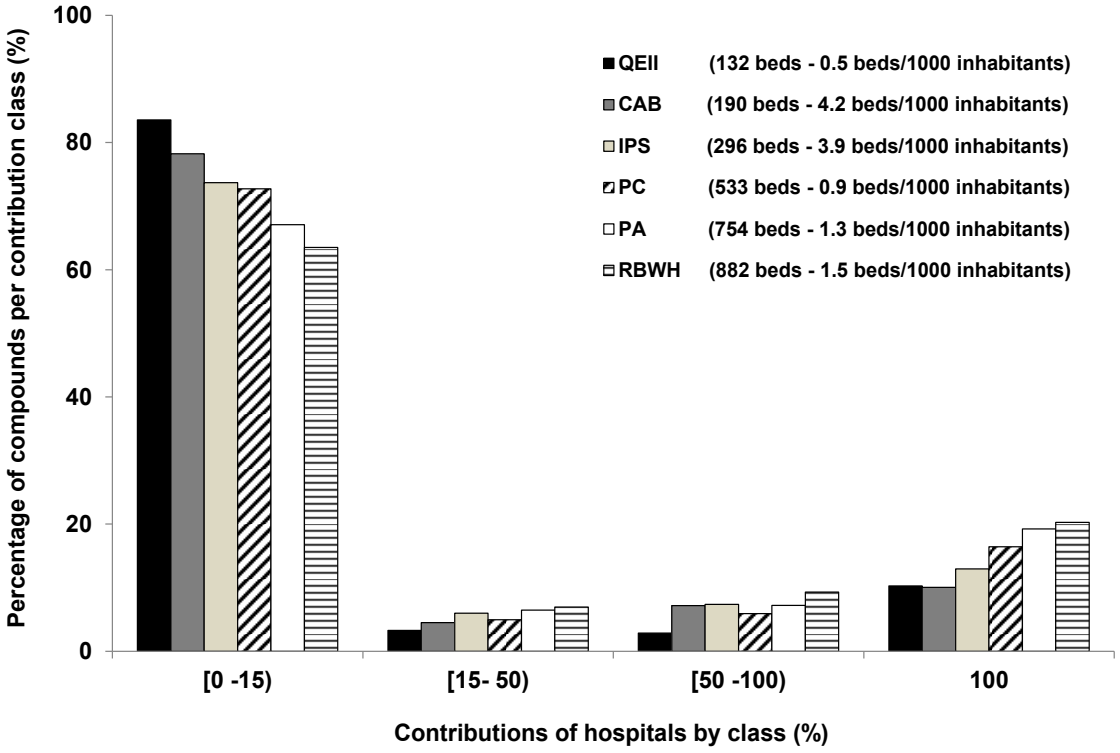


Figure 4. Distribution of the contributions of the six hospitals investigated (Queen Elizabeth II (QEII), Caboolture (CAB), Ipswich (IPS), The Prince Charles (PC), Princess Alexandra (PA) and the Royal Brisbane and Women's Hospital (RBWH)) towards the load of pharmaceuticals in the corresponding STP. (*For each contribution class, inwards-pointing square brackets indicate the inclusion of the value; inwards-pointing round brackets indicate the exclusion of the value*)

For 16 to 37% of the evaluated compounds, the predicted contributions of the six hospitals varied between 15 and 100% (supporting information B – Table SI 8 and Table SI 9). The percentage of compounds solely used at a hospital, (i.e. returning a 100% contribution; Figure 4 and supporting information B -Table SI 7) was 10% at the smallest hospital (i.e. QEII - 132 beds) and 20% at the largest (i.e. RBWH - 882 beds) confirming the belief that a higher number of hospital-specific compounds are used at larger hospitals.

Predicted contributions obtained for a set of eight pharmaceuticals from the percentage contribution class [0-15%) were compared with experimental data available in the literature (Table 5). Predicted and measured contributions correlate well in view of comparing catchments with different hospital bed density in different countries. For instance, predicted contributions for the beta-blocker metoprolol ranged from 0.4% (QEII) to 2.5% (CAB), while contributions measured at two hospitals in Norway by Langford and Thomas (2009) were 0.7% and 0.8%. For carbamazepine, contributions predicted in the six hospitals investigated range from 0.7% (QEII) to 1.8% (CAB). For that compound, Heberer and Feldmann (2005a, b) reported measured weekly loads of 3.60 g in the effluent of a 300-bed military hospital in Germany. Compared to the weekly load of 2192 g carbamazepine measured at the

corresponding STP, this is a 0.1% contribution for that hospital. In comparison, hospital contributions for carbamazepine measured by Langford and Thomas (2009) and Ort *et al.*, (2010a) varied from 0 to 1.3% (Table 5).

Table 5. Comparison of predicted contributions below 15% with values available in the literature.

		Measured Contributions (% of total STP Influent)				Predicted Contributions (% of total STP Influent)					
Reference		Langford and Thomas (2009)		Ort *et al.* (2010a)	Heberer and Feldmann (2005a, b)	This Study					
Hospital		Riks-Hospitalet *Norway*	Ulleval Hospital *Norway*	CAB *Australia*	Military Hospital *Germany*	QEII	CAB	IPS	PC	PA	RBWH
Number of Beds		585[b]	1200	190	300	132	190	296	533	754	882
Compounds	Therapeutic group[a]										
Carba-mazepine	AC	0.9	0.8	0.0-1.3[c]	0.2	0.7	1.8	1.6	1.0	0.8	1.0
Paroxetine	AD	0.5	ND	NM	NM	0.5	1.0	0.9	0.4	0.4	0.3
Sertraline	AD	0.1	ND	≥5[d]	NM	0.7	1.2	0.9	0.5	0.5	0.5
Tamoxifen	AN	0.01	ND[f]	NM[f]	NM	0.2	0.6	0.5	0.2	0.5	0.4
Metoprolol	βB	0.7	0.8	2.0-7.0[c]	NM	0.4	2.5	2.5	1.0	1.4	1.2
Atenolol	βB	0.5	2	0.9-3.5[c]	NM	0.2	0.5	0.6	0.4	0.5	0.5
Simvastatin	HL	0.2	1	ND	NM	0.2	0.6	0.7	0.3	0.3	0.3
Atorvastatin	HL	0.5	2.1	3[e]	NM	0.5	1.0	0.5	0.6	0.6	0.7

[a] AC= anticonvulsant; AD= Antidepressant; AN= Antineoplastic; βB = Beta-blocker; HL=Hypolipidemic.
[b] In 2005.
[c] Results are min and max values.
[d] Maximum contribution when assuming the STP concentration = limit of detection.
[e] Based on average load measurements.
[f] NM: Not measured; ND: Not detected.

At CAB hospital, one of the hospitals included in the current audit data evaluation, Ort and co-workers (2010a) showed that measured contributions for 75% of the compounds investigated were in good agreement with predicted contributions using the same consumption-based approach. For example, a contribution of 2.5% and 0.5% was predicted in our study for CAB hospital for metoprolol and atenolol which correlates well with the results obtained experimentally by Ort *et al.* (2010a) for the same hospital (Table 5). Other compounds investigated by Ort *et al.*, (2010a) at CAB hospital included trimethoprim and roxithromycin. These were the only two substances measured in both the hospital and corresponding STP resulting in contributions above 15%. When using a conservative approach to account for experimental uncertainties a maximum contribution of 18% for trimethoprim and 56% for roxithromycin was determined. The predicted average contributions obtained in our study for these two compounds at CAB hospital were 13% for trimethoprim and 19% for roxithromycin. These contributions are close to the average results of 10% for trimethoprim and 26% for roxithromycin experimentally determined by Ort *et al.* (2010a).

Overall, the six hospitals investigated contribute from 1% in the catchment of QEII hospital to 9% in the catchment of PA, PC and RBWH hospitals to the total pharmaceutical load at the corresponding STP. Reducing pharmaceutical loads in municipal wastewater through on-site treatment of these hospitals effluent would be limited. In fact, Lienert *et al.* (2011) conducted a comprehensive multiple-

criteria decision analysis in two hospitals: a large general hospital (6.2 beds/1,000 inhabitants) and a small psychiatric hospital (14.4 beds/1,000 inhabitants). The general hospital makes up for 38% of the total load in the influent of the corresponding municipal STP while the psychiatric hospital, contributes only approximately 5% to another municipal STP. The STP catchment in which the general hospital is located is characterised by a hospital bed density higher than any in our study. Stakeholders from the larger hospital under investigation, wastewater experts and environmental and health authorities were in favour of reducing pharmaceutical loads in the general hospital effluent with on-site treatment. But, for the smaller psychiatric hospital - with a contribution of the same order of magnitude as the hospitals investigated in our study - consensus among stakeholders was not so clear.

2.4.2. Therapeutic Classes

Figure 5 shows the therapeutic classes of the pharmaceuticals, in terms of their mass proportion of pharmaceutical consumed annually, for which the contributions of the hospitals QEII and RBWH fall in the percentage classes (i) [0 -15%]; (ii) [15-97%]; and (iii) [97-100%]. These two hospitals represent both ends of the spectrum of investigated hospitals in terms of number of beds with QEII being the smallest (132 beds) and RBWH the largest (882beds).

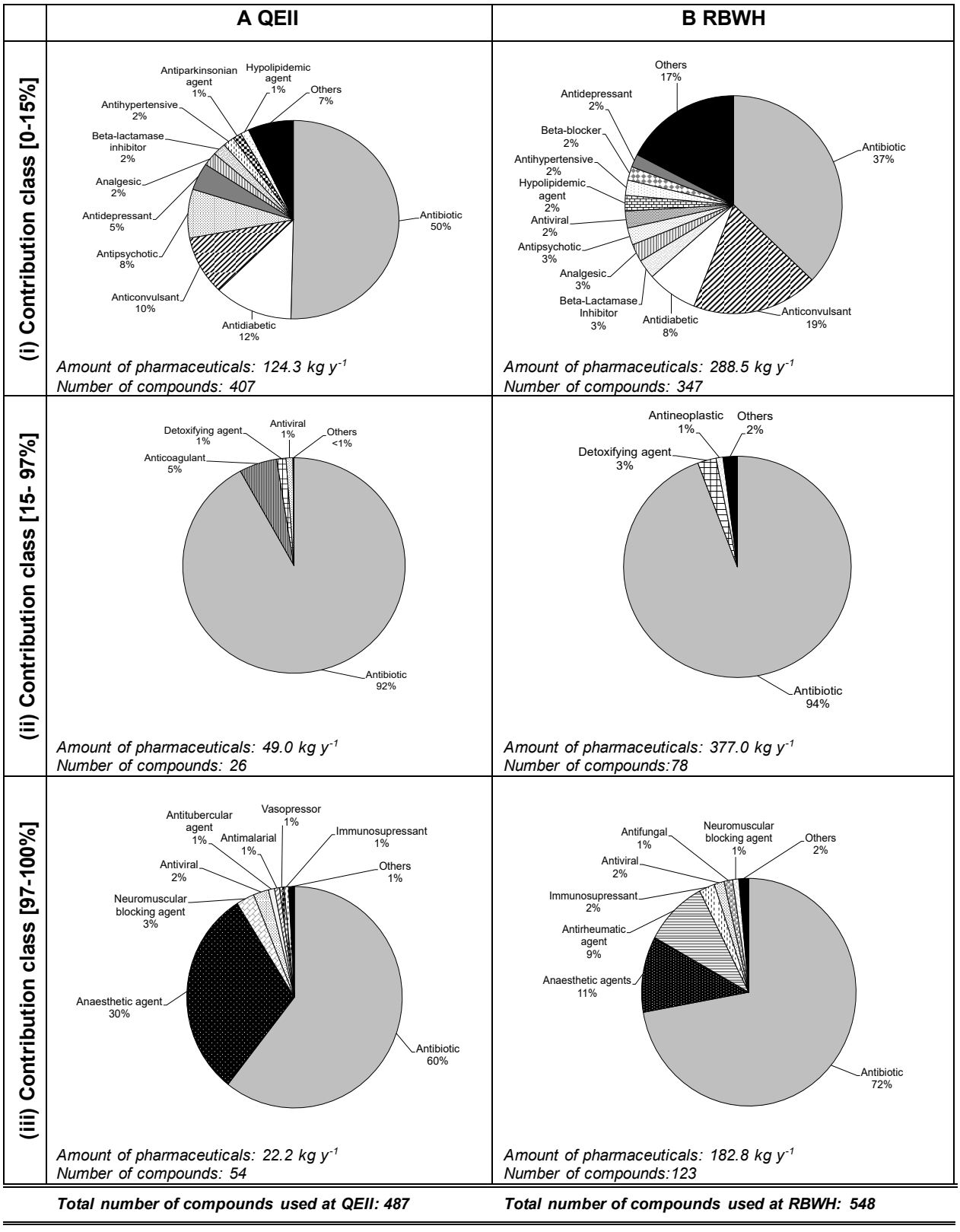


Figure 5. Therapeutic classes covered per contribution class at QEII and RBWH hospitals in terms of annual pharmaceutical consumption.

Overall, there were 109 distinct therapeutic classes figuring in the list of compounds to evaluate. The substances for which these two hospitals would be only a minor point source (i.e. contribution <15%) were very diverse, they covered 97 distinct therapeutic classes (details in supporting information B3 and B4). These classes ranged from common antibiotics (e.g. ciprofloxacin), antidepressants (e.g. paroxetine) and beta-blockers (e.g. atenolol) to more specific classes such as antineoplastics (e.g. fluorouracil, tamoxifen) and antivirals (e.g. lamiduvine). At both hospitals, antibiotic, anticonvulsant and antidiabetic drugs were in the top 3 of the most consumed substances. For instance, the annual amount of pharmaceuticals used in that contribution category at QEII was 124.3 kg, of which antibiotic contributed to 50%, antidiabetic to 12% and anticonvulsant 10% (Figure 5A, (i)). In comparison, the annual amount of pharmaceuticals used in that contribution category at RBWH was 288.5 kg, of which antibiotic contributed to 37%, anticonvulsant to 19% and antidiabetic to 8% (Figure 5B, (i)).

In the contribution category [15-97%], the number of compounds was typically lower (i.e. 26 to 78 substances), so was the number of drug classes. This contribution category encompassed 11 and 31 distinct therapeutic classes at QEII and RBWH respectively. However, at both hospitals, antibiotics nearly cover the overall mass of pharmaceuticals used in that contribution range, with 92 and 94% of the 49 and 377 kg of drugs consumed at QEII and RBWH (Figure 5(ii)). Finally, for compounds solely used in hospitals (97-100% contribution), once again antibiotics were the most consumed substances with 1.3 kg y^{-1} at QEII and 131.9 kg y^{-1} at RBWH. However, the therapeutic classes also covered more specific substances (Figure 5(iii)). These include substances only administered and excreted in hospitals such as anaesthetic agents (e.g. 30% and 11% of the annual mass of pharmaceuticals at QEII and RBWH in that contribution category) and muscle relaxants (e.g. rocuronium) used in surgery. To a lesser extent, substances such as antivirals used in HIV treatment (e.g. abacavir and ritonavir) are also found in this contribution category as they are only prescribed in hospitals, but these are most likely to be excreted at home.

2.4.3. Hospital-Specific Compounds

2.4.3.1. Comparison of Predicted Concentrations with Effect Thresholds

The results show that 153 distinct pharmaceuticals returned contributions between 97 and 100% across the six hospitals investigated. According to this first prioritisation step, these compounds would then be the ones requiring specific attention. However, high hospital contributions may not be necessarily associated with high consumption values and excretion in the hospital. This is illustrated with the antiviral abacavir which returned a 100% contribution at four of the six hospitals. For this compound, consumptions varied from 0.06 g y^{-1} bed^{-1} at IPS hospital to 0.3 g y^{-1} bed^{-1} at QEII hospital. Based on water consumption of the hospitals, assuming no metabolism, concentrations expected in these hospitals effluents would be 0.3 µg L^{-1} and 1.0 µg L^{-1}. As a comparison, concentrations for abacavir in influent of the STP to which these hospitals discharge their effluent would range from 0.003 to 0.004 µg L^{-1}.

A 100% contribution for HIV antiretroviral drugs is not surprising. Indeed in Australia, these types of pharmaceuticals are subsidised under the "Highly Specialised Drug Program" and as such can only be prescribed by qualified medical practitioners through hospital-based pharmacies (HSDP, 2011). However, treatments with antiretroviral drugs are not curative but help managing HIV infections, and are long-term treatments (Anderson and Lennox, 2009). Consequently, such substances are more likely to be excreted at home rather than in hospitals, suggesting that the contributions of hospitals for this type of drugs are extremely overestimated. In fact, a recent report by McArdell *et al.* (2011) experimentally quantifying mass flows of 100 pharmaceuticals in wastewater of a Swiss hospital and municipal wastewater showed that the contribution of that hospital for ritonavir (one of the top 100 of compounds prescribed in that hospital) was only 0.9%.

In order to assess if hospital-specific compounds could have an impact on risks of human exposure to these substances, concentrations in all hospital effluents and municipal wastewater were predicted based on water consumption and compared to effect threshold (ET) concentrations.

The comparison of concentrations predicted in hospital effluents with the calculated effect thresholds for compounds solely originating from hospitals show that - depending on the hospital investigated - between 54 and 75% of these compounds are expected in concentrations more than 100-fold lower than the calculated ET values. In STP influents, the percentages of compounds for which MOEs would be more than 100-fold lower than ET values increases to values in the range of 90 to 100%. This indicates that only a small percentage of compounds originating from hospitals may be of concern.

Figure 6 and Figure 7 illustrate the results obtained at the hospitals QEII and RBWH. At the smallest of the hospitals, QEII, 15 of the hospital-specific compounds concentrations in hospital effluents were less than 100-fold lower than the calculated ET values (Figure 6(A) and supporting information B5 - Table SI 18). MOEs for these compounds varied from 1 for the local anaesthetic agents ropivacaine and oxybuprocaine to 70 for the antibiotic meropenem and the anaesthetic agent ketamine. However, when determining MOE values in the influent of the corresponding STP, none of these 15 compounds returned a MOE below 100 (Figure 6 (B) and supporting information B5 -Table SI 18). The expected concentrations in STP influent (based on flow rate data and assuming no metabolism) would all be more than 500 times lower than the ET values, making these compounds unlikely to increase risks of human exposure to any of these hospital-specific substances.

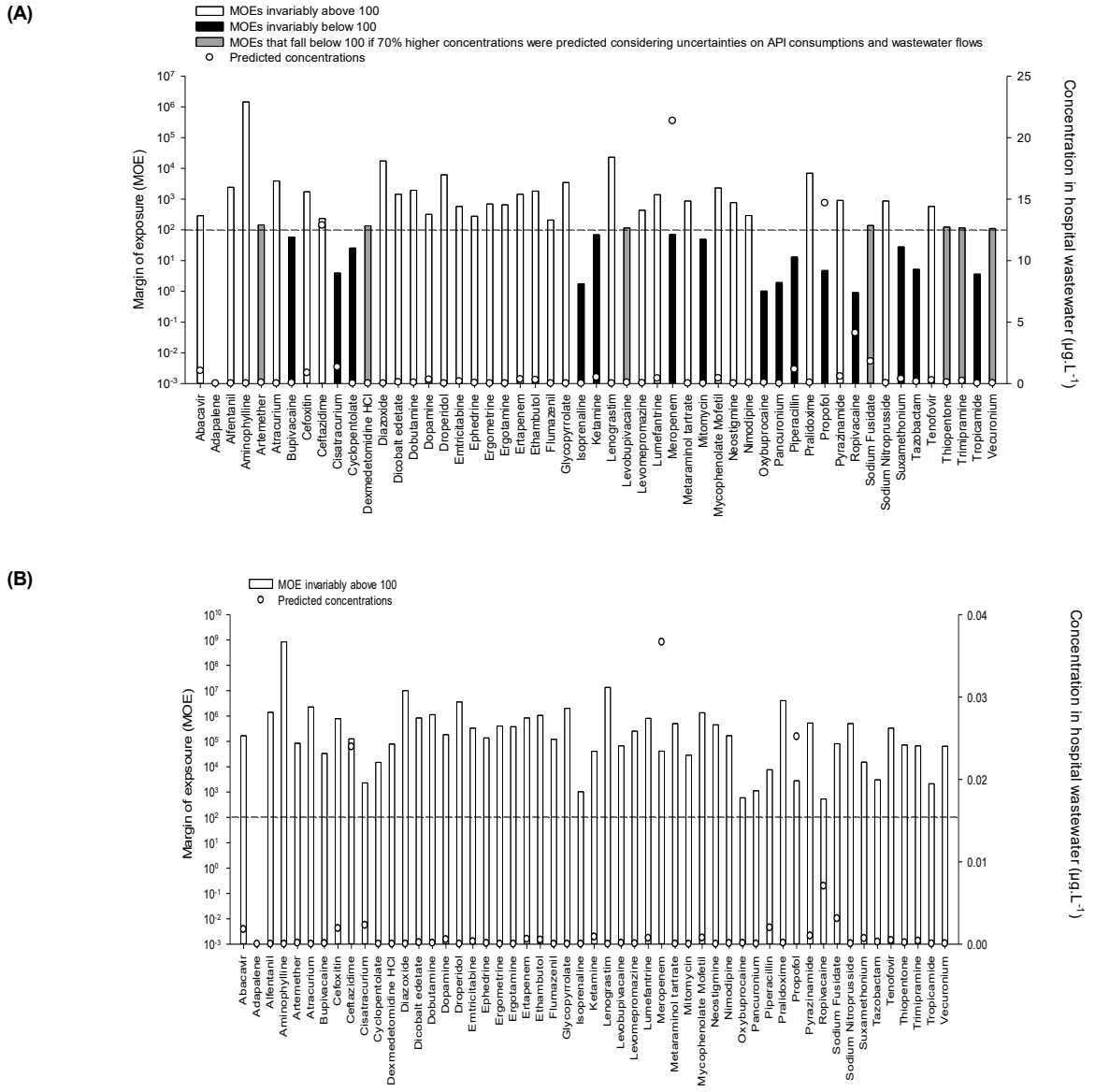


Figure 6. Predicted concentrations and MOE in (A) the effluent of QEII hospital and (B) in the influent of the corresponding STP for hospital-specific compounds (i.e. 97 - 100% contribution). The dashed line corresponds to a MOE of 100.

At the largest hospital, RBWH (Figure 7 and Table SI 19), MOEs in hospital effluent were below 100 for 41 out of the 123 hospital-specific compounds. At this hospital, the antineoplastic vincristine sulphate, the beta-lactam inhibitor tazobactam, the general anaesthetic agent propofol, the local anaesthetic agents bupivacaine and oxybuprocaine were among the compounds with the lowest MOE (<1), while the analgesic alfentanil, the antineoplastic agent anagrelide and the muscle relaxant atracurium had MOEs just below 100 with 85, 92 and 98 respectively (Figure 7 (A)). In the influent of the STP to which RBWH hospital discharges its effluents, 9 out of 123 hospitals-specific compounds with MOE below 100 remained (Figure 7(B) and Table SI 19). These included vincristine sulphate (MOE=0.4), tazobactam (MOE=3) and piperacillin (MOE=8).

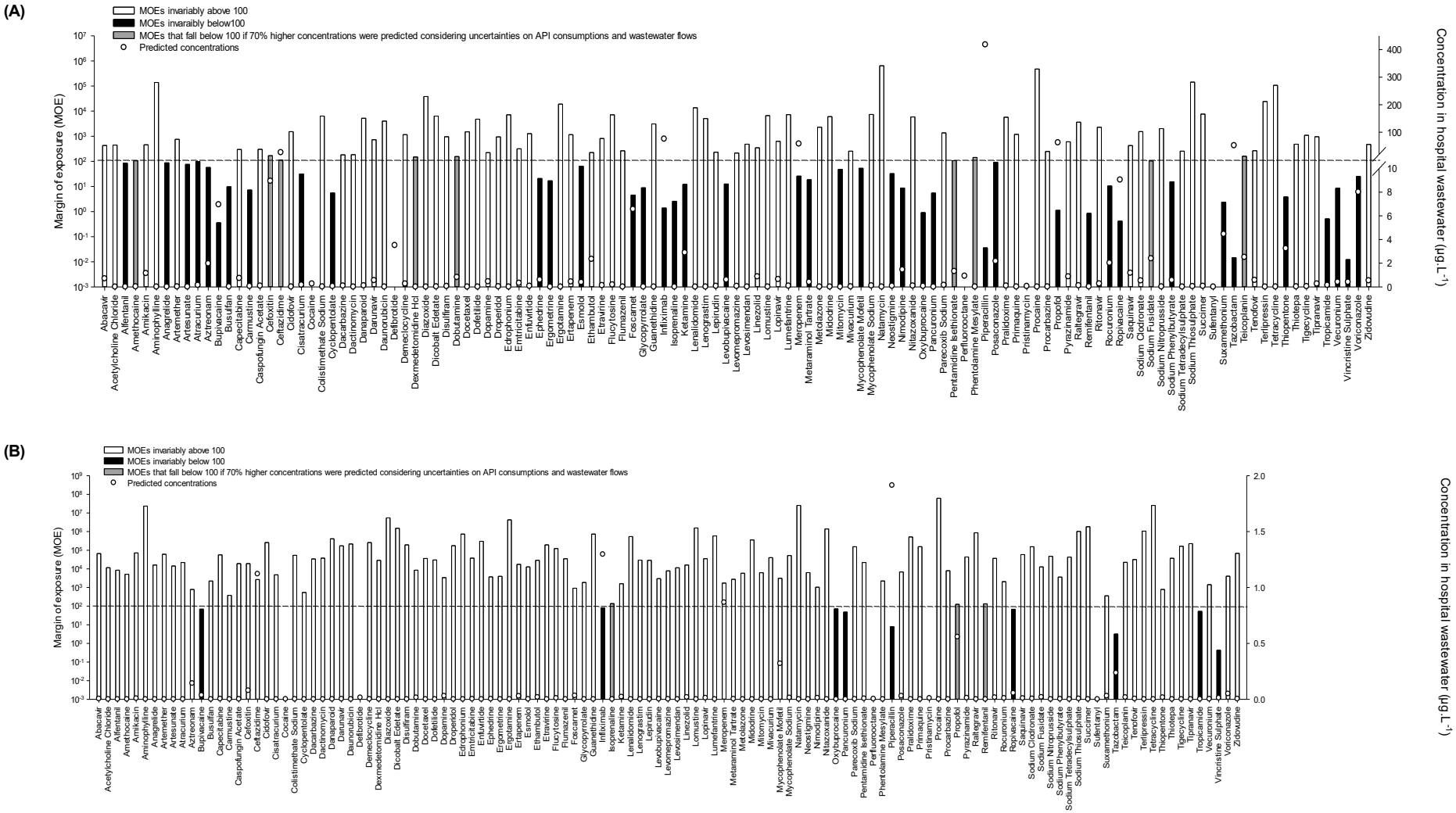


Figure 7. Predicted concentrations and MOE in (A) the effluent of RBWH hospital and (B) in the influent of the corresponding STP for hospital-specific compounds (i.e. 97 - 100% contribution). The dashed line corresponds to a MOE of 100.

2.4.3.2. Compounds of Potential Concerns for Human Health

Table 6 and Table 7 show the final lists of compounds for which hospital-specific substances would result in MOE values below 100 in the hospital effluents and influents of the corresponding STPs. As can be seen, the "top-down" methodology used here allowed the segregation of a limited number of hospital-specific pharmaceuticals present at concentrations which may be of concern and warrant further investigations. This list includes 57 distinct substances across the six hospitals investigated for MOEs predicted in hospital effluents and 12 distinct compounds when MOEs were predicted for influents of the corresponding STPs.

Anaesthetic agents (local or general) were among the compounds most frequently listed and for which MOEs in hospital effluent would be the lowest. For example, concentrations predicted in hospital effluent for the general anaesthetic propofol were very close to the calculated ET values (Table 6). Concentrations in STP influents to which the hospitals investigated discharge, however, were at least 125-fold lower than the calculated ET values. Although 90% of this drug is known to be excreted unchanged, it is believed that propofol is biodegradable in STPs (Kümmerer, 2001). The presence of propofol is thus likely to be very low in treated wastewater. Overall, the presence of anaesthetic agents in the environment has rarely been investigated. To the authors' knowledge, the study by Mullot *et al.* (2010) is the only one reporting information on the presence of anaesthetics and more specifically propofol in hospital and municipal wastewater. In their study, propofol was detected in the effluents of three French hospitals with an average measured daily load of 0.6±0.3 g d^{-1}, but not in influents of the STPs receiving the hospital's wastewater. Although the propofol loads predicted in the current study were significantly higher than the loads measured in hospital effluent by Mullot *et al.* (2010) (i.e. from 8.6 g d^{-1} to 39.1 g d^{-1}), corresponding MOEs would be at the same order of magnitude. For example, the mean concentrations measured in the effluents of the three French hospitals investigated by Mullot and co-workers (2010) ranged from 1.1 to 10.1 µg L^{-1} which would results in MOEs of 64 and 7 respectively. These results also suggest that propofol is unlikely to be discharged to the environment at levels of concern.

The presence of antibiotics in various water sources has been widely investigated as these compounds are the most commonly used in modern medicine (Hawkshead, 2008), and as such are most likely to reach the aquatic environment and be of potential concern for aquatic species and/or human health. Here, eight distinct hospital-specific antibiotics (ampicillin, aztreonam, cefazolin, ceftazidime, ertapenem, meropenem, piperacillin and tazobactam) were used at one or more hospital and found to have MOEs below 100 based on concentrations expected in hospital effluents (Table 6). As shown in Table 7, the list of distinct hospital-specific antibiotics was further reduced when MOEs were estimated in STP influents. The compounds remaining were: cefazolin, piperacillin and tazobactam. Tazobactam is a "potent inhibitor of several beta-lactamases including the plasmid and chromosomally mediated enzymes" (MIMS Australia, 2011). It is mainly used in combination with piperacillin to extend the spectrum of this antibiotic. Tazobactam and piperacillin are mainly excreted in urine with 80 and 69% respectively of the doses administered being excreted unchanged (MIMS Australia, 2011).

Although several antibiotics such as ciprofloxacin (Hartmann *et al.*, 1998), lincomycin (Chang *et al.*, 2010; Watkinson *et al.*, 2009), trimethoprim (Ohlsen *et al.*, 2003) or sulfamethoxazole (Lindberg *et al.*, 2004; Sim *et al.*, 2011) have been detected in hospital wastewater in concentrations in the ng L^{-1} to µg L^{-1} range, data on the antibiotics listed above are sparse. Kümmerer (2003) reported concentrations of ampicillin in effluent of a German hospital ranging from 20 to 80 µg L^{-1}. This would result in MOEs in the range 6 - 480 when applying equation 8, which is above the MOE predicted for that same compound at the hospitals CAB and IPS (Table 6). However, such a comparison has to be taken with precaution. Indeed, the variability of the site characteristics (hospital size, pharmaceutical consumptions) and water consumptions vary significantly from one country to another. Furthermore, experimentally measured concentrations depend on sampling protocols and frequency of analyses which, when reported, vary significantly from one study to another and can cause significant variations of results (Ort *et al.* 2010c).

Table 6. List of hospital-specific compounds* with a MOE below 100 in hospital effluents (*values in grey are MOE values above 100, while numbers in brackets are MOE values that drop below 100 if 70% higher concentrations were predicted considering uncertainties on API consumptions and wastewater flows).*

Hospitals		QEII	CAB	IPS	PC	PA	RBWH
Number of Hospital-Specific Compounds		**54**	**56**	**74**	**92**	**112**	**123**
Number of Compounds with a MOE ≤100 *[number of compounds with a MOE ≤100 when considering 70% higher concentrations]*		**15** [22]	**26** [28]	**32** [35]	**32** [39]	**28** [35]	**41** [50]
Generic Name (API)	**Therapeutic Class**	**MOE**					
Bupivacaine	AA	**58**	**1**	**1**	**4**	**3**	**0.4**
Isoprenaline	BD	**2**	**2**	**3**	**1**	**3**	**3**
Ketamine	AA	**70**	**24**	**20**	**59**	**24**	**12**
Oxybuprocaine	AA	**1**	**2**	**1**	**16**	**1**	**1**
Piperacillin	AB	**13**	**0.6**	**24**	**0.5**	**2**	**0.04**
Propofol	AA	**5**	**2**	**1**	**1**	**3**	**1**
Ropivacaine	AA	**1**	**3**	**10**	**1**	**6**	**0.4**
Suxamethonium	NB	**28**	**2**	**1**	**5**	**37**	**2**
Tazobactam	AB	**5**	**0.2**	**10**	**0.2**	**1**	**0.01**
Tropicamide	MY	**4**	**11**	**6**	**73**	**0.5**	**1**
Cisatracurium	NB	**4**	**39**	**22**	**49**	*148126*	**31**
Cyclopentolate	CM	**25**	*135* **[79]**	**11**	*NU*	**5**	**5**
Glycopyrrolate	Ach	*3467*	**19**	**9**	**62**	*3358*	**9**
Meropenem	AB	**70**	*840*	**73**	**12**	**47**	**26**
Metaraminol tartrate	VP	*867*	**26**	**20**	**33**	*208*	**19**
Mitomycin	AN	**49**	*NU*	**46**	*17080*	**72**	**48**
Pancuronium	NB	**2**	*NU*	**11**	**0.2**	**31**	**5**
Remifentanil	AA	*NU*	**3**	**2**	**2**	**5**	**1**
Rocuronium	AA	*NU*	**20**	**21**	**48**	*1943*	**10**
Thiopentone	AA	*124* **[73]**	**4**	**3**	**29**	**98**	**4**
Vecuronium	NB	*110* **[65]**	**26**	**20**	**59**	**36**	**8**
Alfentanil	AG	*2427*	**68**	**37**	*153* **[90]**	*117* **[68]**	**85**
Aztreonam	AB	*NU*	*NU*	**58**	**3**	*197*	**57**
Ephedrine	VP	*278*	**50**	**15**	*110* **[64]**	*1251*	**21**
Foscarnet	AV	*NU*	*NU*	*NU*	**47**	**59**	**5**
Levobupivacaine	AA	*116* **[68]**	**3**	**1**	*NU*	*NU*	**13**
Mycophenolate Mofetil	IM	*2312*	*4173*	*NU*	**27**	**49**	**53**
Metolazone	DI	*NU*	*NU*	**100**	**31**	**98**	*2288*
Neostigmine	NB	*771*	**32**	**26**	*126* **[73]**	*11756*	**32**
Nimodipine	VA	*291*	**62**	*1173*	*62201*	**11**	**9**
Amethocaine	AA	*NU*	**92**	*1285*	**24**	*1411*	*105* **[62]**
Ampicillin	AB	*NC*	**2**	**2**	*NC*	*NC*	*NC*
Atracurium	NB	*3884*	*NU*	**27**	*1382*	*26333*	**98**
Carmustine	AN	*NU*	*NU*	*NU*	*NU*	**8**	**7**
Cefazolin	AB	*NC*	*NC*	*NC*	**0.1**	*NC*	*NC*
Dobutamine	VP	*1942*	*385*	**75**	**54**	*220*	*156* **[92]**
Ergometrine	OA	*693*	**61**	*151* **[89]**	*NU*	*NU*	**17**
Infliximab	ARh	*NU*	*NU*	*NU*	*NU*	**4**	**1**
Ketorolac	AI	*NC*	**11**	**12**	*NU*	*NC*	*NC*
Posaconazole	AF	*NU*	*NU*	*NU*	**68**	*108* **[59]**	**92**
Vincristine Sulphate	AN	*NU*	*NU*	*NU*	*NU*	**0.02**	**0.01**
Voriconazole	AF	*NU*	*1765*	*612*	**76**	*166* **[98]**	**25**
Midazolam	AX	*NC*	*NC*	**3**	*NC*	*NC*	*NC*
Anagrelide	AN	*NU*	*NU*	*NU*	*263*	*NU*	**87**
Artesunate	AM	*NU*	*NU*	*NU*	*NU*	*353*	**76**
Busulfan	AN	*NU*	*NU*	*NU*	*207*	*NU*	**10**
Capecitabine	AN	*NU*	*3309*	**15**	*1017*	*NU*	*297*
Ceftazidime	AB	*233*	*168* **[99]**	*385*	**13**	*122* **[71]**	*113* **[66]**
Dopamine	VP	*318*	*578*	*281*	**15**	*126* **[74]**	*222*
Esmolol	βB	*NU*	*220*	*210*	*314*	*4608*	**64**
Ertapenem	AB	*1445*	*NU*	*378*	*2350*	**100**	*1155*
Ivabradine	VA	*NU*	*NU*	*NU*	*NU*	**83**	*NC*
Levomepromazine	Apsy	*433*	*NU*	*NU*	**34**	*70536*	*213*
Levosimendan	CaS	*NU*	*NU*	*NU*	**69**	*197*	*481*
Procarbazine	AN	*NU*	*NU*	*NU*	*NU*	**48**	*249*
Sodium Phenylbutyrate	-	*NU*	*NU*	*NU*	*NU*	*NU*	**15**
Trometamol	AI	*NU*	*NU*	*NU*	*NU*	**97**	*NU*
Dexmedetomidine Hydrochloride	CNS	*135* **[79]**	*536*	*749*	*768*	*2469*	*150* **[88]**
Phentolamine Mesylate	Ahyp	*NU*	*NU*	*NC*	*150* **[87]**	*NC*	*143* **[84]**
Sodium Fusidate	AB	*139* **[82]**	*717*	*698*	*173*	*306*	*104* **[62]**
Artemether	AM	*144* **[85]**	*NU*	*1338*	*2057*	*573*	*753*
Caspofungin acetate	AF	*NU*	*NU*	*NU*	*109* **[64]**	*1082*	*301*
Cefoxitin	AB	*1733*	*362*	*NU*	*387*	*519*	*168* **[99]**
Dofetilide	AR	*NU*	*NU*	*NU*	*NU*	*160* **[94]**	*4768*
Fosfomycin	AB	*NU*	*NU*	*NU*	*107* **[63]**	*NU*	*NU*
Lenograstim	IS	*23072*	*NU*	*4363*	*NU*	*157* **[92]**	*5076*
Linezolid	AB	*NU*	*NU*	*NU*	*162* **[95]**	*189*	*347*
Mivacurium	NB	*NU*	*602*	*109* **[64]**	*471*	*7406*	*256*
Pentamidine Isethionate	AB	*NU*	*NU*	*NU*	*2048*	*1254*	*107* **[63]**
Teicoplanin	AB	*NU*	*1480*	*5839*	*497*	*491*	*160* **[94]**
Trimipramin	AD	*115* **[68]**	*NU*	*NU*	*NU*	*NU*	*NU*

AA= Anaesthetic agent; AB= Antibiotic; Acog= Anticoagulant; AF= Antifungal; AG= Analgesic; Ahyp= Antihypertensive; AI= Anti-inflammatory; AM= Antimalarial; Amig= Antimigraine agent; AN= Antineoplastic; Apsy= Antipsychotic; AR= Antiarrhythmic agent; ARh= Antirheumatic agent; ASp= Antispa smodic; AV= Antiviral; βB= beta-blocker; BD= Bronchodilator; CaS= Calcium sensitizer; CM= Cycloplegic and mydriatic agent; CNS= Central nervous system agent; DI= Diuretic; IM= Immunosuppressant; IS= immunostimulator; MS= Muscular stimulant; MY=Mydriatic; NB= Neuromuscular blocking agent; VP= Vasopressor; VA= Vasodilator. NU = Not Used at the hospital; NC: not considered (i.e. contribution <97%). *Contributions comprised between 97 and 100 % were taken into account.

Table 7. List of hospital-specific compounds* with a MOE below 100 in influents of the STPs to which the hospitals investigated discharge their effluents. (*values in grey are MOE values above 100, while numbers in brackets are MOE values that drop below 100 if 70% higher concentrations were predicted considering uncertainties on API consumptions and wastewater flows).*

Hospitals		QEII	CAB	IPS	PC	PA	RBWH
Number of Hospital-Specific Compounds		54	56	74	92	112	123
Corresponding STP		Oxley	Caboolture	Ipswich	Luggage Point		
Number of Compounds with a MOE ≤100 *[number of compounds with a MOE ≤100 when considering 70% higher concentrations]*		0 [0]	3 [3]	3 [6]	8 [11]	9 [12]	9 [12]
Generic Name (API)	**Therapeutic Class**	**MOE**					
Bupivacaine	AA	*33663*	**71**	**47**	**69**	**69**	**69**
Piperacillin	AB	*7599*	**79**	*2058*	**8**	**8**	**8**
Tazobactam	AB	*3030*	**32**	*820*	**3**	**3**	**3**
Oxybuprocaine	AA	*594*	*248*	*126* **[74]**	**71**	**71**	**71**
Pancuronium	NB	*1122*	*NU*	*912*	**48**	**48**	**48**
Ropivacaine	AA	*532*	*365*	*892*	**68**	**68**	**68**
Tropicamide	MY	*2121*	*1415*	*519*	**53**	**53**	**53**
Cefazolin	AB	*NC*	*NC*	*NC*	**32**	*NC*	*NC*
Infliximab	IM	*NU*	*NU*	*NU*	*NU*	**81**	**81**
Vincristine Sulphate	AN	*NU*	*NU*	*NU*	*NU*	**0.4**	**0.4**
Levobupivacaine	AA	*67325*	*447*	**100**	*NU*	*NU*	*2978*
Suxamethonium	AA	*15213*	*256*	**98**	*371*	*357*	*357*
Propofol	AA	*2779*	*269*	*122* **[72]**	*125* **[74]**	*125* **[74]**	*125* **[74]**
Remifentanil	AA	*NU*	*381*	*136* **[80]**	*134* **[79]**	*134* **[79]**	*134* **[79]**
Isoprenaline	BD	*1030*	*291*	*226*	137 **[80]**	*137* **[80]**	*137* **[80]**

AA= Anaesthetic agent; AB= Antibiotic; Acog= Anticoagulant; BD= bronchodilator AN= Antineoplastic; ARh= Antirheumatic agent; MY= Mydriatic; NB= Neuromuscular blocking agent; NU = Not Used at the hospital; NC: not considered (i.e. contribution <97%). *Contributions comprised between 97 and 100 % were taken into account.

Although risks of direct human exposure to traces of antibiotics in the environment have not been demonstrated yet, major health concerns resides in the possible development of antibiotic-resistance bacteria, hence antibiotic-resistant genes that may transfer to human pathogens (Fick *et al.*, 2009). In that context, Reinthaler *et al.* (2003) investigated the resistance of *E. coli* strains isolated in sewage and sludge to a set of 24 antibiotics. These included five of the hospital-specific antibiotic drugs remaining here with MOEs below 100 in hospital wastewater (e.g. ampicillin, ceftazidime, meropenem, piperacillin and tazobactam). For these, they obtained resistance rates of 18 % and 4 % against ampicillin and piperacillin for *E.coli* strains isolated from the influent of a STP conjointly treating municipal and hospital wastewater. On the contrary, no resistance was found against ceftazidime, meropenem and the combination piperacillin/tazobactam. The latter was one of the only hospital-specific antibiotic of potential concern remaining in our final list of substances (Table 7). Additionally, the highest degree of *E.coli* resistance that they observed was for the antibiotic tetracycline. Among the six hospitals investigated in our study, RBWH was found to be a major contributor to the loads of tetracycline in municipal wastewater but corresponding MOEs in both hospital effluent and municipal wastewater were well above 100,000. This suggests that if our approach helps screening antibiotics for which hospitals would be major contributors and of potential concern for human health, further investigations on potential human health risks resulting from the spread of antibiotic-resistant bacteria that may originate from hospitals are warranted.

Risks associated with the presence of some of the antibiotics listed above in hospital wastewater have also been investigated but in the context of environmental risk assessments. De Souza *et al.* (2009) have recently assessed environmental risks associated with highly consumed antibiotics in a small intensive care unit of a Brazilian hospital. Meropenem, cefazolin, piperacillin, ampicillin, ceftazidime and tazobactam were found among the 21 antibiotics the most consumed in that intensive care unit.

They showed that these compounds were potentially of environmental concern as ratios between Predicted Environmental Concentrations (PEC) and the corresponding Predicted No Effect Concentration (PNEC) were above 1, with values ranging from 2 for tazobactam to 147 for ampicillin. Similarly, Kümmerer and Henninger (2003) investigated bacterial resistance to antibiotics originating from hospitals in Germany. These antibiotics included all the compounds listed above except ertapenem and tazobactam. In their study, they predicted environmental concentrations (PEC) for hospital effluents and municipal sewage. The results of their predictions showed that for all these compounds the ratios PEC/PNEC were all above 1 in hospital effluent. These ratios were also above 1 for a majority of these antibiotics when predicted in municipal sewage suggesting a potential risk of effects to the environment. The PEC/PNEC ratios were higher than the MOE in the present study because: (a) the PNEC were lower than the ET because they were generally derived from the most sensitive environmental species, which are often bacteria in case of antibiotics; and (b) because the effluent concentrations in the Brazilian hospital were generally higher.

Finally, seven antineoplastic agents (anagrelide, capecitabine, procarbazine, carmustine, vincristine, busulfan and mitomycin) presented MOE values below 100 in the hospital effluents at four of the six hospital investigated (Table 6). For these substances, consumption values led to concentrations varying from a minimum of 1.10-5 $\mu g\ L^{-1}$ predicted for mitomycin in the effluent of PC hospital to a maximum of 14 $\mu g\ L^{-1}$ for capecitabine at IPS hospital. However, concentrations in the corresponding STPs dropped significantly making vincristine the only cytotoxic compound remaining with a MOE below 100 in the catchment of PA and PC hospitals (Table 4) with concentrations below 0.012 $\mu g\ L^{-1}$. Although such a concentration seems low and in accordance with low concentrations typically observed for this category of substances in the environment (Webb, 2004), it would deserve additional investigations. Indeed, anticancer drugs are among the most toxic substances used in medicine and are known to be poorly biodegradable (Aherne *et al.*, 1990; Kümmerer, 2004b). Vincristine sulphate belongs to the chemical group of vinca alkaloids which are cytotoxic substances acting as inhibitor of cancer cells division and have been found to be potentially fetotoxic and embryotoxic (Al-Ahmad and Kümmerer, 2001). Vincristine sulphate is mainly used in the treatment of acute leukaemia as a component of various chemotherapeutic regimens and adverse effects include neurotoxicity. It is highly metabolised and excretion rates reach approximately 80% of an injected dose in faeces and 10 to 20% in urine (MIMS Australia, 2011; AMH, 2011). In 2001, Al-Ahmad and Kümmerer have demonstrated that vincristine is not toxic towards bacteria in wastewater and not readily biodegradable - only 30% biodegradability after 28 days - suggesting that it may not be readily removed in conventional STPs.

Despite the current study showing that anticancer drugs are exclusively used in hospitals, the real impact of hospital effluents on the load of these compounds in municipal wastewater is difficult to assess. The administration of some of these compounds to out-patients as well as the slow excretion of some of these substances (i.e. capecitabine, fluorouracil) means that significant fractions of antineoplastic drugs are excreted at home (Johnson *et al.*, 2008). A trend towards home-based administration of anticancer treatments has been recently confirmed in France by Besse *et al.* (2012). Their analysis of consumption data from a local chemotherapy centre showed that 50% of the antineoplastic agents consumed in that centre were prescribed to out-patients and that only 20% of the drugs prescribed to out-patients were excreted onsite. This trend implies that hospitals may no longer be a major source of chemotherapeutic drugs. In that case, if hospital wastewater was treated independently from domestic wastewater, little reduction of anticancer drug levels in municipal wastewater would be achievable. As an alternative, urine separation from patients under chemotherapeutic treatment may help reducing the amount of some of the hospital-specific cytotoxic drugs discharged into wastewater (Lienert *et al.*, 2007). However, Kümmerer and Al-Ahmad (2010) reported that excreta separation was not an option to recommend due to higher risks of exposure to these substances by hospitals employees during collection than by the general population if excreta were discharged in hospital wastewater.

2.4.4. Impact of Uncertainty Evaluation on Prioritised APIs

If 70% higher API concentrations are considered to account for uncertain or variable pharmaceutical consumption and wastewater flows the number of additional hospital-specific compounds that may require further investigation - because the MOE would drop below 100 - proved to be limited.

For the smallest hospital (QEII), Figure 6(A) shows that MOEs of seven hospital-specific APIs (artemether, dexmedetomidine hydrochloride, levobupivacaine, sodium fusidate, thiopentone, trimipramine, and vecuronium) in the hospital effluent could fall below 100. When looking at the list of hospital-specific compounds with MOE below 100 values (Table 6), among these seven substances, three APIs were already listed as of potential risk at other hospitals (i.e. thiopentone, vecuronium and levopubivacaine). Four other APIs are new on the list. Overall, in the effluent of QEII, the MOEs of these newly listed substances would range between 65 for vecuronium and 85 for artemeter, which are relatively close to the 100 limit of "no concern". In influents of the corresponding STP, all seven APIs would invariably remain in concentration more than 100-fold lower than the ET values (Figure 6 B).

At RBWH, the largest hospital, the MOEs of nine hospital-specific compounds would fall below 100 when assuming 70% higher concentrations in the hospital effluent with values ranging from 62 for the anaesthetic agent amethocaine to 99 for the antibiotic cefoxitine (Table 6). Among these nine substances, six APIs would be newly listed as of potential risk. However, in the influent of the corresponding STP, the MOE of only three APIs would drop below 100. These are propofol, remifentanil and isoprenaline. It has to be noted that MOEs for these three compounds would be close to 100 with values of 74, 79 and 80 respectively. Therefore, despite requiring further investigations, it is expected that these would unlikely be present in STP effluents at levels representing a risk to humans.

Overall, the implication of the estimation of a degree of uncertainty associated with concentration predictions are limited to only three additional hospital-specific compounds listed across the six hospitals. In another country with other prescription habits, the picture may look different. However, we can assume that for urban Australian catchments, not many catchments would show a different picture as we have already considered quite specialised hospitals and catchments with varying degrees of hospital contribution.

2.5. Conclusion

- **Predicted Contributions of Hospitals to the Loads of Pharmaceuticals in Municipal Wastewater**

The consumption-based approach presented in this study comprises 589 pharmaceuticals. Despite several conservative assumptions, the results suggest that the contribution of hospitals towards the total load of pharmaceuticals in the influent of STPs is limited: compared to the consumption by the general population the six hospitals over all contribute 6% of the mass of pharmaceuticals (i.e. 1% in the catchment of QEII hospital to 9% in the catchment of PA, PC and RBWH hospitals).

- **Hospital-Specific Pharmaceuticals**

Concentrations of pharmaceuticals in raw wastewater (from hospital or domestic sources) are expected to be significantly reduced after conventional wastewater treatment and advanced water treatment. Therefore, the results obtained for hospital-specific compounds indicate that these are unlikely to be present in STP effluents at levels representing a risk to humans. Nevertheless, 12 compounds were identified which are less than 100 times below a concentration "of no concern" in the influent of STPs. They warrant more detailed investigations including environmental and human toxicity, biodegradation and treatment or source control options.

Based on the results obtained at the six Australian hospitals investigated, the implementation of decentralised treatment systems for hospital wastewater as a strategy to reduce pharmaceutical residues in municipal wastewater seems not efficient. This may be different in STP catchments with substantially higher numbers of hospital beds relative to the general population. Furthermore,

additional aspects, among others the impact of hospital wastewater on the propagation of antibiotic resistant bacteria, will require specific attention to fully evaluate whether source treatment of hospital wastewater is relevant or not.

- **Strengths of the Consumption Based Approach**

The current approach offers a unique opportunity of efficiently screening pharmaceuticals used in hospitals and identifying potential compounds of concern that may require monitoring and specific treatment or disposal. Being based on consumption and loads discharged by hospitals into municipal wastewater, this approach is not limited by: 1) the big effort to obtain representative samples from sewers; 2) the availability of sensitive chemical analysis; or 3) a pre-selection of consumption data (e.g. ranked top one or two hundreds consumptions by volume). It represents an additional step towards prioritisation of pharmaceuticals originating from hospital wastewater that is transferrable to other countries depending on availability and quality of audit data.

3. EXPERIMENTAL APPROACH (II): DETERMINING THE FRACTION OF PHARMACEUTICAL RESIDUES IN WASTEWATER ORIGINATING FROM A HOSPITAL - IPSWICH CASE STUDY

3.1. Introduction

Over the past sixty years, the consumption of pharmaceuticals in Australia has increased significantly as illustrated by the increase in the number of prescriptions per capita from 0.4 in 1948 (Costanzo and Watkinson, 2007) to 12.4 in 2008 (Australian Statistics on Medicines, 2009, Australian bureau of statistics, 2008). This major increase in pharmaceutical consumption has been synonymous of a simultaneous increase in the release of pharmaceutical residues in the environment. Indeed, as explained in previous chapters, the primary source of pharmaceuticals in municipal wastewater is excretion from humans. Since STPs were not originally designed to deal with pharmaceutical contamination (Petrovic *et al.*, 2003) these emerging contaminants and their metabolites can find their way to the environment through the discharge of treated municipal wastewater. In Australia, as in a majority of countries, hospital wastewater is directly discharged along with domestic wastewater in sewers. Due to localised intense medical activities, hospitals have therefore been seen as major point sources of pharmaceuticals, hence major contributors to pharmaceutical contamination in municipal wastewater. However, as illustrated in Chapter 1, the detailed experimental study carried out in Caboolture by Ort *et al.* (2010a) showed that due to the high amounts of pharmaceuticals being consumed by the general population and therefore excreted at home, hospitals were unlikely to contribute largely to pharmaceuticals loads in municipal wastewater.

However, this study was performed on a limited set of 59 analytically measurable pharmaceutical compounds. These findings had then to be extrapolated to a larger set of compounds and additional hospitals sites. This was done using a consumption-based approach (Le Corre *et al.*, 2012 / Chapter 2). As discussed in Chapter 2, this study covered six hospital located in SEQ for 589 substances consumed over a year-long period. The outcomes of this study confirmed that hospitals were unlikely to contribute significantly to loads of pharmaceuticals in municipal wastewater since, for a majority of the compounds investigated (63% to 84%), hospitals would contribute less than 15%. Seventy-five per cent of the contributions measured at Caboolture hospital and STP were also found to compare well with predicted contributions for that hospital. But the approach also identified 12 pharmaceuticals exclusively used in hospitals that could be discharged in municipal wastewater and potentially reach the environment at concentration of potential concern for human health if not treated by conventional wastewater treatment. Overall, these results suggested that the predictive approach was a good tool to screen pharmaceuticals used in hospitals and prioritise potential compounds of concern that may require monitoring and specific treatment or disposal. But further experimental investigations were required to fully validate these outcomes.

The experimental quantification of pharmaceutical residues in sewers around hospital premises can be challenging and prone to uncertainties. However, through a good understanding of flow dynamics at the sampling site and the establishment of a thorough sampling protocol, it can be used to evaluate hospital contribution to loads of pharmaceuticals in municipal wastewater (Ort *et al.*, 2010a). In the current study, we used the knowledge gained from the experimental study organised at Caboolture hospital and corresponding STP to perform a similar sampling campaign at a larger hospital, namely Ipswich hospital and its corresponding STP, Bundamba.

The objective of this study was then threefold:

- To experimentally evaluate the contribution of Ipswich hospital to the loads of 34 pharmaceuticals in the influent of the STP to which it discharges (Bundamba STP);
- To confirm the results obtained at Caboolture hospital and STP;
- To validate the predictive approach developed for hospitals in SEQ.

3.2. Material and Methods

3.2.1. Hospital and Sewage Treatment Plant Characteristics

3.2.1.1. Hospital and Sewage Treatment Plant Characteristics

Ipswich General Hospital is located in a catchment including 75,000 inhabitants. It comprises 296 beds resulting in a bed density of 3.9 beds per 1,000 inhabitants. This hospital provides a wide range of services to the Ipswich and surrounding community including general medicine and surgery, paediatrics, intensive care, orthopaedics, maternity, obstetrics and gynaecology, ophthalmology and emergency. Additional services such as cancer treatment and allied health services (e.g. physiotherapy, dietetics and nutrition, dialysis, psychology etc.) are also available. Ipswich Hospital discharges its effluent to Bundamba STP which in May 2012 (time at which the following sampling campaign was performed) treated on average 17,748 m^3 d^{-1}. In May 2012, the volume of water consumed at this hospital was 144 m^3 d^{-1}which corresponded to 0.8% of Bundamba influent wastewater flow for that month.

3.2.1.2. Sewer Network Characteristics and Sampling Location

(a) Sampling Point Location

The principal sewerage system of Ipswich Hospital is located beneath Chelmsford Avenue (Figure 8). This system was inspected to determine the feasibility of sampling from either of the manholes constituting the network linked to the hospital.

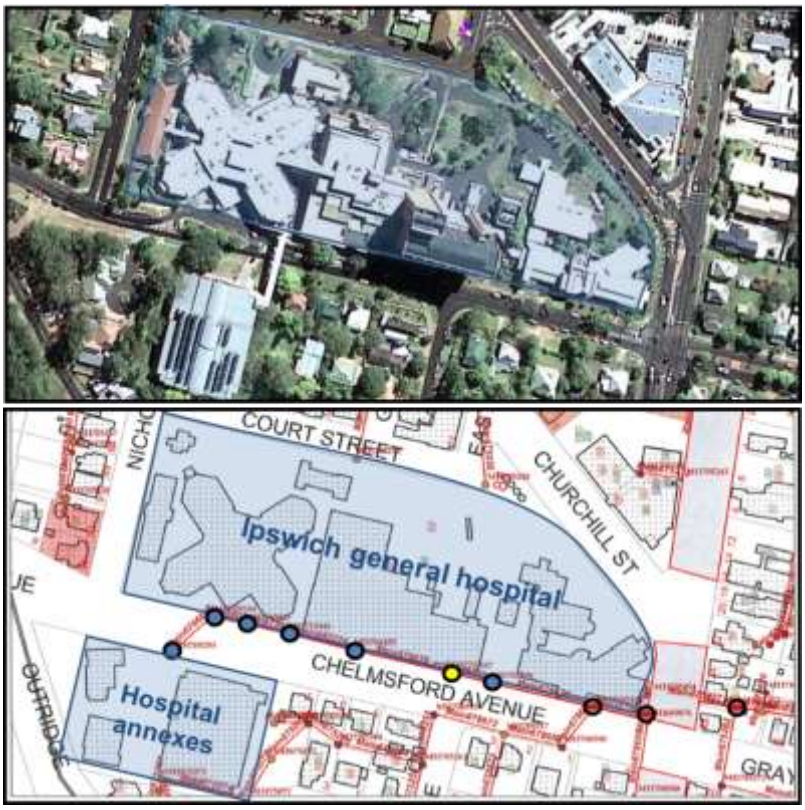


Figure 8. Aerial view of Ipswich Hospital and its corresponding sewerage network map. (*The blue dots correspond to sewers exclusively collecting hospital wastewater, the reds dots to sewers collecting hospital wastewater along with domestic wastewater from households nearby and the yellow dot the sewer chosen for sampling*).

Among the ten manholes inspected, seven exclusively collect effluents from Ipswich Hospital (Figure 8) while three of the manholes collect the effluents from the hospital and a fraction of domestic effluent from nearby private houses.

Among the seven manholes solely collecting hospital effluents, only one proved suitable for sampling (Figure 8 - highlighted in yellow). Indeed the majority of the sewers located along Chelmsford Avenue are either on the pavement or in driveways. Unlike other manholes, this manhole is located on a patch of grass off the pavement (Figure 9) and close to one of the hospital buildings that allow access to a source of power for all electrical equipment required for sampling.

Figure 9. External and internal view of the selected manhole (0.8mx0.4mx1.0-1.3m) in front of Ipswich Hospital.

According to the hospital maintenance service, the manhole selected (indicated as yellow in Figure 8 and 9) from which wastewater samples were collected receives the effluent from theatres, day procedures, outpatients, pharmacy, a surgical ward, an orthopaedics ward, birthing suites and a renal unit. Also going into this sewer are effluents from the hospital kiosk and several office areas.

(b) Distance and Wastewater Travel Time between the Hospital and Bundamba STP

The sewer network between the hospital and Bundamba STP is composed of two gravity lines sections and two rising mains sections (Table 8) separated by two pumping station (Roseberry Parade and Tantivy). The total distance between the hospital and the STP is 10.7km.

Table 8. Sewer network between Ipswich hospital and Bundamba STP.

Section	Type	Total Length	Slope
1 "Ipswich hospital to Roseberry Parade pumping station"	Gravity	1.8 km (41 pipes of various length and diameter)	2.9% (average)
2 "Roseberry Parade pumping station to The Terrace"	Rising main	0.7 km (22 pipes of various length and diameter)	NA
3 "The Terrace to Tantivy pumping station"	Gravity	1.9 km (38 of various length and diameter)	1%
4 "Tantivy pumping station to Bundamba"	Rising main	6.3 km (38 of various length and diameter)	NA

In order to estimate the starting time of wastewater collection at the STP and ensure that the "water packets" collected at the STP would correspond to the one collected at the hospital; the time for wastewater to travel between the two locations was estimated based on the above sewer network description.

- Travel time estimation for gravity line sections:

For these parts of the sewer, the Manning equation for velocity estimation was used (equation 9):

$$V = \frac{1}{n} \times r^{2/3} \times S^{1/2} \quad (9)$$

Where: n is the roughness coefficient, typically 0.013;
S is the slope;
R is the hydraulic radius (i.e. the ratio of the wet cross sectional area by the wet perimeter)

For these sections, it was assumed that the pipes were half-full (i.e. wet radius = radius of the pipe). The manning equation was applied to each sub-section of the gravity line, and times were then added up for the full length of the gravity section. Based on these assumptions it was found that it would approximately take 30 min and 41 min for the wastewater to travel along section 1 and section 2 respectively (Table 8).

- Travel time estimation for rising mains sections:

For these sections, pumping stations data were provided in the form of discharged graphs (Figure 10) over a day where the "y" axis represents the well level as a percentage and the "x" axis the time.

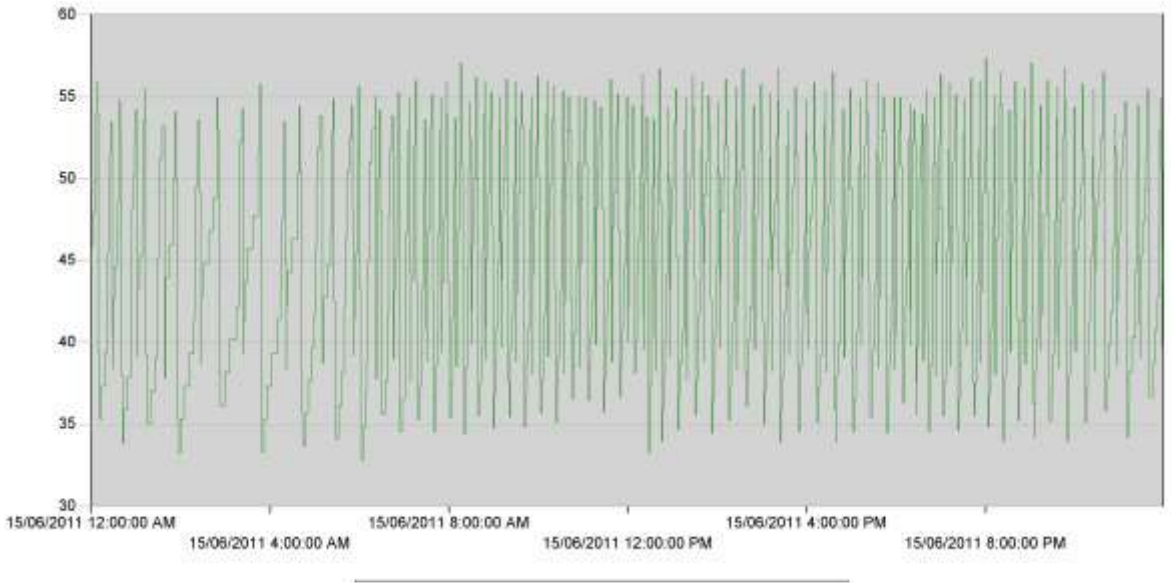


Figure 10. Example of discharged graph provided by Queensland Urban Utilities for Tantivy pumping station.

The first step was to determine the percentage variation for each pumping event, hence the corresponding volume of wastewater being pumped based on percentage conversion tables provided by Queensland Urban Utilities (QUU). The second step was to determine the gap (min) between each pumping event. This also had to be done on the discharge graphs provided. The number of sections crossed (as volume) for each pumping event was then determined, starting with events from 6am onwards. This allowed determining the travel time over the full length of the rising mains sections.

Based on this methodology, it was found that it would take approximately 1h50m for the wastewater to cover the full length of section 3 between Roseberry Parade pumping station and The Terrace, while it would take around 9h30m for the 6.3km long section between Tantivy pumping station and Bundamba.

Overall the time for a water packet from the hospital to reach the incoming raw wastewater channel of the STP would be around 12h30m. This implies that sampling at the STP would have to be started around 12h after the sampling was started at the hospital site to capture at the STP the wastewater generated at a given time at the hospital.

3.2.2. Sampling

As explained in details in Chapter 1, in order to obtain representative samples and limit errors associated with sampling of wastewater for pharmaceutical analysis, a continuous flow-proportional sampling mode was applied at both locations (Ort *et al.* 2010a, 2010b and 2010c). Wastewater was collected at both sites over three days with 12h interval between the wastewater collection start at the hospital and the collection start at the STP.

Access to sewers in Queensland is subject to strict regulations. As the collection of wastewater at the hospital site was carried out in an open-channel located in a closed manhole, it therefore required occasional entry in the manhole which is considered as a confined space. This space is under the control of QUU and as such all on site procedures had to follow the Queensland Work Health and Safety Act (2011). In addition, all activities carried out in the manhole (system installation – sampling) had to conform to the requirements of the AS/NZS 286 2001 - Safe Working in a Confined Space - Act.

3.2.2.1. Sampling Protocol for Ipswich Hospital

The flow of wastewater in the manhole was shallow and as expected subject to high variations over 24h cycles. Therefore, in order to use a flow meter capable of measuring the hospital wastewater flow rate, a 60°C v-notch weir was designed to fit in the manhole open-channel (Figure 11). The presence of the weir allows increasing the depth of wastewater behind the weir, hence variation in wastewater level that can then be converted into flow.

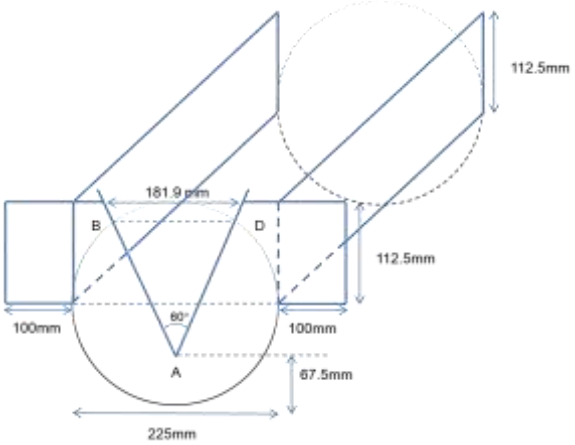


Figure 11. Design of the v-notch weir and weir tank.

To measure the variation in water level, that is to say the hospital wastewater flow rate, a non-contact ultrasonic water level sensor with a 4-20 mA output (WL700 Ultrasonic level sensor, Global water, USA) was used. An open channel flow monitor couple with a data logger preloaded with 60°C v-notch rating (FC220-DC-D Open Channel Flow Monitor, Global water, USA) was then used to record the wastewater flow. The flow monitor was wired to transmit the 4-20 mA signal proportional to the flow

to the sampling pump (Watson Marlow 520UN, programmable interface, water proof casing, equipped with a 520R2 pump head and 3.2 mm tube bore) to control its speed.

For a 4 mA signal transmitted from the flow meter to the pump which corresponded to the minimum wastewater flow of 0 $L.s^{-1}$, the speed of the pump was set at 0 rpm (i.e. 0 $mL.min^{-1}$). Similarly, for a 20 mA signal, which corresponded to the maximum wastewater flow measurable by the flow meter when using the designed weir (i.e. 8 $L.s^{-1}$), the speed of the pump was set at 37 rpm (*i.e.* 74 $mL.min^{-1}$).

Using this set up, approximately 8 L of wastewater were collected each day over three days. The wastewater was collected in 10 L glass bottles left in the manhole over 24h cycles (Figure 12). Over the duration of the sampling campaign, full bottles were collected daily at 6:00 pm and transferred within 40 min to the laboratories for immediate filtration using 0.45µm filters (Nylon 47 mm, 0.45 µm membranes, PM separations, Australia). All samples were then refrigerated before analysis.

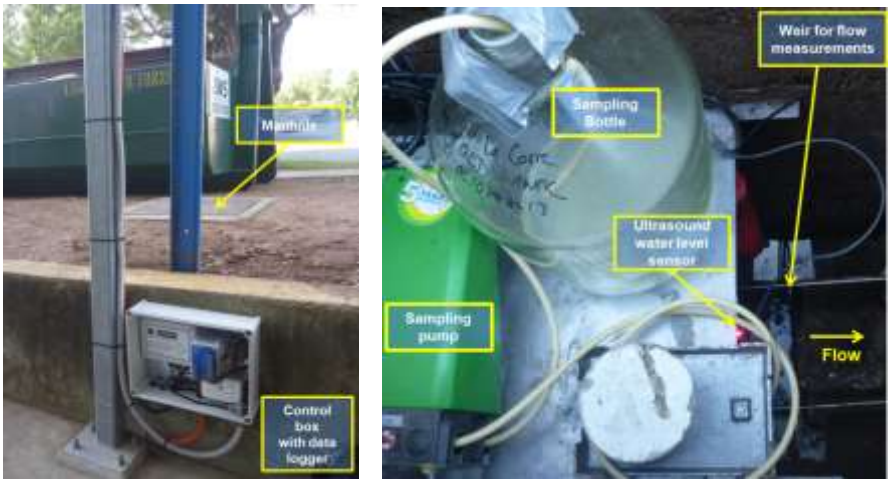


Figure 12. Flow proportional sampling set up. (Left view of the sampling site – right: view of the sampling system in the manhole).

3.2.2.2. Sampling Protocol at the Sewage Treatment Plant

The collection of raw wastewater from Bundamba STP was performed using a similar set-up as the one used at the hospital site. Flow measurements were obtained from the main inflow meter of the STP located above the open channel carrying the raw influent to the STP (Figure 13). The analog 4-20 mA signal emitted by the flow meter was tapped into to control the speed of the sampling pump (Watson Marlow 520UN, programmable interface, water proof casing, equipped with a 520R2 pump head and 3.2 mm tube bore). For a 4 mA signal transmitted from the flow meter to the pump which corresponded to the minimum wastewater flow of 0 $L.s^{-1}$, the speed of the pump was set at 0 rpm (*i.e.* 0 $mL.min^{-1}$). Similarly, for a 20 mA signal which corresponded to the maximum wastewater flow measurable by the flow meter (*i.e.* 1,000 $L.s^{-1}$), the speed of the pump was set at 15.3 rpm (*i.e.* 30.7 $mL.min^{-1}$). Using this set up, approximately 8L of raw wastewater were collected each day over 3 days. The wastewater was collected over 24h cycles in 10 L glass bottles placed in a cool box (Figure 13) Over the duration of the sampling campaign, full bottles were collected daily at 6:00 am and transferred within 40 min to the laboratories for immediate filtration using 0.45 µm filters (Nylon 47mm, 0.45um membranes, PM separations, Australia).

Figure 13. Flow proportional sampling set up at Bundamba STP and sampling bottle after a 24h cycle.

3.2.3. Chemical Analyses

As mentioned previously, all samples were filtered prior to all measurements using 0.45 mm filters (Nylon 47mm, 0.45um membranes, PM separations, Australia) and stored in amber glass bottles.

100 mL duplicates of the filtered hospital wastewater (HWW) and raw municipal wastewater (STPinf) collected each day over three days were then extracted on a Visiprep manifold system (Sigma Aldrich, U.S.A.) using Oasis HLB cartridges (200 mg, 6 mL) from Waters Corporation (U.S.A.), previously conditioned with 10 mL of methanol and 10 mL of deionised water (HPLC grade).

Liquid chromatography-mass spectrometry (LC-MS) analyses were performed using a Shimadzu Prominence ultra-fast liquid chromatography (UFLC) system (Shimadzu, Japan) coupled with a 4000 QTRAP hybrid triple quadrupole-linear ion trap mass spectrometer (QqLIT-MS) equipped with a Turbo Ion Spray source (Applied Biosystems-Sciex, U.S.A.). Chromatographic separation was achieved with an Alltima C18. Column (250 x 4.6 mm, particle size 5 mm) run at 40 °C, supplied by Alltech Associates Inc (USA).

The multi-residue method used is described in supporting information C2, Table SI 22 and Table SI 23. The quantification of the targeted compounds in the extracts was performed using 7-point calibration curves in the range from 1 to 200 µg L^{-1}. Method detection limits (MDLs) and method quantification limits (MQLs) for the analysed samples were calculated by a signal-to-noise ratio (S/N) 3 and 10, respectively. MQLs determined for hospital wastewater (HWW) were in the range 0.2–18.5 ng/L, and 0.1–65.9ng/L for the STP influent (STPinf). Recoveries of the method for HWW and STPinf were determined by analysing fortified samples of each type of wastewater spiked in triplicate to 1 µg/L. The recoveries determined for HWW and STPinf were in the range from 38.0 ± 1.4 % to 160.5 ± 5.5 %, and 43.1 ± 4.2 % to 178.5 ± 10.5 %, respectively, whereas generally they were over 50%. In order to compensate matrix effects from sample matrices internal standard calibration and adequate dilution of sample extracts (i.e. 1:2) were applied (Gros *et al.*, 2006). The recoveries and method quantification limits (MQLs) are summarised in (Table SI 21).

3.3. Results and Discussion

3.3.1. Volume of Wastewater Discharged by the Hospital

The flow measurements performed over the duration of the campaign revealed that on average the flow in the manhole was 0.5 L s^{-1}, that is to say 44.4 kL per day.

According to meter readings provided by the maintenance team of Ipswich Hospital, this flow would represent 31% of the volume of freshwater consumed daily by the hospital (143.9 kL/day in May 2012), hence about one third of the overall wastewater volume discharged by the hospital.

Determining the load of pharmaceuticals released by Ipswich Hospital based on concentrations and flow measured in the sampled sewer may then lead to underestimations of its contribution to the loads of pharmaceuticals in the influent of Bundamba STP. Nevertheless, as sampling from the manhole selected here was the most adequate option, loads and contributions of Ipswich Hospital for the compounds investigated were then determined using the flow measured in the sewer. In addition loads and contributions values determined using measured flows were extrapolated using the freshwater consumption volumes. It was assumed in that case that all the pharmaceuticals detected in this study would be proportionally found in the totality of the effluent discharged by the hospital.

Therefore, section 3.3.3 discussing the contribution of Ipswich Hospital has been divided in two parts; the first one evaluating the contribution of the hospital based on flow measured at the sampling site, and the second based on wastewater volumes discharged by the hospital using the volume of freshwater consumed in May 2012, period at which the sampling was performed.

3.3.2. Detection of Pharmaceuticals in HWW and STPinf

Figure 14 shows the 34 compounds that were detected at both sites over the limits of detection. Unsurprisingly, high concentrations of pharmaceuticals were found in the hospital effluent. Of all the compounds investigated, acetaminophen was detected at the highest concentrations with values ranging from 590 to 733 µg L^{-1} in HWW. Other substances found at the highest average concentrations in HWW were the X-ray contrast agent iopromide at 90 µg L^{-1}, the anti-inflammatory ibuprofen at 23 µg L^{-1}, and the antibiotics norfloxacin, enrofloxacin, trimethoprim and ciprofloxacin, with respective average values of 17, 16, 15 and 14 µg L^{-1}. It must be noted, however, that for two of these antibiotics, high variations in concentrations were observed over the three days of sampling. This was the case for norfloxacin which was detected at a minimum concentration of 0.4 µg L^{-1} and a maximum concentration of 47 µg L^{-1}, and enrofloxacin with a concentrations range from 0.1 µg L^{-1} to 49 µg L^{-1}. A possible explanation to such variations is the variation in consumption, hence excretion of these compounds at the hospital. Among the other substances, 12 were found in HWW in concentrations in the range 1-10 µg L^{-1} including the beta-blocker propranolol, the antibiotic erythromycin and anti-inflammatory diclofenac. The concentration levels of the 14 remaining compounds were in the range 0.04 to 10 µg L^{-1} such as for the antibiotics sulfamethoxazole and roxithromycin, the beta-blocker atenolol and the anti-convulsant carbamazepine.

In comparison, concentrations detected in the influent of the Bundamba STP (to which Ipswich Hospital discharges its effluent) were typically lower than the ones detected in HWW (Figure 14). For 26 of the compounds investigated, levels detected in STPinf were from two times lower for atenolol up to 61 times lower for enrofloxacin. Levels of diclofenac, and doxylamine were similar in both types of wastewater, while for the six remaining substances, including sulfamethoxazole and naproxen, levels detected at the STP were slightly higher (1.8 and 5.7µg L^{-1} respectively) than in HWW (1.0 and 2.6 µg L^{-1}). Overall for the majority of the pharmaceuticals investigated, levels in STPinf were below 10 µg L^{-1}. Only iopromide and acetaminophen returned values above 10 µg L^{-1}. Iopromide was detected in STPinf at concentrations between 13 and 18 µg L^{-1}, while acetaminophen, which was also the pharmaceutical substance detected at the highest concentration was found in concentrations between 70 and 156 µg L^{-1}. However, the concentrations of both substances were nearly six times lower than the ones detected in HWW.

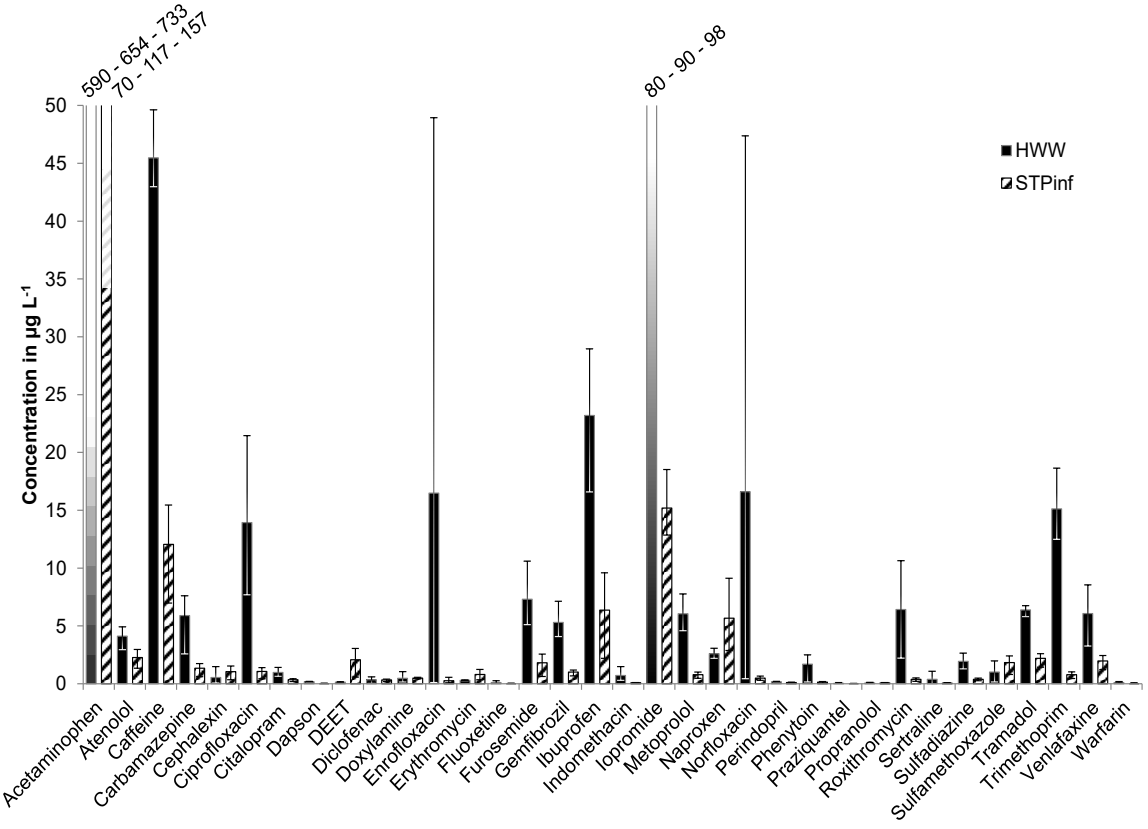


Figure 14. Average concentrations for pharmaceutical compounds detected in the hospital wastewater (HWW) and influent of the sewage treatment plant (STPinf). The error bars represent the minimum and maximum concentrations measured over three days. For acetaminophen and iopromide labels for values out of scale have been added on the graph (minimum-average-maximum).

When compared to experimental data available in the literature, concentrations detected at Ipswich Hospital generally fit in the ranges measured in various hospital wastewater sources. For instance, Thomas *et al.* (2007) investigated the presence of 20 pharmaceuticals in the effluent of two Norwegian hospitals located in a catchment with a bed density of 4.4 beds per 1,000 inhabitants and including 585 and 1200 beds. For acetaminophen, they reported measured concentrations at the hospitals investigated ranging from a minimum of 5.4 µg L^{-1} to a maximum of 1368 µg L^{-1} over a twelve week period. The average concentration measured for that compound in Ipswich HWW was 657 µg L^{-1}. In their study on mass flows of X-ray contrast media and cytostatics in the effluent of a Swiss hospital (485 beds), Weissbrodt *et al.* (2009) detected iopromide at levels ranging from 5 to 1390 µg L^{-1}, that corresponds to a maximum of 2.9 µg.L^{-1} per bed. At Ipswich Hospital, a maximum concentration of 98.4 µg L^{-1} was found for this compound, corresponding to 0.3 µg L^{-1} per bed. Kovalova et *al.* (2012) investigated the presence of 68 micropollutants in the effluent of a 346 beds hospital. Among the compounds tested over a five-week period, they reported an average concentration of propranolol of 0.12 ± 0.04 µg L^{-1} which is comparable to the values in the range 0.04 - 0.13 µg L^{-1} detected in HWW. In contrast, carbamazepine was detected in much lower concentrations (i.e. 0.22 ± 0.11 µg L^{-1}) than in our study (from 2.58 to 7.61 µg L^{-1}). However, care should be taken when considering such comparisons due to the specificity of the sites investigated in terms of water consumption, size of the hospital and catchment investigated, bed density, population and also diversity of sampling protocols.

3.3.3. Evaluation of Ipswich Hospital Contribution to Pharmaceutical Loads Entering Bundamba STP

Hospitals are by definition locations where pharmaceutical substances are consumed in high amounts on a daily basis, and therefore known to release high levels of pharmaceuticals in sewers (Verlicchi *et al.*, 2010). The results described in section 3.3.2 are therefore not surprising. However, what these

results do not show is to what extent Ipswich Hospital contributes to the loads of pharmaceutical residues entering Bundamba STP. Indeed, if the analysis of levels of pharmaceuticals in HWW can help in the design of eventual decentralised treatment options, the efficacy of such options to reduce pharmaceutical pollution in municipal wastewater may be questionable. To address this question, it is necessary to evaluate the contribution of hospital to loads of pharmaceuticals in municipal wastewater.

3.3.3.1. Contributions Derived from Onsite Wastewater Flow Measurements

Daily flow rates and concentrations measured in the sewer were used to evaluate the loads of pharmaceutical released daily at the sampling site while a daily average influent flow rate (based on the total inflow measured for the month during which the campaign was performed) was used for the STP. For a given pharmaceutical, the contribution of Ipswich Hospital is then the ratio of the load measured at the hospital manhole and the load of this pharmaceutical in the influent of the STP.

The results show that overall the contribution of Ipswich Hospital to the loads of the 34 compounds investigated in the influent of Bundamba STP is low (Table 9). These contributions are on average below 5% for 32 of the compounds investigated. For the two remaining compounds, namely norfloxacin and enrofloxacin, Ipswich Hospital average contributions were respectively 8.6 % and 15.7%. When using a worst case scenario (i.e. maximum contribution), the contribution of Ipswich Hospital would be above 5 % for only six pharmaceuticals: one anticonvulsant (phenytoin (13.0%)); and five antibiotics (trimethoprim (10.5%), ciprofloxacin (11.0%), roxithromycin (13.0%), norfloxacin (41.4%) and enrofloxacin (100%)). For the latter, the maximum contribution of Ipswich Hospital may be biased by extremely high variations of loads over the three days of sampling. Indeed loads of enrofloxacin at the hospital varied from 0.004g d^{-1} to 2.24 g d^{-1} and from 1.50 to 9.82 g d^{-1} at the STP. Monitoring of enrofloxacin and norfloxacin in HWW and STPinf over a longer period of time would be necessary to evaluate if the extreme values recorded for both of these compounds were occasional or on the contrary if they reflected the consumption pattern of these compounds at the hospital.

Table 9. Contributions measured at Ipswich Hospital for the 34 compounds investigated. The compounds are classified by increasing maximum measured contributions.

Contribution Classification According to Maximum Measured Contribution	Substance	Therapeutic Class	Measured Contribution of Ipswich Hospital to Bundamba STP (% of Total STP Influent)		
			Min*	Mean	Max**
Max ≤ 5%	DEET	Insect repellent	0.0	0.0	0.1
	Naproxen	Anti-inflammatory	0.1	0.1	0.2
	Erythromycin	Antibiotic	0.1	0.1	0.2
	Doxylamine	Sedative/ Antihistaminic	0.1	0.2	0.6
	Propranolol	Beta-blocker	0.1	0.2	0.7
	Sulfamethoxazole	Antibiotic	0.0	0.1	0.7
	Diclofenac	Anti-inflammatory	0.0	0.3	0.7
	Perindopril	Antihypertensive	0.2	0.4	0.9
	Atenolol	Beta-blocker	0.2	0.5	1.0
	Cephalexin	Antibiotic	0.0	0.1	1.1
	Tramadol	Analgesic	0.6	0.7	1.3
	Caffeine	-	0.7	0.9	1.7
	Warfarin	Anticoagulant	0.2	0.6	1.8
	Venlafaxine	Antidepressant	0.4	0.8	1.8
	Iopromide	X-ray contrast agent	1.0	1.5	2.0
	Citalopram	Antidepressant	0.3	0.7	2.0
	Sulfadiazine	Antibiotic	0.7	1.2	2.3
	Gemfibrozil	Hypolipidemic agent	0.8	1.3	2.7
	Acetaminophen	Analgesic	0.9	1.4	2.8
	Ibuprofen	Anti-inflammatory	0.4	0.9	2.9
	Carbamazepine	Anticonvulsant	0.4	1.1	3.2
	Fluoxetine	Antidepressant	0.1	0.8	3.5
	Dapsone	Antituberculotic and antileprotic	0.6	1.4	4.0
	Indomethacin	Anti-inflammatory	0.7	1.8	4.1
	Metoprolol	Beta-blocker	1.0	1.9	4.5
	Furosemide	Diuretic	0.5	1.0	4.5
	Praziquantel	Anthelmintic	0.0	0.7	4.6
	Sertraline	Antidepressant	0.0	1.5	4.8
5% <Max <15 %	Trimethoprim	Antibiotic	2.7	4.8	10.5
	Ciprofloxacin	Antibiotic	1.4	3.2	11.0
	Roxithromycin	Antibiotic	1.2	4.0	13.0
	Phenytoin	Anticonvulsant	0.2	2.9	13.0
Max > 15 %	Norfloxacin	Antibiotic	0.2	8.6	41.4
	Enrofloxacin	Antibiotic	0.0	15.7	100*

* The minimum measured contributions correspond to the ratio of the minimum load measured at the hospital to the maximum load measured at the STP.

** The maximum measured contributions correspond to the ratio of the maximum load measure at the hospital to the minimum load measured at the STP.

\# A maximum contribution of 100 is given when the maximum loads at the hospital site was significantly above the minimum load measured at the STP.

When compared to the experimental results obtained at Caboolture Hospital which is located in a catchment with a bed density (3.9 beds per 1,000 inhabitants) comparable to the bed density (4.2 beds per 1,000 inhabitants) in the catchment of Ipswich Hospital (Chapter 1, Ort *et al.*, 2010a), the contributions obtained for similar compounds are generally of the same order of magnitude (Table 10). For instance, the compounds which returned an average contribution below 5% at Ipswich Hospital also returned an average contribution inferior to 5% or really close to that limit value (5.1% for acetaminophen and 5.8% for furosemide) at Caboolture Hospital. The exception to this observation was for trimethoprim and roxithromycin, for which the average contributions obtained at Ipswich were much lower with 4.8 % and 4.0%, while the average contribution obtained for these two compounds at Caboolture were the highest with respectively 10.1% and 25.7%. At Ipswich, the only two compounds for which average contributions were found above 5%, norfloxacin (8.6%) and enrofloxacin (15.7%), were compounds not detected at Caboolture.

Table 10. Comparison of contributions measured at Ipswich Hospital with contributions measured at Caboolture Hospital and contributions available in the literature.

		Measured Contributions at Selected Site				
		This study	Ort et al. (2010a)	Langford and Thomas (2009)	Thomas et al. (2007)	Verlicchi et al. (2012)
Hospital		Ipswich, Queensland, Australia	Caboolture, Queensland, Australia	Oslo, Norway	Oslo, Norway	Italy
Number of Beds		296	190	1785 (2 hospitals)		900
Bed Density		3.9	4.2	4		6.7
Substance	**Therapeutic Class**	Min - Mean - Max	Min - Mean - Max	Mean	Mean	Mean
DEET	Insect repellent	0 - 0 - 0.1	0.1 -0.2-.0.3			
Naproxen	Anti-inflammatory	0.1 - 0.1 - 0.2	0.8 – 2.3 – 4.4			3.9
Erythromycin	Antibiotic	0.1 - 0.1 - 0.2	0.8 -2.6 – 5.5			7.7
Doxylamine	Sedative/ Antihistaminic	0.1 - 0.2 - 0.6	ND			
Propranolol	Beta-blocker	0.1 - 0.2 - 0.7	ND	11.4		4.7
Sulfamethoxazole	Antibiotic	0 - 0.1 – 0.7	0.2 – 0.8 – 2.2			6.1
Diclofenac	Anti-inflammatory	0 - 0.3 - 0.7	NA - 1 - NA		1.6	2.1
Perindopril	Antihypertensive	0.2 - 0.4 – 0.9				
Atenolol	Beta-blocker	0.2 - 0.5 - 1.0	0.9 – 1.8 – 3.5	2.52		4.7
Cephalexin	Antibiotic	0 - 0.1 – 1.1	0.0 – 0.4 – 1.2			
Tramadol	Analgesic	0.6 - 0.7 – 1.3	1.2 – 2.5 – 6.0			
Caffeine	-	0.7 – 0.9 – 1.7	1.4 – 2.6 – 4.4			
Warfarin	Anticoagulant	0.2 - 0.6 – 1.8	ND			
Venlafaxine	Antidepressant	0.4 - 0.8 – 1.8	0.9 – 2.0 – 5.0			
Iopromide	X-ray contrast agent	1.0 - 1.5 - 2.0	1.4 - 2.1 – 3.2			
Citalopram	Antidepressant	0.3 - 0.7 – 2.0	NA - 4 - NA			
Sulfadiazine	Antibiotic	0.7 - 1.2 - 2.3	ND			19
Gemfibrozil	Hypolipidemic agent	0.8 - 1.3 – 2.7	0.7 - 4.1 -10.0			1.2
Acetaminophen	Analgesic	0.9 - 1.4 – 2.8	2.8 – 5.1 -9.8		11.7	4.2
Ibuprofen	Anti-inflammatory	0.4 – 0.9 – 2.9	2.7 -4.8 – 8.5		0.7	4.0
Carbamazepine	Anticonvulsant	0.4 - 1.1 - 3.2	0.0 – 0.4 – 1.3	1.7		2.5
Fluoxetine	Antidepressant	0.1 – 0.8 – 3.5				
Dapsone	Antituberculotic and antileprotic	0.6 - 1.4 - 4.0	ND			
Indomethacin	Anti-inflammatory	0.7 – 1.8 – 4.1	ND			6.2
Metoprolol	Beta-blocker	1.0 – 1.9 – 4.5	2.0 – 4.1 – 7.0		1.5	5.7
Furosemide	Diuretic	0.5 - 1.0 – 4.5	2.6 – 5.8 – 13.7			21
Praziquantel	Anthelmintic	0 - 0.7 – 4.6	ND			
Sertraline	Antidepressant	0 - 1.5 – 4.8	ND			
Trimethoprim	Antibiotic	2.7 – 4.8 – 10.5	5.7 – 10.1 – 18.3		14.2	3.2
Ciprofloxacin	Antibiotic	1.4 – 3.2 – 11.0	ND		310.4	15.5
Roxithromycin	Antibiotic	1.2 - 4.0 – 13.0	11.7 – 25.7 – 56.0			2.1
Phenytoin	Anticonvulsant	0.2 – 2.9– 13.0	ND			
Norfloxacin	Antibiotic	0.2 – 8.6 – 41.4	ND			4.6
Enrofloxacin	Antibiotic	0.0 – 15.7- 100*	ND			

ND: Not detected

Only a few studies available in the literature have investigated experimentally the contribution of hospitals to loads of pharmaceuticals in influent of the corresponding STP. Table 10 provides a list of the contributions reported by Thomas *et al.* (2007), Langford and Thomas (2009) and Verlicchi *et al.* (2012). In the studies from Thomas *et al.* (2007) and Langford and Thomas (2009), which both looked at the contributions of two hospitals in the vicinity of Oslo, contributions reported are generally below 15% and of the same order of magnitude as in Ipswich. For example, they report a contribution of 1.7% for carbamazepine, while for that substance the contribution of Ipswich Hospital was in the range 0.4 - 3.2%. They obtained one of the highest contributions for trimethoprim with an average contribution of 14.2% compared to contributions ranging from 2.7 to 10.5% at Ipswich. However, it has to be noted that the contributions listed in Table 10 for the two Norwegian studies take into account the input of two hospitals to the same STP. If taken individually, the contribution of each Norwegian hospital to the STP would therefore be lower. For instance, the contribution reported by Thomas *et al.* (2007) for acetaminophen at the STP is 11.7 %, which is much higher than the contributions of Ipswich which ranges from 0.9 to 2.8%. But if taken individually, the contributions of each Norwegian hospital for acetaminophen were approximately 5.8%.

When compared to the contributions determined by Verlicchi *et al.* (2012), Ipswich average contributions are typically lower with the exception of a few substances such as norfloxacin, trimethoprim and roxithromycin. However, values obtained at the Italian hospital for these substances fit in the ranges measured at Ipswich (Table 10). These differences may be explained by the higher bed density, 6.7 per 1,000 inhabitants, in the catchment of the Italian hospital compared to the 3.9 beds per 1,000 inhabitants in the catchment of Ipswich Hospital.

Overall, it is interesting to notice that in all studies mentioned here, the contributions of the hospitals to the loads of the substances investigated are mainly below 15%. In a worst case scenario (i.e. maximum measured contribution), the contribution of Ipswich Hospital would be below 15% for 94% of the compounds detected, while the contribution of the Italian hospital would be below 15% for 82% of the compounds investigated (Verlicchi *et al.,* 2012) and the contributions of the Swiss hospitals would be below 15% for 90% of the compounds detected (Thomas *et al*., 2007; Langford and Thomas, 2009). This suggests that a hospital is unlikely to be a major contributor to the loads of pharmaceutical in influent of the corresponding STP with the exception of a few compounds. However, these results only apply to a selection of pharmaceuticals quantifiable experimentally and largely consumed by the general population. As suggested in previous chapters, further attention should be paid to the development of analytical techniques for the detection of hospital-specific substances. This would provide more information regarding compounds exclusively used in hospital that could be of greater concerns than the ones analysed for in experimental studies. But due to the large number and diversity of compounds consumed in hospitals, this implies the development of prioritisation methods such as the one presented in chapter 2 to select pharmaceuticals of potential concern.

3.3.3.2. Extrapolation of Ipswich Hospital's Contribution based on Freshwater Consumption

Contributions based on flow measurements at the sampling site showed that Ipswich Hospital would not be a major point source. However, as explained in section 3.3.1, despite collecting wastewater from a large variety of wards and services (see section 3.2.1.2, a), the manhole selected for the sampling campaign does not collect the total amount of wastewater discharged in the sewer network by the hospital. The loads and contributions determined in sections 3.3.3 have therefore been extrapolated using an average daily volume of freshwater used by the hospital at the time of sampling (143.9 kL/day in May 2012) (Table 11).

Table 11. Contributions measured at Ipswich Hospital for the 34 compounds investigated based on loads derived from Ipswich hospital water consumption volumes. The compounds are classified by increasing maximum measured contribution.

Contribution Classification According to Maximum Contribution	Substance	Therapeutic Class	Measured Contribution of Ipswich Hospital to Bundamba STP (% of Total STP Influent))		
			Min*	Mean	Max**
Max ≤ 5%	DEET	Insect repellent	0.0	0.1	0.3
	Naproxen	Anti-inflammatory	0.2	0.3	0.8
	Erythromycin	Antibiotic	0.2	0.4	0.9
	Doxylamine	Sedative/ Antihistaminic	0.2	0.8	1.9
	Propranolol	Beta-blocker	0.1	0.4	2.0
	Sulfamethoxazole	Antibiotic	0.3	0.7	2.1
	Diclofenac	Anti-inflammatory	0.1	0.8	2.6
	Perindopril	Antihypertensive	0.7	1.3	2.8
	Atenolol	Beta-blocker	0.8	1.5	3.0
	Cephalexin	Antibiotic	0.0	0.4	3.5
	Tramadol	Analgesic	1.8	2.4	3.8
5% <Max <15 %	Caffeine	-	0.7	1.9	5.5
	Warfarin	Anticoagulant	1.1	2.5	5.6
	Venlafaxine	Antidepressant	2.3	3.1	5.8
	Iopromide	X-ray contrast agent	1.2	2.3	6.1
	Citalopram	Antidepressant	3.5	4.8	6.2
	Sulfadiazine	Antibiotic	2.1	3.9	8.2
	Gemfibrozil	Hypolipidemic agent	3.1	4.5	8.5
	Acetaminophen	Analgesic	2.8	4.2	8.5
	Ibuprofen	Anti-inflammatory	1.2	3.5	10.2
	Carbamazepine	Anticonvulsant	1.4	3.0	10.6
	Fluoxetine	Antidepressant	0.2	2.6	10.9
	Dapsone	Antituberculotic and antileprotic	2.2	4.4	12.1
	Indomethacin	Anti-inflammatory	2.2	5.7	12.5
	Metoprolol	Beta-blocker	3.7	6.2	13.5
	Furosemide	Diuretic	1.6	3.3	13.6
	Praziquantel	Anthelmintic	0.0	2.0	13.8
Max > 15 %	Sertraline	Antidepressant	0.1	4.8	15.2
	Trimethoprim	Antibiotic	9.9	15.3	33.0
	Ciprofloxacin	Antibiotic	4.5	10.6	39.8
	Roxithromycin	Antibiotic	3.6	13.0	40.8
	Phenytoin	Anticonvulsant	0.6	9.7	41.0
	Norfloxacin	Antibiotic	0.5	27.1	100*
	Enrofloxacin	Antibiotic	0.1	49.3	100*

* The minimum measured contributions correspond to the ratio of the minimum load measured at the hospital to the maximum load measured at the STP.

** The maximum measured contributions correspond to the ratio of the maximum load measure at the hospital to the minimum load measured at the STP.

\# A maximum contribution of 100 is given when the maximum loads at the hospital site was significantly above the minimum load measured at the STP.

It was found that the contribution of Ipswich Hospital would remain below 15% for a majority of the compounds investigated. For instance, when considering maximum contributions, 27 substances returned a value below 15%. This number increased to 31 substances when considering average contributions (Table 11). In comparison, when using the wastewater flow measured in the sewer, the maximum contributions of 32 substances were found below 15%.

Overall, the maximum contribution of seven substances would be above 15% using freshwater volume for loads calculation (Table 11) instead of two using wastewater flow measurements (Table 10). The five additional substances that would fall in that contribution category are sertraline (15.2%), trimethoprim (33.0%), ciprofloxacin (39.8%), roxithromycin (40.8%), and phenytoin (41.0%). However when considering average contributions, only trimethoprim with a value of 15.3% would remain in his category along with norfloxacin and enrofloxacin which were the only two substances for which contributions above 15% were found previously (Table 10).

These results show that, under the hypothesis that the pharmaceutical substances discharged in the manhole selected for this study are proportionally discharged in the entire volume of wastewater generated by the hospital, the contribution of Ipswich Hospital would remain limited.

However, it is possible that pharmaceuticals detectable at our site would not be found at other wastewater discharge point of the hospital, or inversely could be found in much higher concentrations. In that case, evaluating the hospital contribution for the pharmaceuticals detected at our site by using the overall flow of wastewater produced by the hospital is subject to uncertainties. Once more, this highlights the difficulty of obtaining representative samples from sewers around hospital premises as discussed previously by Ort *et al*., 2010 (see Chapter 1) and reinforces the practicality of using other tools such as the consumption-based tool developed in Chapter 2 to assess the impact of hospitals on loads of pharmaceuticals in municipal wastewater.

3.3.4. Measured Contributions versus Predicted Contributions

The contributions determined using measured pharmaceutical loads in the wastewater discharged in the sampled sewer were compared to the contribution predicted for the same compounds using the consumption based methodology developed in Chapter 2 (Figure 15).

Out of the 29 substances for which both measured and predicted contributions were available, for 13 compounds, the predictions were close to the measured one. These included, for example, carbamazepine with measured contributions in the range 0.4 - 3.2% (average=1.1%) and predicted contribution in the range 1.3 - 1.6% (average =1.5%), or the beta-blocker atenolol with measured contributions in the range 0.2 - 1.0% (average=0.5%) and predicted contribution in the range 0.6 - 0.8% (average =0.7%). Overall, out of the 28 substances classified in the contribution class "<5%" based on average measured loads, 21 remained in this category based on average predicted loads. Of the remaining seven compounds, when compared to measured contributions, the corresponding predictions fall in the category 5-15% for sulfamethoxazole (5.4%), furosemide (6.3%), ciprofloxacin (10.4%), acetaminophen (9.8%), roxithromycin (12.1%), trimethoprim (13.2%) and in the category ">15%" for ibuprofen (31%).

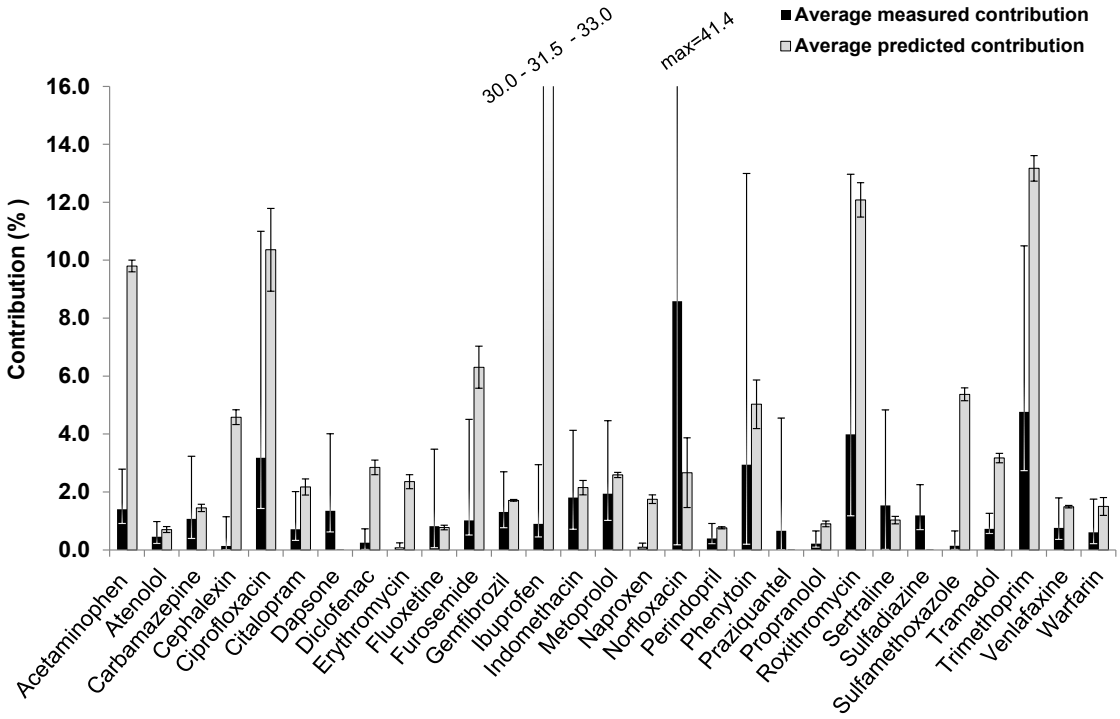


Figure 15. Comparison of contribution measured at Ipswich Hospital with predicted contribution over two years using audit data. The error bars represent the minimum and maximum measured/predicted values. The substances are only displayed when both measured and predicted contributions were available. For predicted contribution, values are only displayed if pharmaceutical consumptions were available in both data sets (i.e. Hospital and National consumption audit data).

As explained in Chapter 2, it should be noted that predicted contributions can be overestimated. For example, average predicted contributions for ibuprofen and acetaminophen were respectively 9.8% and 31.5%, while the corresponding contributions measured at Ipswich were 1.4% and 0.9% (Figure 15). But substances such as acetaminophen and ibuprofen are largely available over the counter (OTC). Therefore, the amount of such substances consumed by the general population is largely underestimated - and the corresponding predicted contribution largely overestimated - since national consumption data in Australia do not take into account OTC drugs (see Chapter 2). For ciprofloxacin and roxithromycin, despite average predictions being above 10%, these are still fitting in the ranges measured at Ipswich. This is not the case for trimethoprim, for which the predicted contributions ranged from 12.7 to 13.6 % (average = 13.2%), while measured contributions were in the range 2.7 - 10.5% (average = 4.8%). However, the maximum contribution measured for trimethoprim is close to the average predicted contribution. A lower average number of patients being treated in hospital over the sampling period or the input from outpatient or visitors treated with trimethoprim to the wastewater discharge at Ipswich Hospital on one specific day of sampling could explain the high variability of loads measured over the duration of the sampling. In contrast, the average measured contribution for norfloxacin was more than three-fold higher than the average predicted contribution. However as mentioned in section 3.3.1, high variations in the concentrations of norfloxacin measured at the hospital from one day of sampling to another were observed (from 0.4 µg L^{-1} to 47.3 µg L^{-1}), hence leading to high loads variations and high variation in contributions. The variation in loads cited above may have been attenuated by sampling over a longer period of time.

3.4. Conclusion

As found at the Caboolture case study site, the results obtained at the Ipswich case study site confirmed that a hospital is unlikely to contribute significantly to the loads of pharmaceuticals in municipal wastewater. In fact, despite slight differences in bed densities and number of beds, the contributions of both hospitals were found to be below 15% for a majority of the compounds investigated. At Caboolture Hospital, only two substances (out of the 28 measured in HWW and STPinf) lead to maximum hospital contributions above 15%. These were roxithromycin (56%) and trimethoprim of 18%. In the current study, of the 34 substances detected at both sites, only two substances resulted in maximum contributions above 15%. These were norfloxacin (41.4%) and enrofloxacin (> 100%). The majority of pharmaceutical residues present in municipal wastewater are then mainly the consequence of high pharmaceutical consumptions by the general population.

When compared to predictions using the consumption-based tool described in Chapter 2, the contributions measured at Ipswich were comparable despite a few exceptions such as, for example, acetaminophen and norfloxacin. However, for substances such as acetaminophen, predicted contributions are likely to be largely overestimated since they are widely available over the counter. Indeed, the national consumption database used in the predictive approach only takes into account amounts of subsidised medicines consumed by the general population and not mass amount sold over the counter. Uncertainties resulting from high flow and concentrations variations that can occur in sewers systems, such as for norfloxacin, can also explain differences between measured and predicted contributions. In that case, long term monitoring of pharmaceutical residues in hospital wastewater originating from hospital would help identifying true outliers from typical loads.

Overall, the results of this study confirmed that predictions based on audit data reflect results obtained experimentally for analytically quantifiable compounds. Hospital and national consumptions data can therefore be used to predict the contribution of a wide range of substances for which no analytical methods are available, hence allowing the prioritisation of compounds never investigated experimentally but that may be of greater importance than the compounds typically analysed for.

4. A REVIEW OF STRATEGIES FOR THE PRIORITISATION OF PHARMACEUTICALS

4.1. Introduction

The presence of pharmaceutical substances in the environment, and more specifically in the aquatic environment, has become the object of a multitude of scientific studies over the past 30 years, with a nearly 40-fold increase in the number of publication on this topic (Figure 16). This exponential trend can be explained by growing concerns regarding the emergence of pharmaceutical contaminants in water and their potential to cause adverse effects on aquatic life and human health since these substances are originally designed to be biologically active (Monteiro and Boxall, 2010). This particular interest is also the consequence of an incessant improvement of analytical equipment which has allowed the detection of more and more pharmaceutical compounds such as antibiotics, analgesics, antidepressants, antineoplastics, beta-blockers and X-ray contrast media at increasingly lower concentrations (down to ng L^{-1}) in municipal wastewater, ground and surface waters and more recently drinking water (Sacher *et al.*, 2001; Fick *et al*, 2009; Busetti *et al.*, 2010; Metcalfe *et al.*, 2010; Vullliet *et al.*, 2011).

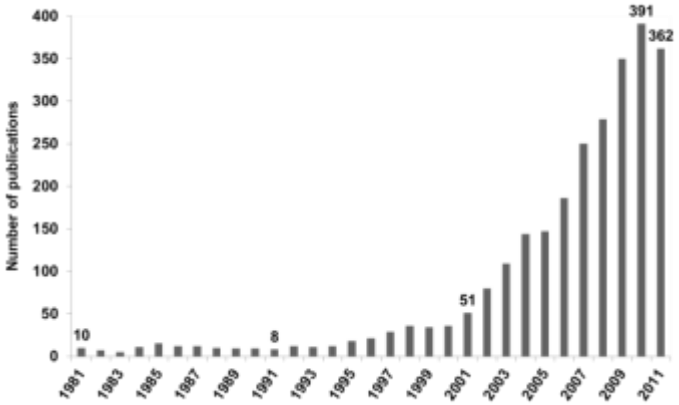


Figure 16. Number of articles related to pharmaceuticals in various sources of the water cycle published in the scientific literature since 1981. (Results extracted from the Scopus International Database in October 2011— including the terms [pharmaceutical OR pharmaceuticals] AND ["drinking water" OR "potable water" OR "wastewater" OR "waste water" OR "hospital wastewater" OR "hospital waste water" OR "hospital effluent" OR "surface water" OR "industrial wastewater" OR "industrial waste water" OR "industrial effluent").

The presence of pharmaceuticals in aquatic systems primarily originates from the discharge of treated municipal wastewater. Sources of pharmaceuticals in municipal wastewater include human excreta (Sanderson *et al.*, 2004), improper disposal of unused medicines (Watts *et al.*, 2007) and in a majority of countries untreated hospital wastewater. Agriculture and industries are also contributing to pharmaceutical pollution in the environment. The latter is getting increasing attention as it was recently shown that this pollution route could be significant in specific locations of the world (Fick *et al.*, 2009).

The number of active pharmaceutical ingredients (API) analysed to date represent only a limited number of the APIs used for human or veterinary medicine. According to Howard and Muir (2011), to date only 275 distinct pharmaceuticals have been detected in the aquatic and terrestrial environment. In comparison, nearly 3000 pharmaceutical compounds were registered in the UK, Germany and more globally over Europe in 2004 (Watts *et al.*, 2007; *Ternes et al.*, 2006) and about 4900 active ingredients are currently authorised by the Therapeutic Good Administration for use in Australia (TGA, 2011). Besides, these numbers are in constant evolution as, with the fast development of modern medicine, every year new substances are commercialised worldwide while others are removed

from the market. For instance in 2010, the European Medicines Agency (EMA) authorised 77 medicines for human use and 24 for veterinary use (EMA, 2011). It should also be noted that all these substances are not only entering the environment as such, but are highly metabolised or transformed in the environment leading to the release of additional molecules that can be as toxic as their parent compound (Escher and Fenner, 2011). Therefore, it is difficult to evaluate if the APIs that have been analysed for in the environment are the most relevant ones in terms of risks to aquatic life or humans. Conversely analysing all APIs and their transformation products is a inconceivable. The development of prioritisation methods to identify compounds of concerns that may require monitoring and eventual regulation is therefore required.

This report focuses on strategies to prioritise research on risks associated with the release of pharmaceuticals in water sources. It provides a summary of these risks and management strategies currently implemented by regulatory institutions. It then reviews prioritisation exercises and methodologies developed by the scientific community to identify pharmaceuticals likely to enter the aquatic environment at concentrations that may present a risk to the aquatic life and/or human health. Advantages and drawbacks of the different methods presented here are analysed and discussed in order to identify future research on the prioritisation of APIs.

4.2. Risks Associated with the Presence of Human Pharmaceutical Residues in the Water Cycle

According to Covello and Merkhofer (1994), a risk is defined as:

> "*a two-dimensional concept involving (1) the possibility of an adverse outcome, and (2) uncertainty over the occurrence, timing or magnitude of that adverse outcome. If either attribute is absent, then there is no risk*".

In the case of the possible occurrence of human and/or veterinary pharmaceutical residues in the environment resulting from the use and disposal of APIs, risks can be divided into two categories: environmental risks and human health risks. Depending upon the risk category investigated, the parameters used to assess and/or predict the effect of these compounds on aquatic or terrestrial organisms then necessarily differ.

4.3. Environmental Risks and Current Management

By definition, pharmaceuticals used in human and veterinary medicine are chemical substances presenting preventive or therapeutic properties or prescribed for diagnosis purposes, and therefore are developed with the aim of triggering a biological effect against targeted organisms (Jones *et al.*, 2001; Halling-Sørensen *et al.*, 1998). The presence of pharmaceutical residues in the aquatic environment may then contribute to the occurrence of specific adverse effects on non-targeted organisms/species exposed to them. For instance, the feminisation of male fish has been observed in surface waters of various countries. Such endocrine disrupting effects are suspected to be closely linked to the presence of estrogenic substances through the discharge of wastewater into the environment (Jobling *et al.*, 2006). Indeed, laboratories studies have shown that endocrine disrupting compounds such as steroids could alter the reproduction system of fish (see review by Mills and Chichester (2005); Vajda *et al.*, 2011). In 2007, Kidd and co-authors demonstrated at a larger scale the effect of chronic exposure of a fish population living in an experimental lake to low concentrations of a synthetic oestrogen. Concentrations of 5-6 ng L^{-1} of 17 α-ethinyloestradiol in the lake over three years lead to the feminisation of the male fathead minnow group, alteration of the reproductive system of the female group and after three years of exposure to an almost extinction of this species. Schultz *et al.* (2011) have also recently showed that antidepressants such as sertraline and venlafaxine could reduce the survival of fathead minnows (*Pimephales promelas*) exposed for 21 days to levels in the nanogram per litre range which could be found in wastewater and surface water. In their study of the biological community structure of a Spanish river, Muñoz *et al.* (2009) showed that concentration variations for the beta-blocker propranolol and the anti-inflammatory indomethacin from one region of the river basin to another correlated with variations in abundance of invertebrates of the benthic zone. Finally, the presence of antibiotics in the water cycle is also thought to contribute to the development of

resistant bacteria. For instance, Reinthaler *et al.* (2003) demonstrated the resistance of *E. coli* strains isolated from three STPs in Austria to a set of 16 antibiotics. Among the samples tested, strains collected in influent of a STP conjointly treating municipal and hospital wastewater presented the highest resistance with 10% for nalidixic acid, 18% for ampicillin, 29% for cephalotin and up to 31% for tetracycline. The highest resistance, 57% *E. coli* resistance to tetracycline, observed across the sites investigated was obtained for strains collected in sludge of the same plant.

Moreover, as underlined by Jones *et al.* (2004), exposure to single ingredients is an unlikely scenario when it comes to assessing toxicity effects on the aquatic fauna and flora. Mixture effects have to be taken into consideration as the presence of multiple API and their transformation products may lead to additive and possible synergetic toxicity effects (Escher and Fenner 2011). However, studies assessing risks for metabolites, transformation products, or mixture of pharmaceutical substances are very limited (Kümmerer, 2010). Richards *et al.* (2004) studied the effect of a mixture of three pharmaceuticals on model aquatic ecosystems including fish, phytoplankton and zooplankton. In this study they showed that exposure of sunfish (*Lepomis gibbosus*) to a combination of the anti-inflammatory ibuprofen, the antibiotic ciprofloxacin and the antidepressant fluoxetine at concentrations of 60, 100 and 100 µg L^{-1} could lead to the death of 47% of the population in 35 days. Similarly, effects on the abundance and diversity of phyto-and zooplankton were also observed under such conditions. However, this study did not investigate effects resulting from the presence of groups of substances with similar modes of action, while as mentioned by Jones *et al.* (2004) any risk assessment should take into consideration effects of groups of compounds sharing similar modes of action and the possibility of additive or synergetic effects.

With the detection of an increasing amount of substances of pharmaceutical origin in the environment, a number of countries have developed legal and regulatory documents for the environmental risk assessment (ERA) of pharmaceutical substances prior to commercialisation. However a majority of these documents focus on products used in veterinary medicine, while only a few countries have developed guidelines regarding environmental risk assessment of human pharmaceuticals (Koschorrecck and Apel, 2006). In 1998, the US Food and Drug Administration (FDA) established regulations on the "*environmental assessment of human drugs and biological application*", while the European Medicines Agency (EMA) released a few years later a guideline document on "*the environmental risk assessment of medicinal products for human use*" (EMA, 2006). The methodologies used for ERA in both documents are similar to some extent and based on the determination of the expected concentrations of specific chemical compounds in the environment, the so called Predicted Environmental Concentration (PEC) in Europe or Expected Introduction Concentration (EIC) in the US (Table 12 and 13). The determination of PEC or EIC is then used to determine if further eco-toxicity testing of the substance investigated is required, and ultimately decides if a substance presents an environmental risk. In essence, these regulatory tools thus help prioritising compounds of concerns that may require monitoring and subsequently adequate treatment.

Table 12. Example of environmental risk assessment approaches and action thresholds in Europe (EMA, 2006) and the United States (FDA, 1998).

EUROPE (EMA, 2006)			
Risk Assessment Phase	**Methodology**	**Tests / Data Requirements**	**Threshold Value and Decisions**
Phase I **Pre-Screening: Exposure Estimation**	Initial prediction of risk: PEC calculation in surface water (PEC_{SF}) Action limits determination	Consumption data; Log Kow*; Maximum daily dose consumed per patient Fraction of market penetration; Amount of wastewater per inhabitant per day; Dilution factor in receiving water.	If **PEC_{SF} <0.01µg L^{-1}**, and no other environmental concerns are apparent, the substance is unlikely to present a risk for the environment; If **PEC_{SF}>0.01µg L^{-1}**, environmental fate and effect analysis is required (Phase II) In case of known environmental concerns, and irrespective of the PEC_{SF} values, if the log Kow* of a substance is equal or superior to 4.5, screening for persistence, bioaccumulation and toxicity is required.
Phase II, A **Risk Screening**	PNEC calculation Calculation of the PEC to the Predicted no effect concentration (PNEC) ratio in surface water, groundwater and microorganism in water, also termed risk quotient (RQ) Readily biodegradability determination (Adsorption – desorption ratio, Koc)	Base set aquatics toxicology and fate: Standard acute toxicity tests on algae, daphnia and fish;	If RQ<1, the substance does not present an environmental risk; If RQ>1, the substance present a risk for water organisms and should be further assessed in Phase II B. or If logKow (phase I)>3 and Koc>4, environmental risk assessment for terrestrial compartment is required in Phase II B
Phase II, B **Risk Refinement Phase**	Chronic toxicity test; Microorganism specific test; Bioaccumulation evaluation Refined PEC	Extended data set on emission, fate and effects.	Report to the committee for medical product for human use (CHMP)

*Logarithm of the Octanol-Water partition coefficient which indicates the potential of a substance to bio-accumulate (lipophilicity).

Table 13. Example of environmental risk assessment approaches and action thresholds in the United States (FDA, 1998).

USA (FDA, 1998) – (Pharmaceutical Used for Human Medicine)			
Risk Assessment Phase	**Methodology**	**Tests / Data Requirements**	**Threshold Value and Decisions**
Phase I **Pre-screening Investigation of the Compounds Depletion Mechanisms**	Hydrolysis, aerobic and soil biodegradation analysis	Physical properties (Water Solubility, Dissociation Constant(s), Octanol-Water Partition Coefficient, Vapour Pressure or Henry's Law Constant).	If depletion mechanisms are rapid or complete, no further assessment is required except a microbial inhibition test. If depletion mechanism are not complete or slow, further assessment is required (Phase II).
Phase II **Risk Screening**	Expected Introduction Concentration (EIC) calculations in surface water	Consumption data; Treatment work flows.	If **EIC_{SF} <0.1µg L^{-1}** and the substance would not inhibit microorganisms and subsequently disrupt waste treatment process (microbial inhibition tests), no further assessment is required. If **EIC_{SF}>0.1µg L^{-1},** further fate and effect analysis is required (Phase III, A). If Log K_{ow}>3.5 (*i.e.* bioaccumulation risk), further fate and effect analysis is required (Phase III, C).
Phase III, A **Risk Refinement**	Acute toxicity test on minimum one organism. Assessment factor determination (AF), or ratio of the median effective concentration (EC_{50}) or median lethal concentration (LC_{50}) to the Maximum expected environmental concentration (MEEC)	MEEC calculation; EC_{50} or LC_{50} determination on minimum one species.	If AF≥ 1,000, and no further testing should be conducted unless sub lethal effects are observed at the MEEC. If the AF< 1,000, further testing is required (Phase III, B).
Phase III, B **Risk Refinement**	Acute toxicity tests on a base set (fish, aquatic invertebrate, and algae bioassays); Assessment factor determination (AF),	MEEC calculation; EC_{50} or LC_{50} determination on minimum one species.	If AF ≥ 100 for the most sensitive organism in the base set, no further testing should be conducted unless sub-lethal effects are observed at the MEEC. If AF < 100, further testing is required (Phase III, C)
Phase III, C **Risk Refinement**	Chronic toxicity test	MEEC calculation; EC_{50} or LC_{50} determination on minimum one species.	If AF≥ 10 no other effects are observed and the assessment can be concluded. If AF< 10, reporting to the Centre for Drug Evaluation and Research (CDER) and Centre for Biologics Evaluation and Research (CBER) is required.

As illustrated in Table 12, the EMA guidance document provides general principles for the assessment of potential risk of human pharmaceuticals following a two phase approach: a pre-screening phase which estimates the potential exposure of the environment to the drug considered (PEC) and a second phase to estimate the fate and effects of a substance in the environment. For instance, if the PEC predicted in surface water for a specific medical substance is lower than 0.01 µg L^{-1}, and no other environmental concerns are apparent, the substance is considered as unlikely to present a risk for the environment. Conversely, if the PEC of this substance in surface water is larger than 0.01 µg L^{-1}, further testing is required and includes the determination of a risk quotient which is the ratio of the PEC to the concentration below which exposure to a substance is not expected to cause adverse effects (Predicted No Effect Concentration, PNEC). This involves a battery of biodegradability, aquatic toxicity and microbial toxicity tests. The US used a similar approach as the EMA (Table 13), with the distinction being made between pharmaceuticals used in human medicine and pharmaceuticals used for veterinary purposes. Another difference concerns the use of a higher threshold value of 0.1 µg L^{-1} for the EIC in surface water which determines the need for full environmental risk assessment.

Despite the development of such regulations, the knowledge available to date on the influence of APIs onto aquatic and terrestrial organisms remains limited. This is not only due to the large number of compounds available on the market but also because the majority of the studies on this topic focuses on short-term toxicity testing (i.e. acute toxicity) for practical and financial reasons when information on chronic toxicity would be needed to truly assess adverse effect on the aquatic life (Kümmerer, 2010). To illustrate, in his compilation of available eco-toxicity data, Webb (2004) identified 107 human pharmaceuticals for which acute toxicity values performed on algae, fish and macro-invertebrate were available. 35% of these values were in the range 100-1,000 mg L^{-1}, that is to say, well above concentrations detected in the environment. This was, for example, the case for naproxen, for which the lowest acute toxicity reported by Webb (2004) was 140 mg L^{-1} for Daphnia (corresponding to a 24h EC_{50} value for immobilisation), while, for example, concentrations measured in the environment were found in the range of "not detected" (ND) – 400 ng L^{-1} in surface water and 3,500 ng L^{-1} in the effluents of a STP (Öllers, *et al.*, 2001) and in the range 698 -18,100 ng L^{-1} for grab samples of hospital wastewater collected in Taiwan by Lin and Tsai (2009) and in Spain by Suarez *et al.* (2009). In comparison, in the same review, Webb (2004) only identified 20 human API for which chronic toxicity tests on aquatic organisms had been carried out. These tests mainly focused on algae. This highlighted the need for additional work on chronic effects of pharmaceuticals, specifically on fish.

As mentioned by Götz *et al.* (2009), although ERAs can be a useful tool to the prioritisation and ranking of pharmaceutical substances, they are based on PNEC while toxicity data and environmental concentrations are lacking to confirm PNEC values. This partly explains why a limited number of pharmaceutical compounds have been evaluated using this procedure (Lienert *et al.*, 2007). Ankley *et al.*, (2007) also emphasised the limitations of the ERA methodology, as the action thresholds set for further environmental risk assessment may not be protective for the environment. They cite the example of Ethinyloestradiol (EE2) for which concentrations below 0.01 µg.L^{-1}, the actual action limit in the EMA guideline, would be sufficient to have adverse effects on fish. Finally, one of the aspects not accounted for in the guidelines available to date are the effects of complex mixtures of pharmaceuticals. The combination effect of individual pharmaceuticals present in the environment may have the potential to increase toxic effect on aquatic species. For example, Schnell *et al.* (2009), showed that the combined toxicity of pharmaceuticals belonging to the same therapeutic class (i.e. anti-inflammatory drugs) on the rainbow trout liver cell line was additive. For mixtures composed of pharmaceuticals belonging to different classes, the combined toxicity was more than additive suggesting that toxic effect of pharmaceuticals as mixtures could occur at concentration lower than expected for individual compounds.

4.4. Human Health Risks and Current Management

Evidences of contamination of wastewater, surface water and groundwater by various types of pharmaceutical compounds such as analgesics, antibiotics, anti-epileptics, antineoplastics, anti-inflammatories, beta-blockers, and contraceptives, suggest that indirect human exposure to these

compounds via drinking water is a possible pathway (Daughton and Ternes, 1999). Consequently the analysis of emerging contaminants in drinking water sources has become a point of attention in the past ten years, especially in countries where drinking water is produced from surface water receiving wastewater and/or countries planning water recycling for indirect potable reuse (Jones *et al.*, 2005).

One of the first compounds identified in drinking water was bleomycin, a cytotoxic antineoplastic agent, detected in 1990 in the UK at a maximum concentration of 13 ng L^{-1} (Aherne *et al.*, 1990). However, as stated in Aherne's *et al.* study, such a concentration would correspond to a level ingested one million times below the daily therapeutic dose assuming a drinking water consumption of 2 L d^{-1}. In the early 1990s, clofibric acid, a lipid regulator, raised concerns since it was measured in Berlin tap water at a maximum concentration of 165 ng L^{-1} (Heberer *et al.*, 1998). More recently, compounds such as the beta-blocker atenolol, the anticonvulsant carbamazepine, the anxiolytic diazepam and the antibiotic sulphamethoxazole have been regularly found in finished water produced from surface water sources despite undergoing full drinking water treatment (Table 14). To illustrate, atenolol has been recently detected in France (2 ng L^{-1}), the US (18 ng L^{-1}) and Spain (23 ng L^{-1}) despite chlorination and oxidation steps prior to distribution. However concentrations measured in drinking water are usually very low when compared to therapeutic doses. For example, one of the highest concentration measured in finished water for carbamazepine was recorded by Stackelberg *et al.* (2004) with 258 ng L^{-1}, but as mentioned in their study such a concentration is $4x10^4$ lower than a single therapeutic dose used for humans and is $5x10^4$ lower than the maximum possible intake of carbamazepine in a lifetime based on a drinking water consumption of 2 L d^{-1} over 70 years.

Table 14. Recent examples of maximum concentrations detected in finished water in France, Spain and the US.

Pharmaceutical	Drug Class	Location	Source Water	Concentration in Finished Water (ng L^{-1})	Reference
Atenolol	βB	France	Urban Dam	2.0	Vullliet *et al.* (2011)
		Spain	River	23.0	Huerta-Fontela *et al.* (2011)
		USA	River[a]	18.0	Benotti *et al.* (2009)
Bezafibrate	AH	France	River	2.2	Vullliet *et al.* (2011)
Carbamazepine	AC	France	Lake	32	Vullliet *et al.* (2011)
		USA	Well	6.9	Wang *et al.* (2011)
		USA	River or lake	5.7	Snyder and Benotti (2010)
		USA	Reservoir[b]	18	Benotti *et al.* (2009)
		USA	Streams[e]	258	Stackelberg *et al.* (2004)
Carbamaz epoxide	AC	Spain	River	2	Huerta-Fontela *et al.* (2011)
Diazepam	AL	USA	River[a]	0.33	Benotti *et al.* (2009)
Diclofenac	AG	France	Lake	1.00	Vullliet *et al.* (2011)
Erythromycin	AB	USA	River or lake	1.3	Snyder and Benotti (2010)
Fenofibric acid	AH	France	Urban Dam	0.2	Vullliet *et al.* (2011)
Fluoxetine	AD	USA	Reservoir [c]	0.82	Benotti *et al.* (2009)
Gemfibrozil	AH	USA	River or lake	6.5	Vullliet *et al.* (2011)
		USA	Reservoir[b]	2.1	Benotti *et al.* (2009)
Hydrochlorothiazide	DI	Spain	River	7	Huerta-Fontela *et al.* (2011)
Ibuprofen	AI	France	Urban Dam	1.3	Vullliet *et al.* (2011)
		USA	River or lake	32	Snyder and Benotti (2010)
Ketoprofen	AG	France	River	0.9	Vullliet *et al.* (2011)
Lyncomycin	AB	USA	Wells	4.4	Wang *et al.* (2011)
Levonorgestrel	Cv	France	Urban Dam	10	Vullliet *et al.* (2011)
Meprobamate	AL	USA	Lake[d]	42	Benotti *et al.* (2009)
		USA	River or lake	13	Snyder and Benotti (2010)
Metoprolol	βB	France	Lake	1	Vullliet *et al.* (2011)
Naproxen	AG	France	Lake	0.5	Vullliet *et al.* (2011)
		USA	River or lake	8	Snyder and Benotti (2010)
Norethindrone	Cv	France	Urban Dam	6.8	Vullliet *et al.* (2011)
Oxazepam	AL	France	Urban Dam	2.5	Vullliet *et al.* (2011)
Paracetamol	AG	France	Lake	45	Vullliet *et al.* (2011)
		USA	River	9.5	Wang *et al.* (2011)
Phenytoin	AC	Spain	River	10	Huerta-Fontela *et al.* (2011)
		USA	River[a]	19	Benotti *et al.* (2009)
		USA	River or lake	6.7	Snyder and Benotti (2010)
Pravastatine	AH	France	Lake	0.2	Vullliet *et al.* (2011)
Salycilic acid	AG	France	Lake	19	Vullliet *et al.* (2011)
Sotalol	βB	Spain	River	3	Huerta-Fontela *et al.* (2011)
Sulphamethoxazole	AB	USA	River	4	Wang *et al.* (2011)
		USA	Reservoir[c]	3	Benotti *et al.* (2009)
Triclosan	AS	USA	Lake	7.3	Wang *et al.* (2011)
		USA	Reservoir [c]	1.2	Benotti *et al.* (2009)
Trimethoprim	AB	France	Lake	1	Vullliet *et al.* (2011)
		USA	Wells	4.7	Wang *et al.* (2011)
		USA	River or lake	1.3	Snyder and Benotti (2010)

AB: Antibiotic; AC: Anticonvulsant; AD antidepressant; AG: analgesic; AH: Anti-hyperlipidemic; AI: anti-inflammatory AL: Anxiolytic; AS: Antiseptic; βB: βeta-Blocker; Cv: Contraceptive; DI: Diuretic.

a: with upstream wastewater source); b: containing wastewater from upstream tributaries; c: no direct wastewater input; d: receiving water from two tributary lakes and treated effluent from a wastewater treatment plant.; e: streams receiving effluent from 50 STPs.

Adverse effects on human health resulting from the presence of pharmaceutical residues in drinking water sources have not been demonstrated (Benotti *et al.* 2010). However, in 2006, Pomati and co-authors investigated the effects of a mixture of 13 API including common antibiotics, diuretics and beta-blockers but also more specific substances such as the antineoplastic cyclophosphamide on human embryonic cells. They showed that this mixture of individual APIs in ng L^{-1} concentrations caused the inhibition of human embryonic cell growth. This suggested potential health effects from exposure to low environmental concentrations. Studies have also focused on antibiotic and their potential impact on human health. Indeed as stated by Fick *et al.* (2009), the presence of antibiotic resistant bacteria is one of the greatest health concerns for humans as they can lead to the development of antibiotic resistant bacteria and "*possibly horizontal transfer of resistance factors to human pathogens*".

Furthermore, health impacts of long-term consumption of trace levels of APIs and their metabolites, or by-products generated during drinking water treatment (Mompelat *et al.*, 2009), on healthy or sensitive categories of the population (*i.e.* infants, elderly people) remain unknown (Bruce *et al.*, 2010).

Although, the very low concentrations observed in drinking and surface water to date are unlikely to cause concern for human health, a recent report on "Pharmaceuticals in Drinking-water" published by the World Health Organisation (WHO, 2011), emphasises the needs for further research on potential human health risk that could result from long-term exposure to trace levels of pharmaceuticals (or combination of pharmaceuticals) in potable water. However, as mentioned in this report, one of the key challenges consists in developing prioritisation methods to determine pharmaceuticals of potential concerns that may require future monitoring and or treatment.

Despite the increasing detection of traces of pharmaceuticals in drinking water, parameters related to pharmaceuticals have not been added to existing guidelines for drinking water quality (WHO, 1993; EU, 1998). Indeed, according to the WHO (2011) report, given the very low levels of pharmaceuticals detected in drinking water, thus their limited impact on human health, monitoring is not warranted. One exception is Australia, where the NHMRC, the EPHC and the NRMMC established guideline values for a list of 86 pharmaceutical compounds used in human and veterinary medicine for drinking water production from sources receiving recycled municipal effluent (NRMMC- EPHC - NHMRC, 2008). The concentration limits set for these compounds are based on the calculation of acceptable daily intakes (ADI) obtained by dividing the lowest therapeutic dose of a compound by a safety factor ranging from 1,000 for antibiotics to 10,000 for cytotoxic drugs. The drinking water guidelines (in µg L^{-1}) are then derived for adults with a bodyweight of 70 kg drinking on average 2 L of water per day and the proportion of the pharmaceutical allocated to the water intake (as opposed to other possible pathways such as food) (Figure 17). For example, the guideline value given for carbamazepine is 100 µg L^{-1}, which is 388 times higher than the maximum concentration published by Stackelberg *et al.* (2004).

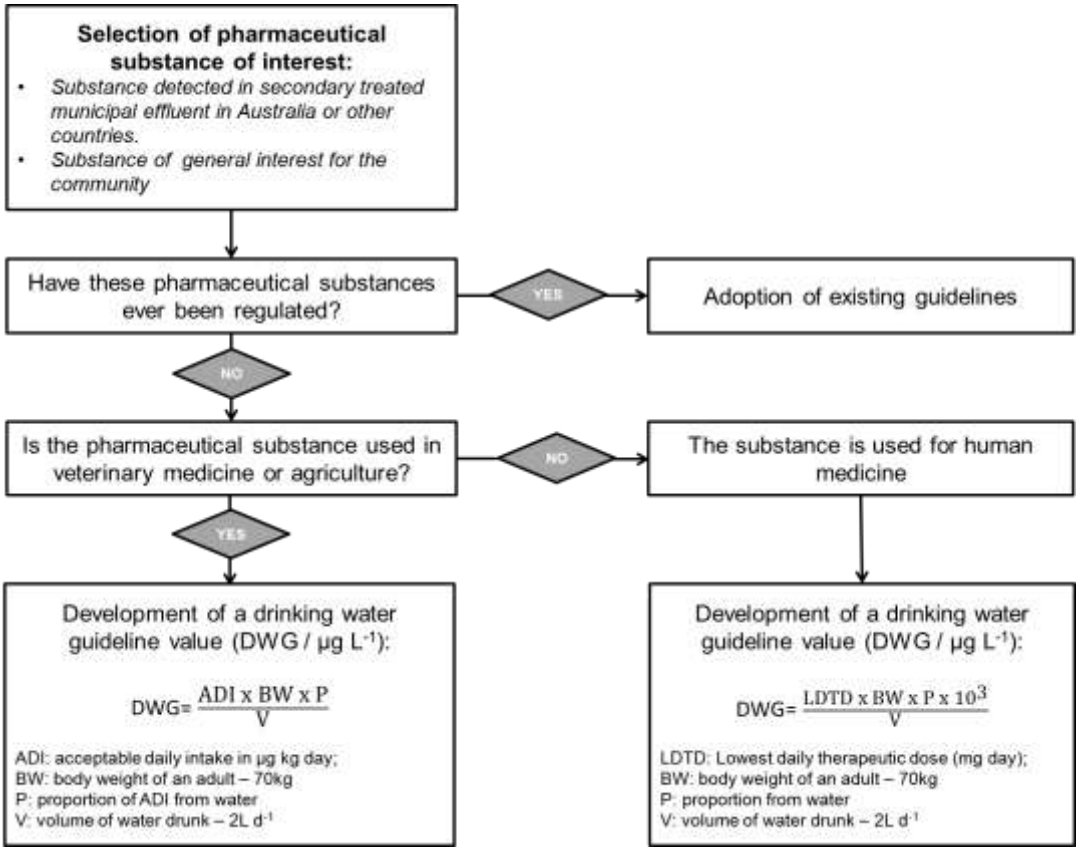


Figure 17. Decision diagram to set guidelines for pharmaceutical substances in recycled water to be used for indirect drinking water purposes in Australia (adapted from NRMMC- EPHC - NHMRC, 2008).

Although the establishment of such guidelines is a first and should help water utilities deciding which compounds should be monitored, the number of pharmaceuticals and their metabolites potentially present in the environment is vast and in constant evolution and it may be possible that some compounds not accounted for in such guidelines are or could become contaminants of greater concerns. One of the solutions now investigated by numerous studies consists in finding ways of assessing the potential exposure to active ingredients in order to prioritise future monitoring.

Finally, all studies mentioned above are based on risks of exposure through ingestion of drinking water. However, it has to be taken into consideration that this pathway is not the only one through which humans could be exposed to pharmaceuticals since drinking water is by definition used for domestic purposes including showering and bathing, swimming and gardening, so long-term exposure by contact is also a possibility (Jones *et al.*, 2005). Further research is then required to fill these knowledge gaps.

4.5. Prioritisation of Pharmaceutically Active Compounds

4.5.1. Targeting Pharmaceuticals in Water Sources: Current Practices

A review of 55 experimental studies on the presence/detection of pharmaceuticals in various water and wastewater sources worldwide published between 2009 and 2010 (Supporting information D, Table SI 24) revealed that pharmaceutical compounds investigated in various waters and wastewaters are often similar from one study to another (Figure 18). Overall, 282 distinct compounds (excluding metabolites) were mentioned in the 55 articles reviewed. Out of these 282 substances, 95 were analysed in three or more than three articles. The most frequently analysed substances belong to therapeutic classes commonly used in human medicines such as anti-inflammatories, anticonvulsants, beta-blocker and antibiotics. Among these substances, the ten most frequently analysed substances are

ibuprofen, diclofenac, naproxen, carbamazepine, ketoprofen, sulfamethoxazole, gemfibrozil, atenolol, paracetamol and trimethoprim with a number of occurrences in 20 to 35 articles (Figure 18).

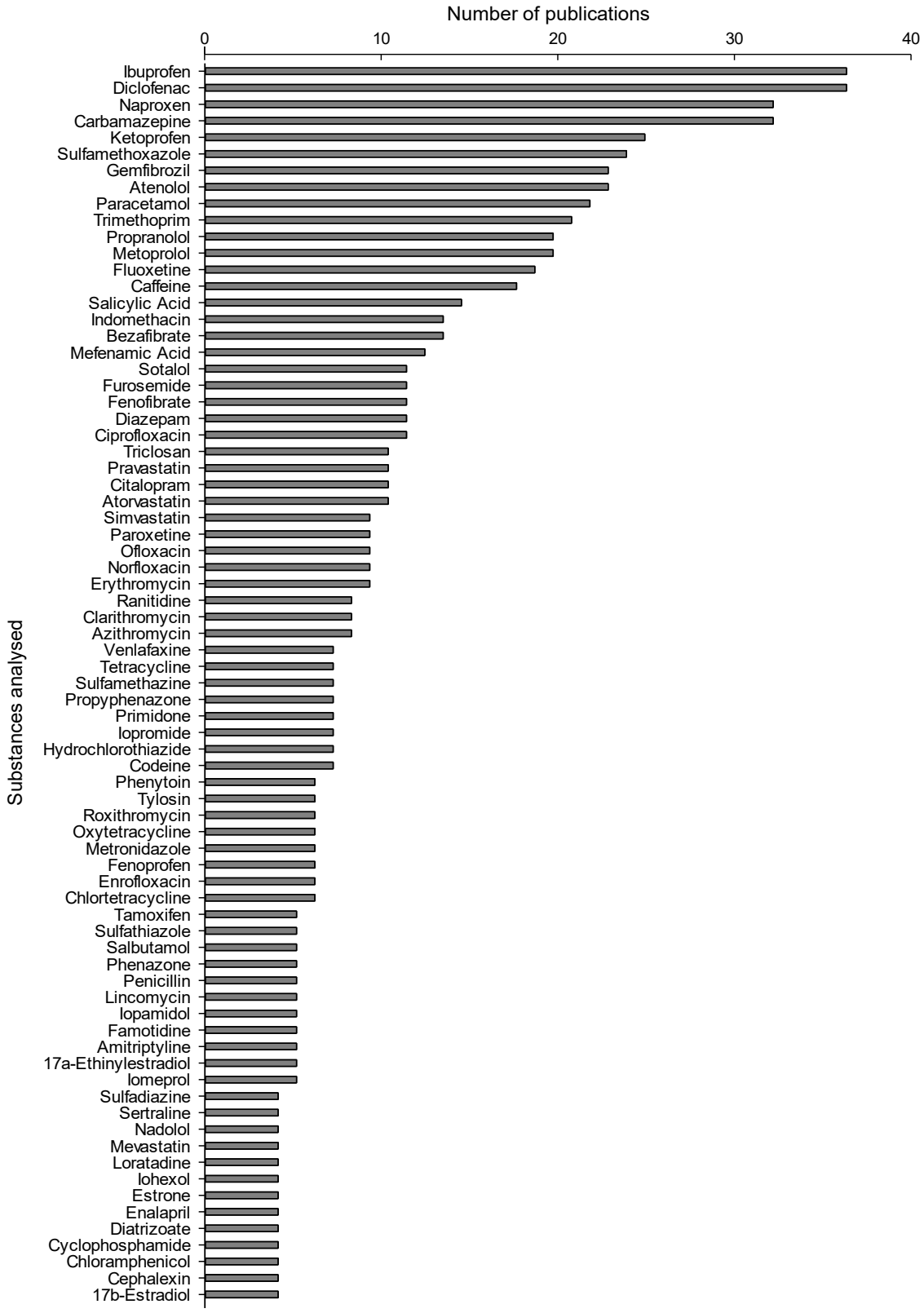


Figure 18. Pharmaceuticals analysed in more than three articles out of 55 publications reviewed for the year 2009-2010.

It is not surprising to find these compounds in a majority of these studies. Indeed, the occurrence and fate of these pharmaceuticals in the environment have been widely studied by the scientific community for the past 10 years as they are among the most consumed substances in numerous countries worldwide. As such, they are more likely to be ubiquitous in the aquatic environment at detectable levels and be of potential concern for aquatic species and/or human health. For example paracetamol, ibuprofen, naproxen and carbamazepine figure in the 2004 top 20 most consumed substances per inhabitant in the UK, France and Australia (Supporting information D, Table SI 25). Furthermore, some of them belong to therapeutic groups which have received increased attention such as antibiotics which are highly prescribed in human and veterinary medicine and the release of wastewater is suspected to contribute to the development of antibiotic-resistant bacteria in the environment (Jury *et al.*, 2011). This is the case for sulfamethoxazole and trimethoprim, typically used conjointly. The antiepileptic carbamazepine and the anti-inflammatory diclofenac are also often part of analytical methods as they are known to be poorly removed by conventional wastewater treatment (10% and between 20 and 40% removals for carbamazepine and diclofenac respectively) and persistent in the environment (Zhang *et al.*, 2008; Yamamoto *et al.*, 2009).

Table 15 summarises the criteria explicitly and/or implicitly mentioned in the 55 reviewed publications to select the pharmaceuticals to be studied. Overall, out of 55 studies, 24 reported the use of a combination of two or more criteria to justify their choice of substances to be analysed, while 19 mentioned only one criterion and 12 did not specify any selection criteria. The study from Mullot *et al.* (2010) is the only one reporting the use of six criteria. These included: location/site specificity (e.g. hospital site), annual consumption, therapeutic dose, metabolism, analytical capabilities and therapeutic classes.

The pharmaceuticals belonging to a specific therapeutic class (42% of the publications) and their usage in terms of quantity or amount prescribed locally, nationally or internationally (31%) were the most frequently cited criteria of selection across studies (Table 15). As expected, consumption is often used for the reason mentioned above, but it is interesting to note that in most studies this criterion is used as a first screening step in combination with at least another screening criteria. Indeed, using consumption/sales volumes as a sole prioritisation criterion would be delicate, as highly consumed substances may not necessarily be the ones likely to present the highest risks for the environment and human health. For instance, Escher *et al.* (2011) estimated that some pharmaceuticals such as amiodarone, clotrimazole and ritonavir, which are moderately used, could pose an environmental risk because they are expected to be highly toxic to aquatic organisms. Although toxicity of the pharmaceuticals investigated should be a priority to assess impact on the environment and human health, only 6% of the papers mention this parameter. However, the publications mentioning therapeutic classes as a criterion may also implicitly take this aspect into account. Finally, it is also interesting to notice that the availability of analytical methods has been reported in 7% of the studies as a factor of selection, or rather "non-selection" of drugs to analyse. This parameter is however a limiting factor rather than a selection criterion.

Table 15. **List of criteria identified and corresponding citation frequency across 55 publications.**

	Number of Criteria Used Conjointly							Percentage of Publications Citing the Criteria
	6 Criteria	5 Criteria	4 Criteria	3 Criteria	2 Criteria	1 Criterion	No Criteria Specified	
Number of publications	1	1	3	6	13	19	12	
Split by criteria								
Drug class	1		2	1	8	11		42
Usage - quantity (consumption (national or regional)/ sales/ prescriptions numbers/ production amount)	1	1	2	4	8	2		31
Occurrence / detection in the environment/ frequency of detection		1	2	3	2	1		15
Physico-chemical properties	1	1	2	1	3			15
Analytical feasibility	1	1		2		1		9
Treatability / Resistance to treatment		1		3				7
Persistence in the environment			1	1	1			5
Eco or human toxicity			2	1				5
"Common compounds" used in human medicine						2		5
Local context	1					2		5
Priority compounds (regulation etc.)					2			4
Predicted environmental concentration			1		1			4
Potential and or known effects on the environment or human health				1	1			4
Therapeutic dose	1							2
Mode of action				1				2

4.6. Targeting Pharmaceuticals in Water Sources: Prioritisation

As illustrated in section 3.1, studies on the impact of pharmaceutical substances in the water cycle are focusing on a limited fraction of the extremely wide range of active ingredients that may reach the environment. When researching the presence of pharmaceutical substances in various water sources and potential risk associated with their release in the environment, choices for studying one set of compounds over another, when justified, was based on the use of one or two selection criteria at a local scale. And ultimately this choice was restricted by the availability of analytical techniques. Because such approaches do not guarantee that the pharmaceutical studied in the aquatic environment are of most concern, the development of methods to identify research priorities are becoming a necessity. As a result, a number of screening and prioritisation exercises for pharmaceuticals have been developed in various countries. The following section provides information on a selection of these methods focusing on pharmaceuticals used for human medicine.

4.6.1. Examples of Prioritisation Methodologies

4.6.1.1. Australia

- *Modelling of pharmaceutical residues in Australian sewage by quantities of use and fugacity calculations, (Khan and Ongerth, 2004).*

The aim of this study was to identify pharmaceutical compounds likely to be found in raw and treated wastewater. For this purpose the authors developed a model to predict concentrations in raw influent of STPs but also concentrations and fate of these compounds during primary and secondary treatment. In the former case, the model was based on a set of parameters including mass of the top 50 pharmaceuticals prescribed in Australia, metabolism, excretion rates, STP influent flow rates, population, and eventual disposal of pharmaceuticals down the drain. In the latter case, the model incorporated fugacity principles that is to say an evaluation of the pharmaceutical distribution between the various biological and chemical phases (biomass, liquid and gaseous) based on transport and transformation processes occurring during treatment.

The prioritisation method developed by Khan and Ongerth (2004) followed five steps:

1. A ranking of the "top 50 pharmaceuticals by mass" for the year 2008 excluding inorganic salts, naturally produced hormones. Masses of pharmaceuticals dispensed were deduced from prescription numbers and average quantities per prescription.
2. Metabolism data was then taken into account. For this purpose, the proportions of parent drugs excreted unchanged and as hydrolysable conjugates were considered.
3. Chemical and physical properties of the compounds were then analysed. Regarding the hydrolysis of simple conjugates in sewage, it was assumed to be 100%. In addition, it was assumed that the proportion of pharmaceuticals discharged down the drain was low (0.01%).
4. Parameters for the evaluation of the distribution of pharmaceuticals during treatment (fugacity model) were then taken into account. These included partitioning of the compounds to the atmosphere and biodegradation.
5. As mass amounts were derived from national consumption, the population in the catchment of the STP under consideration and characteristics of the STP (influent flow rates) were then used to predict concentrations in influent of the STP.

Results showed that 27 compounds and two metabolites could be present in concentrations equal to or above 1 µg L^{-1} in raw sewage. The compounds with the highest predicted concentrations in raw wastewater were paracetamol (100 µg L^{-1}), metformin (40 µg L^{-1}), amoxicillin (10 µg L^{-1}) and cephalexin (10 µg L^{-1}).

The model is claimed by the authors to be limited by a number of uncertainties notably regarding the quality of the consumption data collected which do not take into consideration the pharmaceuticals sold over the counter (OTC). For instance, paracetamol, which resulted in the highest predicted concentration in raw influent, is likely to be underestimated as it is largely sold without prescriptions. Furthermore, concentrations predictions after primary treatment are limited by a number of factors such as limited data on biodegradation rates of compounds.

4.6.1.2. North America

- *Identifying new persistent and bioaccumulative organics among chemicals in Commerce II: Pharmaceuticals, (Howard and Muir, 2011 - USA).*

This recent study aims at generating a list of priority pharmaceuticals that might be persistent in the environment (P) and have a potential to bioaccumulate (B). The method used by Howard and Muir (2011) is a multistep approach to screen commercial pharmaceuticals listed in specific databases using Quantitative Structure Property Relationships (QSPR). QSPR provides estimations of physical and chemical properties (Log K_{ow}, bioconcentration factors) of these pharmaceuticals.

The prioritisation exercise carried out in this study involved:

1. Compiling pharmaceuticals commercially available in the US (2700 compounds used either in human or veterinary medicine), high production volume pharmaceuticals (300), the top 200 best-selling drugs, 29 pharmaceuticals sold in France, UK and Spain, and finally 375 veterinary drugs (note that some compounds may be present in more than one data base).
2. Listing pharmaceuticals for which parameters such as chemical name, structure, formula, log octanol/water partition coefficient (Log Kow), log bioconcentration factor were available or predictable using modelling software (i.e. (EPI) suite, EPA (2011)). Overall, 3193 distinct drugs constituted the final database.
3. Identifying pharmaceuticals that had been detected in the environment.
4. Analysing the potential for the 3193 pharmaceuticals of being persistent (i.e. log $K_{ow} \geq 3$), and/or to bioaccumulate in the environment.
5. Screening of compounds to identify those produced in large amounts but not yet detected in the environment, and which have both the potential to bioaccumulate and being persistent in the environment. These are considered by the authors as potential "emerging contaminants", hence priority compounds.

In their database, the authors identified a total of 399 pharmaceuticals produced in high volumes. Among these and based on the review of 31 references available in the literature, 102 pharmaceuticals had been detected in environmental media (including ground and surface water, raw and treated wastewater, swine manure). For the remaining substances (297), the method allowed prioritising 58 drugs as having both the potential to bioaccumulate and to persist in the environment and 48 as being potentially persistent. These included for example the antiarrhythmic amiodarone which returned a log K_{ow} value of 8, suggesting a high bioaccumulative potential. For the authors, these drugs were the one to prioritise as they had not been investigated in the environment despite being produced in large amounts and being potentially persistent and bioaccumulative. The method used by the authors also showed that among the remaining substances that had neither been detected in the environment nor identified as highly produced substances, 364 might both be persistent and bioaccumulative. These could also be defined as emerging contaminants and would deserve further investigations.

- *Toxicological relevance of pharmaceuticals in drinking water, (Bruce et al., 2010 - USA).*

This study looked at potential risks on human health associated with the presence of pharmaceuticals screened using a method consisting of developing health risk levels based on toxicological data (i.e. animal toxicity data, adverse effects at therapeutic doses). Health risks screening levels obtained for prioritised substances were then compared to concentrations detected in either source or finished water across 19 drinking water treatment plants in the United States and distribution water at 15 sites. The main objective of this study was to establish a target list of pharmaceuticals likely to be measured at levels that may present a potential risk to human health.

The screenings steps included:

1. A selection of a set of compounds based on likelihood of presence and potential toxicity, interest among the public and the water utilities which were all among the top 300 most prescribed drugs in the US.
2. A selection of compounds that met at least one of the toxicity criteria at low or chronic exposure reported in the medical literature (potential risk for the foetus; carcinogenicity for animals or humans; reproductive or developmental toxicity on animal or human). Occurrence in wastewater and surface water were also considered.
3. The determination of screening levels based on health risks. For non-carcinogenic substances: thresholds values were determined based on No Observed Adverse Effect (NOAEL) and lowest observed adverse effect (LOAEL) values readily available. Thresholds values were then refined by using uncertainty factors (UF) to take into account effect on sensitive population and quality of the data set used. For compounds having carcinogenic effect on animals, estimates of the probability to develop cancer at a given dose were calculated.
4. The calculation of Drinking Water Equivalent Levels (DWEL). By definition, the DWEL corresponds to an "*estimated lifetime exposure level at which adverse health effects are not anticipated to occur, assuming 100% exposure"* (Pankratz, 2000).

5. The determination of margins of exposure (MOE) as the ratios of the DWEL by the maximum concentrations measured in drinking water from 19 drinking water treatment plants across the US.

A list of 15 APIs and 4 metabolites was obtained using the screening criteria including rate of use in the US, likelihood of exposure in drinking water sources, potential toxicity at low and chronic exposure concentrations. Among the 19 substances targeted by Bruce *et al.* (2010), ten substances were quantified in drinking water in concentrations ranging from 0.00033 µg L^{-1} for diazepam up to 0.042 µg L^{-1} for meprobamate. These concentrations were considerably lower than the DWEL values, leading to margin of exposure (MOE) above 3800. These results suggested that these pharmaceuticals and metabolites were unlikely to cause adverse health effects.

The applied methodology only assumed exposure to pharmaceuticals via drinking water while other routes of exposure may need consideration (i.e. food consumption). However, a wide variety of therapeutic classes and number of compounds were used as a starting point. The use of parameters such as likelihood of presence in water or occurrence data from the literature may be a limit to the method as some compounds that have not been evaluated to date may be more important than detected ones. Therefore, further investigations for a broader range of compounds would be interesting to fully assess this methodology.

- *Toxicological relevance of EDCs and Pharmaceuticals in Drinking water, (Snyder et al., 2008 - USA)*

Due to the vast number of pharmaceuticals available in the US (more than 3000), ways of prioritising compounds of potential concern is a necessity. In this study, the authors investigated the occurrence and risk of exposure to pharmaceuticals and EDCs in drinking water in the US. Potential effects on human health were then evaluated for a selection of compounds. The objective of this study was to identify substances that may require future monitoring and regulation to ensure human health protection. The prioritisation approach used to select pharmaceuticals for evaluation was based on six criteria:

1. Selection of prescription medicines as opposed to OTC drugs which tends to exhibit lower toxicity.
2. Analysis of the mass consumption of this prescription medicines and selection of the top 300 most prescribed (i.e. accounting for 2.2 million prescriptions or more per year).
3. Review of toxicity data for the 300 pharmaceuticals. Compounds presenting the highest potential to cause adverse effect at low dose, or chronic exposure levels were screened (i.e. carcinogenicity, mutagenicity, immunotoxicity).
4. Review of the literature regarding the occurrence and frequency of detection in drinking water sources, although this criterion was not crucial in deciding whether a compound should be excluded or not due to lack of analytical data available.
5. Selection of pharmaceuticals representative of specific pharmaceutical groups such as anticonvulsants, antipsychotic etc. that could be analysed for validation.
6. Exclusion of compounds for which calibrations standards were not available to ensure reliability of analytical measurements.

Based on these six criteria, 16 APIs and four metabolites were selected for evaluation. These included antilipidemic agents such as atorvastatin and gemfibrozil, anticonvulsants including carbamazepine, anti-inflammatory compounds such as diclofenac, the antipsychotic risperidone or antibiotics such as sulphatomethoxazole and trimethoprim.

The occurrence of these 20 pharmaceuticals from source to finished drinking water (after disinfection step) was investigated at 20 drinking water sites. Out of the 20 selected compounds, only phenytoin and meprobramate were consistently detected (frequency of detection > 50%) in drinking waters across the sites investigated. Maximum observed concentrations for these APIs were 32 and 43 ng L^{-1} respectively. Nine other substances were also detected in drinking water in maximum concentrations

ranging from 1 ng L^{-1} (conservative value adopted if the maximum concentration detected was below 1) for diazepam, fluoxetine and norfluoxetine up to 18 ng L^{-1} for carbamazepine.

Risk to human health associated with the presence of these compounds at these maximum detected levels were then evaluated by comparison with DWEL as defined above. According to the results, the volume of drinking water that could be consumed per day without exceeding a dose protective for human health would range from 330 L for risperidone to 5,200 kL per day for triclosan. These results suggest that the presence of pharmaceuticals compound in US drinking water is unlikely to cause concern for human health.

- *Human health risk assessment from the presence of human pharmaceuticals in the aquatic environment, (Cunningham et al., 2009 - USA).*

Cunningham and co-workers evaluated human health risks of 44 APIs produced by a pharmaceutical company taking into account exposure through drinking water and fish consumption. Although the primary aim of this study was not to develop a prioritisation tool, the methodology used in this evaluation of health risks associated with the presence of trace levels of APIs in European and American surface water could be used for prioritisation of pharmaceuticals originating from specific locations such as industries. The authors targeted specific substances based on:

1. Selection of major APIs produced by the industry investigated.
2. Collection of chemical data, pharmacokinetics, toxicity and pharmacology of the substances.
3. Determination of acceptable daily intakes and predicted no effect concentration for human health ($PNEC_{HH}$) (equation 10) taking into account two exposure routes: ingestion via drinking water and through fish consumption:

$$PNEC = \frac{1000 \times ADI \times BW \times AT}{(Ing_{DW} + BCF \times Ing_{RF}) \times EF \times ED} \quad (10)$$

   Where:
   AT: is the averaging time; $IngR_{DW}$ and $IngR_F$ are respectively the drinking water ingestion rate (L person^{-1} d^{-1}) and fish consumption rate (kg person d^{-1}); BCF is the bioconcentration factor for fish (L kg^{-1}); BW is the body weight (kg person^{-1}); EF is the exposure frequency (d y^{-1}); ED is the exposure duration (y); ADI is the acceptable daily intake for an adult (µg kg^{-1} d^{-1}); and 1,000 is the conversion factor from ng to µg.
4. Determination of predicted environmental concentrations (PEC) based on either measured environmental concentrations published in the literature or predicted in surface water using exposure models developed in the US and Europe. The exposure model takes into account parameters such as metabolism, usage and removal by STP.
5. Evaluation of risks to human health by determining $PEC/PNEC_{HH}$ ratios. A ratio below 1 suggest no risk to human health from the consumption of drinking water and fish containing trace levels of the compounds investigated.

Among the 44 APIs investigated, nine substances were measured in the environment in either STP influent, STP effluent, surface water, groundwater or drinking water including albuterol (salbutamol), ranitidine, amoxicillin, hydrochlorothiazide, trimethoprim, cimetidine, digoxigenin, digoxin and metformin. For all other substances, PEC values were predicted based on annual estimates of mass of APIs sold by the pharmaceutical company across the US, UK, Germany, France and Italy. The results showed that none of the compounds investigated presented a risk for human health at levels of exposure measured or predicted with PEC/PNEC risk ratios all below 1. The highest ratio was obtained for amoxicillin with 0.067. Although limited to major products produced and marketed by a pharmaceutical company, such a method could be transferrable to wider lists of substances provided that data on consumption are available and of sufficient quality to predict environmental concentrations. One of the interesting aspects included here is the consideration of human exposure not only through drinking water but also consumption of fish.

- *Risk to aquatic organisms posed by human pharmaceutical use, (Kostich and Lazorchak, 2008 - USA) and Predicting variability of aquatic concentrations of human pharmaceuticals, (Kostich et al., 2010 - USA).*

Kostich and Lazorchak's (2008) work looked at the environmental risks due to human pharmaceuticals residues in wastewater but also potential health risks resulting from human exposure to pharmaceuticals in the environment. The ultimate aim of this study was to develop a method to prioritise compounds based on potential risks of exposure to human pharmaceuticals. For this purpose the potential risks of pharmaceuticals used in human medicine were evaluated based on marketing data and corresponding pharmacological data available in the US.

The criteria used for prioritisation of pharmaceuticals were:

- Annual consumptions calculated according to prescription numbers and/or sales data (in dollars);
- Predicted environmental concentrations (PEC); and
- Therapeutic doses.

The prioritisation method included the following steps:

1. Determination and ranking of the mass amounts of 371 APIs used in the US. Mass amounts were estimated using annual sales data and/or annual prescription rates. For a given generic:
   - A mass amount estimated from sales data was determined by dividing the annual sales values by the lowest available price per unit for that generic.
   - A mass amount estimated from prescriptions data was determined using prescription numbers, maximum daily doses and length of therapy treatment for that generic.
2. Prediction of concentrations reaching the environment (PEC) of the 371 APIs in wastewater by not taking into account metabolic inactivation as per equation 11:

$$PEC = \frac{\text{Anual minimum daily dose equivalent}_{API}}{\text{Annual wastewater volume}} \quad (11)$$

3. Selection of the top 50 APIs from step one and PEC refinement by taking into account metabolic inactivation (fraction inactivated - Fi) and disposal rates (fraction wasted Fw) as follows from equations 12 and 13:

$$\text{Activity}_{API} = \left[\text{Mass}_{API\ dispensed} \times \left(1-F_i\right) \times \left(1-F_w\right)\right] + \left(\text{Mass}_{API\ dispensed} \times F_w\right) \quad (12)$$

$$PEC = \frac{\text{Annual Activity}_{API}}{\text{Annual wastewater volume}} \quad (13)$$

4. Estimation of hazard quotients for microbial exposure for the top 50 compounds as a ratio of wastewater PEC and available minimum inhibitory concentrations (MIC).
5. Assessment of the effect of mixtures of APIs on human health based on modes of action (MOA) and by estimating exposure risks to API groups sorted out by MOA. The exposure is expressed as numbers of days of water consumption required to ingest the equivalent of a single minimum daily therapeutic dose.

This approach is relatively complex as it takes into account potential effects of human pharmaceutical residues in wastewater on humans and non-target organisms. It requires the cross analyses of multiple data bases. The approach is conservative in the sense that rates likely to provoke significant effects on human health are assumed to be similar to minimum therapeutic dose rates. In addition, the authors highlight the fact that risks of human exposure were likely to be overestimated as they were calculated based on the consumption of 2 L d^{-1} of water containing an API concentration equivalent to the concentration predicted for that same API in raw wastewater.

Results of this approach suggested that risk of human exposure to the single APIs investigated were low with exposure rates more than 100 times lower than the minimum therapeutic dose. However for non-human exposure, risk quotients results showed that 11 substances including for example estradiol, atorvastatin and promethazine returned a risk quotient above 1. Such ratios suggest that effects on microorganisms are a possibility.

One of the issues related to the use of marketing data as opposed to consumption data is that sales data (here in dollars) need to be converted to amount of pharmaceuticals prescribed/consumed. Such estimations are highly dependent upon the quality of marketing data provided but also on selling prices of pharmaceuticals. These prices may not only vary from one region to another, but also depend on packaging size. Indeed the price per gram of one substance will vary with quantity sold per package. This then requires averaging pricing to get an estimation of mass consumption and therefore may not be as accurate as readily available mass consumption data. Furthermore the concentrations predicted using marketing data at a national level were compared with measured environmental concentrations (MEC) at a regional scale. The study is limited to the top 50 prescribed APIs out of the 371 initially selected. However, the authors concluded that such an approach based on the assessment of risks of exposure to human pharmaceuticals helped reducing the number of compounds that may require specific attention.

This study is the prequel to a more recent study published by Kostich *et al.* (2010) where PEC values were determined using regulatory data instead of marketing data. The regulatory data were sourced in reports published by the Drug Enforcement Administration (DEA) on 12 classified substances that need to be legally regulated and for which mass amounts are available.

In this study, a national PEC was calculated by dividing the mass of these 12 regulated API (kg y^{-1}) available in the DEA reports by an estimate of the annual production of wastewater in the US. A local PEC was obtained by dividing the local usage rate by API by the rate of wastewater production per capita. These PECs were then converted to exposure as dose per decade values. These correspond to the minimum daily dose of an API that would be consumed over ten years if drinking 2 L of water per day that contained this API in a concentration equivalent to the calculated PEC. The results obtained were then compared to MEC values available in the literature for wastewater, surface water and groundwater. The exposure to multiple APIs with similar mode of action has also been taken into consideration in this study.

Exposures to most APIs investigated in both studies were below levels required for clinical effect. This new prioritisation methodology based on regulatory data highlighted the limitation of marketing databases as overall PEC values derived from sales/ prescription numbers were typically higher than the regulatory derived PEC. However, this also highlighted the conservative characteristics of prediction obtained from sales. Comparison of PECs derived from marketing data at a local scale with MECs available in the literature showed that an adjustment of these PECs by a factor 10 would results in more realistic predicted concentrations.

- *Pharmaceuticals, personal care products and endocrine disrupting chemicals in the US surface and finished drinking waters: a proposed ranking system, (Kumar and Xagoraraki, 2010 - USA).*

The aim of this study was to generate a ranking system to prioritise the monitoring of pharmaceuticals and personal care product in surface and finished drinking water. The ranking system was based on priority scores obtained when using the prioritisation methodology which includes the following set of criteria:

- Occurrence in water;
- Overall removal via drinking water treatment (not considered when ranking is done for stream/ source water);
- Ecological effects (potential to bio-accumulate / ecotoxicity- not considered for ranking in drinking water); and
- Health effects on mammalians.

This prioritisation method used five main steps:

1. The selection of one of the above criteria.
2. Development of attributes per criterion, for instance prevalence, frequency of detection and magnitude for the occurrence criteria, removal via drinking water treatment for the treatment criterion, propensity to bioaccumulate and ecotoxicity for the ecotoxicological effect criterion and finally effect category (carcinogenicity, endocrine effects etc.) and pregnancy category for the health effect criterion.
3. Collection of data based on each criterion. The occurrence data were collected from studies published in the US between 2000 and 2009. The treatment data were obtained from studies published until 2009. Ecological data and health effects data were collected from published studies and available databases.
4. Ranking by criterion.
5. Calculation of an overall score.

The methodology used to rank 100 compounds not only focuses on pharmaceuticals (57) but also on personal care products (43) detected in the US in two different types of water: stream water and drinking water. The higher the ranking of a substance is, the higher the priority. The rank is determined using a complex weighting procedure by attributing numerical values (so-called utility function in the study) to either qualitative or quantitative data from the literature, weighting their importance and combining them for each single compound. The top 20 substances ranked in stream/source water included six pharmaceuticals - mestranol, estrone, bezafibate, atorvastatin, 17β-estradiol and gemfibrozil - while in the finished water, the number of pharmaceuticals present in the top 20 increased to 16. The method offers a unique opportunity to prioritise substances for future monitoring. It provides information on risk associated with the presence of the ranked compounds although this information is only qualitative. Finally, this method is strongly dependent upon availability of data published in the literature which can be site specific.

- *Prioritising research for trace pollutants and emerging contaminants in the freshwater environment, (Murray et al., 2010 - USA).*

The aim of this study was to identify research needs on trace pollutants and emerging contaminants using a prioritisation approach based on occurrence and toxicity data of three different types of micropollutants, including pharmaceuticals, available in the scientific literature. The data compiled by Murray and co-workers (2010) focused on studies performed in the USA, Europe and Asia on the detection of micropollutants in surface water, groundwater or drinking water.

The criteria used in this study for prioritisation of pharmaceuticals compound are:

- Occurrence of pharmaceuticals in the freshwater environment;
- Frequency of detection; and
- Health risks associated with these compounds.

The prioritisation method used by Murray *et al.* (2010) included three main steps:

1. Compilation of frequencies of detection of compounds along with maximum, minimum and median concentrations (μg L^{-1}) in surface water, drinking water and groundwater.
2. Compilation of toxicity data of the compounds based on acceptable daily intakes (ADI) published in the USEPA (2010) and other literature references.
3. When both concentrations in freshwater and ADI values were available, consumption rates posing health risks (CRPHR) for a 70 kg individual were determined according to equation 14:

$$CRPHR_{L\,d^{-1}} = \frac{\left(ADI_{mg\,kg^{-1}d^{-1}} \times 70_{kg}\right)}{(Concentration \times 0.001)} \quad (14)$$

A CRPHR below 2 L d^{-1} for a specific compound means this compound is considered to be of high priority. A CRPHR above 200 L d^{-1} is considered of low priority that is to say unlikely to cause concerns for human health at concentrations measured in freshwater.

In this study, among the 21 pharmaceuticals and hormones for which an ADI value was available, carbamazepine, diclofenac and clofibric acid were found to be the most frequently detected compounds in fresh water environment with respectively 95, 83, and 81% of frequencies of detection across the studies reviewed. Out of these 21 compounds detected in the freshwater environment, 17α-ethinyl estradiol, carbamazepine, 17β-estradiol, triclosan, acetaminophen and estrone were found as potentially representing a risk to human health at the concentration detected in the environment. One of the issues with this method is that compounds either not frequently detected in the environment or with no ADI values (or both) were not considered. This limits the impact of such a prioritisation methodology. Indeed, the measurement of pharmaceuticals in the environment is restricted to analytical feasibility, therefore selecting measureable compounds as a prioritisation starting point excludes a large number of substances that may present higher risks than the ones analysed for.

- *Preliminary risk assessment database and risk ranking of pharmaceuticals in the environment, (Cooper et al., 2008 – USA).*

In this study, Cooper *et al.* (2008) developed a risk ranking system for pharmaceuticals in the aquatic environment (mainly marine and estuary), using a database compiling physico-chemical (K_{ow}, measured environmental concentrations, solubility etc.) and toxicological information (toxicity, environmental half-life, persistence etc.) on the 200 most prescribed pharmaceuticals in the US as a starting point.

The ranking of the pharmaceuticals was established based on potential environmental exposure and risk by categorising data using:

- Annual prescriptions;
- Surface water concentrations;
- Environmental half-life;
- Biological half-life;
- Mammal, crustacean and fish toxicity;
- Kow;
- Solubility;
- Toxicity data estimated using Quantitative Structure Activity Relationship (QSAR)/ Ecological Structure Activity Relationships (ECOSAR)

Five different types of ranking were performed:

1. A ranking considering all the data mentioned above;
2. A ranking only based on toxicity data estimated using QSAR/ ECOSAR;
3. A ranking taking into account all data except the one generated with QSAR/ ECOSAR;
4. A ranking including compounds for which data in most categories were available;
5. A ranking based on data categories likely to be the most characteristic of risk for the aquatic environment.

All rankings were then compared and results analysed by drug class. The class found to present the most important risk for the aquatic environment in the top 100 of the rankings was the Central Nervous System (CNS) class. This category accounted for 71 pharmaceuticals out of the 313 evaluated such as analgesic drugs (paracetamol, naproxen) or antipsychotic (diazepam). When only considering the 5th type of ranking based on risks to the aquatic environment, the top 10 priority pharmaceuticals were erythromycin base, oxytetracycline, sulfamethoxazole, fluoxetine hydrochloride, nitroglycerin, clofibrate, ibuprofen, acetominophen, estradiol and diclofenac sodium. The authors concluded that anti-infective drugs were the class of drug that may present the highest environmental risk considering factors such as environmental transport, fate and aquatic toxicity. The database developed in this study provides a preliminary risk assessment for commonly prescribed pharmaceuticals. The fact that this database is used to rank these pharmaceuticals in five different ways depending on the type physico-chemical and ecotoxicological data considered offers different possibilities of addressing risk depending on the user's need. Overall, as for all environmental risk based methodologies, the assessment of the impact of pharmaceuticals on the aquatic environment is biased by factors such as availability of toxicity data, consideration of acute ecotoxicity rather than

chronic ecotoxicity, and more generally missing information regarding the physicochemical properties of a large number of pharmaceuticals. This can result in large uncertainties on predictions.

- *Ranking and prioritisation of environmental risks of pharmaceuticals in surface waters, (Sanderson et al., 2004 - Canada)*

The methodology developed in this study is focusing on effect assessment rather than exposure assessment. The main objective was to develop a tool to predict acute environmental toxicity, therefore prioritising environmental risks of pharmaceuticals in surface waters. The multiple step process involved:

1. The use of a conservative application of QSAR, where the lowest ecotoxicological values were predicted with ECOSAR (ECOSAR is typically used to predict of acute aquatic toxicity of chemicals). Through this step, the authors prioritised environmental risks related to pharmaceuticals in surface water by ranking predicted hazard quotients (HQ) according to equation 15:

$$HQ = \frac{PEC}{PNEC} \quad (15)$$

   Where: PEC default value is 1 µg L^{-1}; and PNEC is the ratio of the median effective concentration (EC_{50}) to a safety factor of 1,000.

2. A relative ranking of pharmaceutical classes that can be used for prioritisation of environmental risks. For this purpose, authors predicted health quotients for each pharmaceutical class (HQ_{class}). This HQ_{class} is the product of the average HQ obtained for a specific class by the number of HQ values above one in that class.

Through these first 2 steps, 2986 pharmaceuticals among the 4500 extracted from the Martindale (2002) returned QSAR values that could be classified under 51 distinct pharmaceutical classes for further risk prioritisation taking into account the following parameters:

1. Ecotoxicity (algae, daphnia, fish);
2. Potential for bioaccumulation (Log Kow); and
3. Predicted removal of pharmaceuticals in sewage treatment plants.

When taking into account predicted hazard, potential to bioaccumulate and frequency of occurrence per class, results showed that among the 51 drug classes ranked in the initial phase, the gastrointestinal drug class (including 54 compounds) and the cardiovascular drug class (including 271 compounds) were the most hazardous therapeutic classes to the environment. When combining predicted ecotoxicity (algae, daphnia, fish), log Kow and treatability by sewage treatment plants and number of compounds per class, the cardiovascular, anxiolytic, antipsychotic, gastrointestinal, antiviral and corticosteroids drugs represented the most hazardous classes with average HQ per class in the range 3 to 6. However, when considering predicted aquatic toxicity, frequency and potential to bioaccumulate, Sanderson *et al.* (2004) identified the cardiovascular class as representing the highest risk for the aquatic environment.

This complex modelling tool offers an opportunity to rank pharmaceuticals that may have an impact on the environment. However it presents some limitations mainly due to the fact that the environmental effect assessment from which the ranking is obtained is based on short-term toxicity prediction rather than long-term toxicity. Furthermore, results are organised by class of compounds, so the method does not provide information on specific individual substances.

4.6.1.3. Europe

- *Desk based review of current knowledge on pharmaceuticals in drinking water and estimation of potential levels, (Watts et al., 2007 - UK).*

In this report, the authors reviewed information available on pharmaceuticals in raw and treated wastewater to estimate which pharmaceuticals could be found in the UK's surface water - and potentially drinking water. Maximum levels in drinking water for these prioritised compounds were then estimated using a modelling approach based on four drinking water treatment scenarios. The prioritisation procedure was carried out as follows:

1. In a first stage, the authors examined lists of pharmaceuticals that were consumed in the UK in 2004. They removed substances considered to be of minimum risk for the environment and/ or humans. They also omitted substances that were likely to be present or enter the environment in levels much higher than those that pharmaceutical consumption would be responsible for (e.g. plant products and extracts; animal products and extracts (cod liver oil, lanolin); inorganics, vaccines, diet preparations, gaseous substances).
2. The 394 substances screened in the first step were then ranked according to sales amount.
3. They then calculated a margin of exposure (MOE) for these 396 substances by dividing their minimum therapeutic doses (MTD) with a maximum daily intake from drinking water. When not available, MTD were assumed to be 10 mg for topical substances, and 1 mg for others.

   Maximum daily intakes were determined by predicting concentrations in drinking water using two modelling approaches.

   The first approach, which corresponded to a worst case scenario or conservative approach for which no metabolism of the compounds was assumed, no removal was achieved by STP unless data were available in the literature, dilution in river was ignored and no removal through drinking water treatment, unless data were available.

   In the second approach, five scenarios were considered from classic drinking water treatment trains to advanced ones receiving source water from catchments with low to high sewage input. Depending on the drinking water treatment scenario evaluated parameters such as usage, population, wastewater production, metabolism and treatability by sewage treatment plant were taken into account.

4. In either scenarios, the environmental concentrations in drinking water (PEC_{DW}) were determined using an adapted version of the EMA methodology for risks assessment of pharmaceuticals in the environment according to equation 16:

$$PEC_{DW} = \frac{A \times (100-R) \times (100-M) \times (100-W)}{365 \times P \times V \times D \times 100 \times 100 \times 100} \quad (16)$$

Where: PEC_{DW} is the predicted concentration in drinking water (mg L^{-1});
A.is the amount of active ingredient used per year in the catchment (mg y^{-1});
M is the percentage metabolised in humans;
R is the removal rate in sewage treatment (as a percentage);
P is the population under consideration (i.e. 59,600,000 for the UK or the population equivalent for each catchment scenario);
V is the volume of wastewater produced per capita per day (assumed to be 200 L);
W is the removal rate in the appropriate drinking water treatment scenario;
D is the dilution factor in the environment (derived as the 5% flow rate).

Note that the second approach was only performed for substances for which a MOE below 1,000 was obtained using the conservative approach, that is to say 24 substances.

Out of the 396 substances evaluated using the conservative approach, only ten returned MOEs below 1,000 including four illegal drugs (cannabis, cocaine, ecstasy and LSD) and 6 pharmaceuticals or combinations of pharmaceuticals (a combination of 19 non-steroidal anti-inflammatory drugs, oseltamivir (Tamiflu), aminophylline, beclometasone, zidovudine and acamprosate). When using the refined approach considering process treatment, as expected, the predicted concentrations decreased as opposed to the ones obtained using the worst-case scenario modelling approach where parameters such as metabolism and removal ability of STPs were not taken into account. This resulted in increases of MOEs. Therefore, out of the 24 substances targeted in the conservative approach, only 2 substances had MOE below 1,000. These were the illicit drug cannabis and oseltamivir suggesting that

levels at which pharmaceuticals may be found in drinking water are very unlikely to cause health concerns to human (if found).

This prioritisation exercise shows that the conservative approach used is a simple way to screen large numbers of pharmaceuticals without requiring a large amount of data on the substances. This method relies mainly on the accuracy of consumption or mass amount data of substances to predict environmental concentrations and margin of exposure for a rapid human health risk assessment. In the second approach, by taking into account additional parameters, predicted concentrations are refined by considering excretion rates, dilution in receiving water, and removal through sewage treatment and drinking water treatment available in the literature. However, such data are based on values available in the literature which may not always be representative of the context where the evaluation is carried out (i.e. country, treatment characteristics, and source water). Furthermore, data are not always available and in that case authors had to use conservative values which meant that the results were similar to those obtained with the refined approach.

- *Exposure assessment of pharmaceuticals and their metabolites in the aquatic environment; application to the French situation and preliminary prioritisation, (Besse et al. 2008 - France).*

Besse *et al.*, (2008) developed a method to identify human pharmaceuticals that should be monitored in surface waters in France. This prioritisation method relies on the consumption of human pharmaceuticals in France to predict environmental concentrations in surface water (PEC). The method also incorporates the notion of risk quotients to rank compounds of potential concern for the aquatic environment. PEC values were derived from the method proposed by the EMA (EMA, 2006) to establish guidelines for pharmaceuticals in surface water.

The parameters used in this study for prioritisation of pharmaceuticals compound are:

- Consumption/ sales data;
- Occurrence of compounds in surface water (published literature);
- Metabolism and excretion data; and
- Environmental risks associated with these compounds.

The screening of pharmaceuticals was carried out through four main steps:

1. Compilation of consumption/ sales data for human pharmaceutical in France and selection of the top 100 excluding steroids and cytotoxic drugs. Indeed, the authors believe that additional prioritisation schemes dedicated to these types of substances should be developed given their specific toxicity. The data base used included sales data for prescribed and over-the counter pharmaceuticals for both hospitals and pharmacies. 12 compounds previously monitored in surface water were also added to the list of compounds.
2. Prediction of concentrations in surface water (PEC) according to equation 17:

$$PEC = \frac{Consumption \times F_{Excreta} \times F_{STP}}{V_{WW} \times n_{inhab} \times dilution \times 365} \quad (17)$$

Where: V_{ww} is the volume of wastewater generated per person per day (200L d^{-1});

Consumption is the yearly consumption of an active ingredient by the population in a defined zone (mg d^{-1});

F_{exceta} is the excretion fraction of the active ingredient;

F_{STP} is the fraction of emission of the drug from WWTP to surface water;

n_{inhab}: number of inhabitant;

dilution is the dilution factor from WWTP to surface water (10 as default).

Three types of PECs have been predicted: PEC_A not taking into account metabolism and removal by STP, PEC_B taking into account metabolisms but not removal by STPs and PEC_C taking into account both metabolism and removal by STP.

3. Calculation of excretion ratios (sum of excreted proportion of the unchanged active ingredient and proportion of the parent compound as gluconide conjugate). This step involved the review of metabolism pathways of the compounds investigated.
4. Calculation of risk quotients (RQ) to assess environmental risks (RQ=PEC_C/PNEC, if PEC_C was not available, PEC_B and PEC_A were used)

The method used by Besse *et al* (2008) is based on consumption data and data available in the scientific literature regarding metabolisms pathways, removal by STP and ecotoxicity. The calculation of PECs in this study is limited by a number of uncertainties such as the non-consideration of compounds consumed in veterinary medicine, the quality of the consumption data, the fraction of pharmaceuticals removed by STP (which can vary significantly from one site to another), the volume of wastewater produced per capita arbitrarily chosen to determine PECs, and sorption of pharmaceuticals on sediments and biodegradation of some compounds in surface water. However, the PECs correlate well with levels measured in the environment, therefore offering a good opportunity to assess risk of exposure to pharmaceuticals in surface water. Out of the 112 substances investigated, 15 were found with PEC values> 1 µg L^{-1} including paracetamol (75 µg L^{-1}), ibuprofen (5.5 µg L^{-1}), dextropropoxyphene (1.2 µg L^{-1}), amoxicillin (7.6 µg L^{-1}) and aspirin (5.5 µg L^{-1}). However the prioritisation of compounds based on environmental risks (using RQ) was not possible due to limited ecotoxicological data. Only amoxicillin was found with a RQ> 1.

- *Human pharmaceuticals in surface waters: Implementation of a prioritisation methodology and application to the French situation, (Besse and Garric 2008 - France)*

The method investigated in this study relies on the same principle used in the preliminary study by Besse and co-workers in 2008 but includes a ranking of compounds based on their potential environmental risk to the aquatic environment. The ultimate aim of the study was to prioritise pharmaceuticals consumed in France to generate a list of compounds that would require monitoring in French surface waters. The method follows the following steps.

1. Screening and ranking based on exposure assessment:
   - Selection of the top 100 compounds the most consumed along with a set of compounds detected in the aquatic environment. The total number of compounds evaluated was 120 plus 30 metabolites (estrogen and cytotoxic compound were excluded from the compounds evaluated).
   - Calculation of PEC in the aquatic environment using consumption data. Classification of compounds according to their PEC values. Two PEC values are predicted: a conservative one - PEC_A - assuming no metabolism and no removal by STP and PEC_B taking in to account the amount of a compounds excreted unchanged as per equations 18 and 19:

$$PEC_A = \frac{Consumption}{V_{WW} \times n_{inhab} \times dilution \times 365} \quad (18)$$

$$PEC_B = \frac{Consumption \times F_{Excreta}}{V_{WW} \times n_{inhab} \times dilution \times 365} \quad (19)$$

   - Comparison of PECs to threshold values set by the FDA (1998) at 100 ng L^{-1} and by the EMEA (2006) at 10 ng L^{-1}. Establishment of risk classes ranging from “very low risk” for the aquatic environment to “highest risk compounds”.
2. Additional screening and ranking based on effect:
   - Further prioritisation based on ecotoxicological, pharmacological (mechanisms of action, side effects in human), toxicological data (carcinogenicity in rodent), and physiochemical (Log Kow) data was performed:
     - Ecotoxicology: due to the lack of chronic toxicity data that makes the estimation of PNEC values complicated, the authors used no observed effect concentrations (NOEC) and compared them to threshold values derived from persistence,

bioaccumulation and toxicity criterion. Any NOEC values below 10 µg L^{-1} is classified as toxic hence prioritised.

- Pharmacology: due to scarcity of ecotoxicological data, parameters such as mechanism of action, side effects in humans, enzymatic induction or inhibition and modulation in the expression of glycoprotein P (protein playing a significant role in transporting toxins and xenobiotics out of a cell) were used as additional effect criteria.
- Toxicology: available acute toxicity data, carcinogenicity in rodent were also reviewed although not used for prioritisation.
- Physico-chemistry: the potential for a substance to bioaccumulate (Log Kow) was taken into consideration.

3. Final ranking:
   - For prioritised compounds belonging to the same pharmacological and chemical class (i.e. with similar structures and mechanisms of action), further screening was performed based on NOEC, LOEC and potency (as defined daily dose).

Using this prioritisation method, Besse and Garric classified 40 pharmaceuticals as priority compounds to monitor in French surface water. Seven of them were selected as based only on their PEC_B values which were > 100 ng L^{-1}. These included for instance allopurinol (PEC_B = 150 ng L^{-1}) and atenolol (PEC_B = 419 ng L^{-1}). 33 others were prioritised based on a combination of exposure and effect data such as ciprofloxacin which was selected based on his PEC_B value of (PEC_B = 159 ng L^{-1}), its therapeutic class and high ecotoxicity. The interesting part of this approach is that the authors combined the use of available ecotoxicological data (here as NOEC) with pharmacological data to evaluate potential environmental effects. This allowed, for example, the prioritisation of substances such as diclofenac based on its high Kow value and know side effect on kidneys, while it would not have been selected if using a risk ratio (PEC/PNEC) approach as the ERA one as it would have returned a value below 1. Such an approach recouping PEC with pharmacological data allows can compensate for the lack of ecotoxicological data and estimate/rank risks associated with pharmaceuticals.

- *Targeting aquatic microcontaminants for monitoring: exposure categorisation and application to the Swiss situation, (Götz et al., 2009 - Switzerland).*

Götz *et al.* (2009) developed a method for the prioritisation of micro-contaminants based on exposure. This method does not only focus on pharmaceuticals, but also takes into account a variety of chemical substances such as biocides, personal care products and industrial chemicals. The objective of this method is to provide information on compounds that may occur in surface water, select the most relevant ones for water protection and develop new monitoring strategies. The method is applied to the Swiss situation and includes the following steps:

1. The 250 substances investigated were selected based on priority substances listed in the European Water Framework Directive (EU, 2000), substances listed as relevant for the River Rhine and substances that had been detected in surface water in Switzerland. Data were sourced from either national monitoring campaigns or the literature.
2. The 250 chemical substances were then classified in 7 exposure categories according to their distribution behaviour between various environmental media (*i.e.* water, air, sediments and suspended solids), persistence/degradability and input dynamic in the environment (*i.e.* continuous from STPs or complex that is to say *"dependent upon seasonal variation or rain events")*. These categories ranged from: category (i) "*highly persistent chemicals with a complex input dynamic*" to categories (vi) "*rapidly degradable chemicals*" and (vii) "*unclassified chemicals*".
   a. To determine the distribution between media, the authors determined the water-phase fraction of each chemical at equilibrium using a combination of air-water, sediment-water and particle-water partition coefficients.
   b. To determine the persistence of a chemical, they used available data on biodegradability and hydrolysis. If such data were not available the chemical was considered as neither readily biodegradable nor hydrolysable, meaning that it would be moderately to highly persistent.

3. The methodology was then evaluated by comparing average measured water-phase concentrations with consumption data.

Out of the 250 chemicals investigated, 51 pharmaceuticals used in human medicine and seven used in veterinary medicine were categorised. The majority of the human pharmaceuticals (44) were classified in Category (iii), that is too say, as being moderately persistent with continuous input to the environment, while 7 of them figured in Category (i) "highly persistent, continuous input" which counted 11 chemicals. These included five antibiotics (azithromycin, ofloxacin, clarithromycin, erythromycin and roxitrhomycin), one antifungal (fluconazole) and one contrast media (diatrizoate). These pharmaceuticals which are continuously discharged to surface waters are, according to the authors, the compounds that should be part of monitoring programs. This method does not assess risks and as such can also be used as a method to prioritise substances for which full risks assessment should be performed.

- *Development of a common priority list of pharmaceuticals relevant to the water cycle, (De Voogt et al., 2009 - The Netherlands)*

De Voogt *et al.*'s (2009) approach is unique as it provides a priority list of pharmaceuticals established by reviewing prioritisation methods published in the literature and by evaluating the criteria used for prioritisation in each publication reviewed. The list generated identifies compounds that are likely to be present at various points of the water cycle and their potential impact on human health.

The screening step used to generate a ranking of pharmaceuticals consists of the following five steps:

1. Review of the literature and selection of 25 key studies on prioritisation of pharmaceuticals.
2. Identification of key criteria and distinct compounds listed as priority substances.
3. Evaluation of the list of criteria identified by an expert panel and selection of the most relevant ones.
4. Re-evaluation of priority compounds based on the criteria selected.
5. Scoring of the compounds.

In this study, de Voogt *et al.* (2009) selected seven key criteria used for the prioritisation of 153 compounds listed across 25 key publications on the prioritisation of pharmaceuticals. These criteria were: regulatory data (i.e. substances listed in any environmental directives), consumption/sales data, physicochemical properties, degradability and persistence in the environment, toxicity to humans, ecotoxicity and occurrence in surface waters, ground water, drinking water or wastewater. The re-evaluation of the 153 compounds based on these seven criteria allows the creation of three categories:

- High priority pharmaceuticals for the compounds which were mentioned in minimum five articles and were prioritised using at least four of the seven criteria.
- Priority pharmaceuticals for compounds which were mentioned in two to four articles and were prioritised using at least two of the seven criteria.
- Low priority pharmaceuticals for compounds which were mentioned in two articles and were prioritised using at least two of the seven criteria.

Based on this approach, the authors identified ten compounds categorised as high priority pharmaceuticals including carbamazepine, sulfamethoxazole, diclofenac, ibuprofen, naproxen, bezafibrate, atenolol, ciprofloxacin, erythromycin and gemfibrozil. Although presenting some interesting aspects that could help the harmonisation of criteria used for the prioritisation of pharmaceuticals in various water sources, the results of such an approach have to be taken with care. Indeed, this study focuses on published articles, namely on "pre-screened compounds". Furthermore the criteria used have been equally considered while some of them such as consumption data might be more relevant than others.

- *Predicted critical environmental concentrations for 500 pharmaceuticals, (Fick et al., 2010 - Sweden).*

Fick *et al.* (2010) developed a method to prioritise research on pharmaceuticals in the environment based on predicted critical environmental concentrations (CEC) that is to say concentrations in surface water expected to cause a pharmacological effect on fish. The method relies on the fact that the

potency of pharmaceuticals for human is well documented and can be used to assess pharmacological effects on others species. It is an adapted version of the "Fish Plasma Model" (FPM). Assuming that biological drug targets are conserved between humans and fish, the FPM model compares human therapeutic plasma concentrations (HTPC) of an API with estimation of fish steady state plasma concentrations (FssPC) for that same API as a concentration ratio (CR) (CR=HTCP/FssPC). In this study, it is assumed that HTCP=FssPC. The CEC of an API is therefore derived from potency (as HTCP) and bio-concentration factors between water and fish derived from the API's lipophilicity (Log P).

The prioritisation steps included:

1. A selection of the 500 pharmaceuticals based on mass of pharmaceuticals sold over a year in Sweden and availability of HTPC data.
2. Calculation of bio-concentration factors Log P.
3. Calculation of CEC according to equation 20:

$$CEC = \frac{HTCP}{CR \times P} \quad (20)$$

Where CR=1

4. Comparisons of predicted CECs with observed concentrations when available.

Overall, CECs were below 1 µg L^{-1} for 127 of the 500 substances investigated. The ten lowest CECs values (in ng L^{-1}) were obtained for: the vasoldilator iloprost (0.18), four hormones - ethinylestradiol (0.37), estradiol (0.40), etonogestrel (1.6) and medroxyprogesterone (2.1) -, three antihistamine agents - ioratadine (0.56), clemastine (0.74), azelastine (1.0) -, the analgesic buprenorphine (1.0) and the anti-inflammatory misoprostol (1.2). Overall this method allows prioritising pharmaceutical substances that may cause adverse pharmacological effects by comparing CEC predictions with measured environmental concentrations. In addition, the authors indicated that comparing CEC values based on predicted bioconcentrations factors with environmental concentrations can also provide information to readjust limit of detections of some APIs in analytical protocols. For example, the experimental results to which they compared their CEC values showed that 17β-estradiol and 17α-ethynloestradiol were not detected in surface water. However for these substances the limit of detection was 5 ng L^{-1} while the CEC values for these two substances were 0.4 ng L^{-1}. This suggested that for such substances, a low detection limit should be determined / applied.

- *Occurrence and fate of micropollutants in the Vidy bay of Lake Geneva, Switzerland. Part I: priority list for environmental risk assessment of pharmaceuticals. (Perazzolo et al., 2010-Switzerland).*

The objective of this study was to generate a priority list of pharmaceuticals that may require monitoring in Swiss surface waters. The method was derived from the procedure used by Besse and Garric (2008) and the EMA guidelines for environmental risk assessment of pharmaceuticals. The procedure included five main phases:

1. A preliminary screening of the 30 most consumed pharmaceuticals used in Switzerland plus 23 selected pharmaceuticals was performed based on their potential risk for the environment. For this phase, two criteria were used:
   - The first one was the mode of action of groups of substances
   - The second one was the potential of these compounds to bioaccumulate based on their log Kow. (If log Kow ≥ 3 the compounds were prioritised).
2. The second phase consisted of a primary exposure assessment according to equation 21:

$$PEC_A = \frac{Consumption / (n_{inhab} \times 365)}{V_{WW} \times dilution} \quad (21)$$

Where: PEC is in ng L^{-1}.

Consumption is the total consumption of the selected pharmaceutical in one year ng y^{-1};.
n_{inhab} is the number of Swiss inhabitants.
365 is the conversion factor between year and day.
V_{WW} is the amount of wastewater per inhabitant per day [L $inhab^{-1}$ d^{-1}].

Dilution is the standard dilution factor (10) as per the Technical Guidance Document published by the European Commission (EC TGD 2003).

The compounds for which PEC_A were <10 ng L^{-1} were considered unlikely to present a risk for the environment and then removed from the priority list.

3. The third step was used for the remaining compounds. This additional step consisted in a re-evaluation of PEC_A obtained for these compounds taking into account metabolism data according to equation 22:

$$PEC_B = PEC_A \times F_{Excreta} \quad (22)$$

If PEC_B values were <10 ng L^{-1}, compounds were considered as potentially not harmful. If not, PEC_B values were then compared to the PNEC values of these compounds.

4. The compounds with ratios $PEC_B/PNEC \geq 1$ were then further screened by re-evaluating PEC_B values taking into account removals by STPs using equation 23.

$$PEC_C = PEC_B \times (1-R) \quad (23)$$

Where: R is the fraction of pharmaceutical that is removed by STPs.

If ratios $PEC_c/PNEC$ were ≥ 1, compounds were considered as potential not harmful for the environment.

5. For ratios $PEC_c/PNEC \geq 1$, compounds were further evaluated using a final stage where PEC_C values were refined considering dilution of STP effluent in the Vidy bay only and using renewal rates of the water in the bay of 1 day and 90 days to check the impact of two mixing scenario (low and fast).

Using this method, 36 compounds were prioritised including compounds such as gabapentin, amoxicillin and fluoxetine that were never analysed before in that area. The authors have adapted the EMA method for environmental risk assessment by adding additional screenings steps. This allowed reducing the number of compounds requiring a full evaluation, therefore limiting the amount of data needed to evaluate environmental risks. This method is also strongly dependent upon the availability and quality of pharmaceutical consumption data. For instance, in this study the author only had access to sales data for less than 3% of the compounds used in Switzerland. Another constraint associated with this type of method is the lack of ecotoxicological data, the use of national consumption data for environmental risk assessment at a regional level. The authors also highlighted the fact that for a vast number of compounds prioritised using their method, analytical methods were not readily available. Perazzolo *et al.*, 2010 concluded that the use of prioritisation methodologies for future monitoring would need to "*balance theoretical risks predicted and practical feasibility*".

- *A new risk assessment approach for the prioritisation of 500 classical and emerging organic micro-contaminants as potential river basin specific pollutants under the European Water Framework Directive, (von der Ohe et al., 2011 - European project).*

This study does not specifically focuses on pharmaceutical substances, but is investigating a new approach for the prioritisation of 500 organic chemicals (including pharmaceuticals) in four European river basins. These 500 substances were classified under six action categories determined based on availability of exposure data measured at 20 sites. These action categories ranged from "To be included in monitoring program", "Rigorous effect assessment required" to "Reduction of monitoring effort". For each of these substances:

- If exposure data were available, that is to say that a substance had been detected onsite in concentration (MEC_{site}) above the limit of quantification (LOQ), then it was classified in one group that would undergo additional assessment based on availability of sufficient effect data. In that case, 95th percentile of MEC obtained across the sites investigated will be compared to the lowest PNEC value determined for that substance.
- When exposure data were not sufficient (i.e. MEC_{site}< LOQ in the majority of the sites investigated), the authors compared the LOQ of a substance to the lowest PNEC for that substance. If the LOQ < lowest PNEC, then effect data of these substances are investigated.

It should be noted that the PNEC value used was the lowest one obtained following a multiple step evaluation process that takes into account availability of standard test data (acute toxicity) and availability of risk assessment data (i.e. chronic data) according to Table 16.

Table 16. Determination of the lowest PNEC of a substance (adapted from von der Ohe *et al.*, 2011).

Step	Screening of Ecotoxicological Database			
1	Standard test data		Risk assessment	
2	Sufficient acute data?		Sufficient chronic data?	
	No	Yes	No	Yes
3	Prediction of a PNEC	Determination of $PNEC_{acute}$	Determination of $PNEC_{acute}$	Determination $PNEC_{chronic}$
4	Selection of the lowest value			

In each of the six categories, compounds were then evaluated based on frequency with which PNECs exceed maximum MEC values and to what extent. Overall, out of 500 investigated, 190 substances concentrations were found to exceed the corresponding lowest PNEC values. For 16 of them risk ratios MEC/PNEC were superior to 100, which is considered of very high concern. For 42 of substances MEC/PEC ratios were above ten and classified as being of high concern. A majority of these substances were pesticides or biocides such as diazinon, heptachlor, and endosulfan. When looking at pharmaceuticals, only 19 substances were listed in the 500 organic chemicals considered. Ten of them were listed in the category 3 - "substances requiring rigorous effect assessments" - as sufficient exposure data were available but effect data were based on the prediction of a PNEC. The others were in the category 5 - "Perform a rigorous hazard assessment and a screening study" - for which only a few observations were recorded, the LOQ was inferior to the lowest predicted PNEC, but for which effect data were not sufficient. Overall, none of the pharmaceuticals were classified as high priority substances. For example, carbamazepine, in the category 3, was monitored at 1,052 sites since 2005, and was never found at concentrations exceeding the lowest PNEC.

Although not specifically focusing on pharmaceuticals, this new prioritisation approach is interesting as it takes into consideration the lack of data on emerging contaminants by categorising substances. It determines substances for which additional assessments will be required for further prioritisation. One of its specificities is that it is based on environmental observations in four river catchments. Risks are, therefore, assessed using maximum environmental concentrations measured across the sites considered rather than predicted environmental concentrations. However this could also become one of the disadvantages of this methodology if exclusively applied to pharmaceuticals, as first of all, analytical methods are available for only a limited amount of compounds and only a few of them are regularly monitored. Another interesting aspect of using measured concentrations across different sites is that it allows taking into account spatial variation of the measure. Indeed, the authors are not averaging MECs but consider a MEC value below which 95% of all MEC measured across sites fall. However, it should be noted that concentrations, when detectable, are often below the LOQ, which does alter the precision of percentile determination.

4.6.2. Summary of Existing Prioritisation Strategies

The review of the previous selection of published strategies for the prioritisation of pharmaceuticals in the aquatic environment highlighted that they could be classified in three different categories, those prioritising compounds of concerns based on:

- Exposure solely;
- Exposure and potential effects on the environment; and
- Exposure and potential effects on human health.

Parameters used to prioritise pharmaceuticals in each of these categories are summarised in Figure 19.

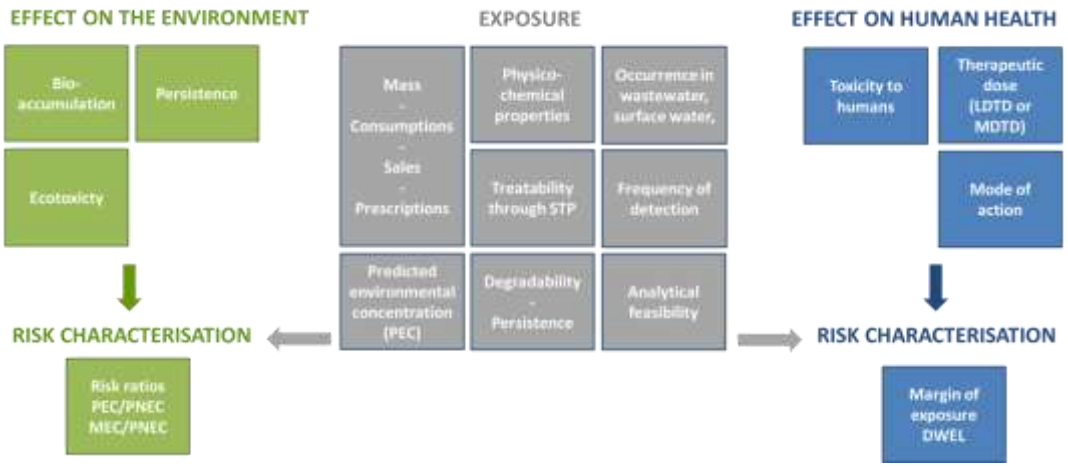


Figure 19. Overview of the parameters listed in the reviewed publications on prioritisation strategies.

4.6.2.1. Exposure

Exposure assessment relies on the prediction of environmental concentrations (PEC) in water. For a single pharmaceutical substance, its PEC is mainly derived from data on annual mass amounts of pharmaceuticals consumed. The accuracy of PEC calculation primarily depends on the quality of data available to determine these mass amounts. In some of the studies, researchers have access to listing of pharmaceuticals published by government and/ or regulatory agencies or alternatively to sales/production reports sold by pharmaceutical companies. In some of these listings, consumption data are available in mass amounts (kg per ton) (Besse *et al.*, 2008) while some other only provide prescription drug sales or prescription volumes which then need to be converted into mass using packaging information (Kostich and Lazorchak 2008). For instance, Kostich and Lazorchak (2008) converted prescription volumes into mass using maximum daily doses or sales of pharmaceuticals in the US into mass by dividing them with the lowest price of a unit. Such conversions require having access to additional sources of information or online databases on values of pharmaceutical substances or manufacturer prescribing information. This can add up to the difficulty of calculating precisely mass consumptions. Other parameters such as the limitation of databases to for example only the 300 most consumed substances or the lack of information on substances sold over the counter can also limit the predictions.

In addition, the accuracy of PEC calculation depends on whether other parameters such as metabolism, degradability/persistence or removal by STPs are taken into account or not. PECs better correlate with measured concentrations if taking into account metabolism and dilution (local or regional) (Besse *et al.*, 2008). However retrieving data on the metabolism of pharmaceuticals is not always simple especially in the first stages of a prioritisation exercise which can cover hundreds of substances. Similarly, data on degradability and or removal of pharmaceuticals by STPs can also help refining PEC values. However, such data are often compiled from the scientific literature and remain limited, case specific, and may therefore not always be representative of the context in which the prioritisation exercise is carried out.

A selection of the highest PEC values obtained when excluding these parameters could be used as a preliminary step approach to identify the compounds most likely to reach the environment. These "worst case scenario" PEC values could then be refined by integrating data on metabolism, degradability and treatability.

Finally, other parameters that are rarely taken into consideration but may affect environmental concentrations predictions are disposal and wash off of various parent molecules and distinctions between regional and national consumptions patterns. Regarding the former parameter, it is known that a portion of drugs enters the environment unchanged not only due to poor metabolic degradation but also to leftover prescribed drugs improperly discarded or substances such as topical treatments being washed off during bathing (Ruhoy and Daughton, 2007). Although such parameters would significantly improve the accuracy of prediction methods, they are rarely used due to a lack of information on amounts of drugs prescribed but not being consumed or difficulty to assess proportion of washed off substance. In their study on risks caused by human pharmaceuticals to aquatic organisms, Kostich and Lazorchack (2008) integrated this aspect in their PEC calculations. They assumed that 5 % of long term therapy drugs may not be consumed while 33% of topical substances may be washed off as the parent compound. They emphasised that taking into account this parameter is all the more important for highly metabolised substances.

Finally, it should be noted that for prioritisation exercises solely based on exposure, compounds that may be consumed in low quantities and would therefore not be prioritised using such methods, could present high toxicity at low exposure levels.

4.6.2.2. Effect

When prioritisation methodologies are targeting the potential impact of pharmaceuticals on the environment or human health exposure assessment is generally considered in the first place and then additional parameters specific to the type of effects targeted are used.

As shown in Figure 19, when targeting risks to the environment, ecotoxicity data and the subsequent prediction of risk quotients as the ratio of PEC to PNEC (Besse *et al.*, 2008) or eventually as a ratio of MEC to PNEC (von der Ohe *et al.*, 2011) are commonly used. However, deriving PNEC values for pharmaceuticals is often challenging. Indeed, it is typically recommended to derive PNECs based on chronic toxicity data, and eventually acute toxicity data but as highlighted in a number of studies, experimental data on chronic or acute toxicity of pharmaceuticals remain limited (Escher *et al.*, 2011). Consequently, a majority of prioritisation exercises based on environmental effect assessment are using QSAR modelling tools to determined risk ratios (Cooper *et al.*, 2008; Sanderson *et al.*, 2004). However, QSAR modelling is also limited since such models are also based on acute toxicity data and chronic effects can only be estimated by extrapolating acute toxicity to chronic toxicity using predefined acute-to-chronic ratios (ACRs) (Dom *et al.*, 2012). In addition, as for any modelling tool, the way QSAR results will describe toxicological effects in a real environment will strongly depend on the number and type of criteria considered to do so (*i.e.* physico-chemical descriptors, mode of toxic action, toxicity endpoints). For instance Escher *et al.*, 2011 explained that QSAR modelling tools have originally been developed for neutral organic compounds while a large number of pharmaceutical active ingredients are acids or bases, and therefore parameters such as bioaccumulation potential cannot be estimated using octanol - water partition coefficients (Kow) as commonly done in various QSAR modelling techniques.

To avoid the issue of the lack of toxicity data, Howard and Muir (2011) based their prioritisation method solely on the pharmaceuticals production volume and their potential to persist and bioaccumulate in the environment. The potential of pharmaceuticals to bioaccumulate is evaluated using log *Kow* or bioconcentration factors as indicators of potential bioaccumulation (*i.e.* for Log Kow < 3 the compounds is considered as potentially bioaccumulative) and persistence in the environment using biodegradability estimates generated from modelling software. Since the potency of chemicals to human is generally well known, Fick *et al.* (2010) used this parameter to assess the potential effect on fish, and derive critical environmental concentrations to prioritise compounds of potential concern.

When targeting potential effect on human health, studies generally focus on the potential presence of pharmaceuticals in drinking water as the main pathway through which human may be exposed to pharmaceuticals residues (Watts *et al.*, 2007; Snyder *et al.*, 2008; Bruce *et al.*, 2010). Cunningham *et al.*, (2009) also considered exposure through consumption of fish in their prioritisation method. Generally toxicity to human (for example carcinogenicity mutagenicity, immune toxicity), therapeutic doses (LDTD or MDTD) and mode of action are the parameters used to classify pharmaceuticals that may be of potential concern for human health. For instance, Bruce *et al.* (2010), use these parameters, to calculate a DWEL for each substance and compare it to the corresponding measured in drinking water in the form of a margin of exposure.

4.7. Pharmaceuticals in Hospital Effluents

4.7.1. Existing Prioritisation Approaches

Hospitals are known to be among the sources contributing to the presence of pharmaceuticals in the environment. However the extent of hospital contributions to pharmaceuticals loads in municipal wastewater, and consequently, the efficacy of a decentralised treatment of hospital wastewater as a way to limit the discharge of pharmaceuticals remain to be evaluated. A number of recent studies (Kovalova *et al.*, 2012; Ort *et al.*, 2010a; Verlicchi *et al.*, 2010; Thomas *et al.*, 2007) have looked at the characteristics of hospital wastewater in terms of pharmaceutical content and potential impact on the environment. Being based on analytical measurements, the majority of these studies are restricted to a certain number of pharmaceuticals, some hospital-specific such as X-ray contrast media and cytostatic drugs (Weissbrodt *et al.*, 2009) others commonly used in both hospitals and by the general population such as antibiotics, analgesics, anti-inflammatories (Thomas *et al.*, 2007). But the analysis of experimentally measurable pharmaceuticals may not be sufficient to fully assess the impact of hospitals on pharmaceutical pollution.

Therefore, as for other sources of pharmaceuticals, there is a need for prioritisation methodologies to identify and quantify pharmaceuticals of concern and to determine if pharmaceuticals consumed in hospitals should receive priority attention when compared to pharmaceuticals used by the general population. Indeed, if hospitals are logically perceived as major point sources due to localised intense medical activities, they are typically situated in dense population areas where a majority of the pharmaceuticals consumed by hospital patients are also largely consumed by the general population. A hospital's contribution to pharmaceutical pollution in municipal wastewater may therefore only be significant for a limited number of substances exclusively used in the hospital. Although most of the prioritisation exercises mentioned in section 3.1 could potentially be transferrable to pharmaceuticals originating from hospital, country-specific prioritisation tools specifically developed for compounds used in hospital would be preferable as hospital consumption data are likely to differ significantly not only from one hospital to another, but also from one country to another depending for example upon consumption data are collected (i.e. hospital consumption data vas national consumption data including or excluding consumption in hospitals). However, such prioritisation tools remain quasi inexistent. To the authors knowledge, only two recent studies have developed such methodologies to prioritise research on hospital wastewater characterisation, one developed by Mullot *et al.* (2011) and the other one by Le Corre *et al.* (2012).

Mullot *et al.* (2010) developed a prioritisation methodology to select pharmaceuticals specifically used in hospitals and predict their corresponding concentrations in hospital effluents. For validation purposes, they compared their predictions to pharmaceutical loads measured in effluents of three French hospitals and municipal wastewater. Overall, the aim of this study was to assess the impact of hospital effluents on loads of pharmaceuticals in municipal wastewater.

The screening methodology was based on consumption data obtained locally regarding the pharmaceuticals used at three selected hospitals organised by therapeutic class. Parameters such as therapeutic dose and metabolism were also used to rank the compounds (Table 17). Using this approach, Mullot *et al.* (2010) identified 13 APIs including: one beta-blocker, atenolol; one anaesthetic agent: propofol; two antibiotics: ciprofloxacin and sulfamethoxazole; three anti-inflammatories: ketoprofen, methylprednisolone, and prednisolone; three antineoplastics:

cyclophosphamide, 5-fluorouracil and ifosfamide; and three X-ray contrast media: gadolinium, iobitridol and iomeprol. The results of this study showed that the contribution of hospitals to the loads of pharmaceuticals in municipal wastewater based on consumption data were in good agreement with loads calculated based on experimental results for the 13 prioritised substances. For instance, the load measured for atenolol was of 0.94 g d^{-1} while the estimated load was 1.04 g d^{-1}. Concentrations of the selected APIs in hospital effluents were typically in the µg L^{-1} range except for the contrast media Iomeprol (1.4 mg L^{-1}) and Iobitridol (0.3 mg L^{-1}). However, in the influent of the corresponding STP, all substances were either not detected or in the low µg L^{-1} range.

These results suggest a limited impact of hospital pollution on municipal wastewater. However, this method is solely based on exposure and further investigations would be required to assess risks associated with priority compounds. Overall, the methodology used in this study to prioritise relevant substances to be measured and quantified in hospital wastewater is relatively simple to implement but limited by a pre-selection step of the most consumed APIs. Although some of the selected compounds, such as the chemotherapeutic agent cyclophosphamide, the anaesthetic agent propofol or iodinated contrast agents (iomeprol, gaddolinium), are identified through this pre-selection step, the method ignores a large number of hospital specific APIs that may be used in lower volumes but more important to prioritise than highly consumed drugs by both hospitals patients and members of the community. For example, among the top 350 most consumed APIs in the three French hospitals, Mullot *et al.* (2010) prioritised substances such as atenolol which is a commonly used beta-blocker so for which hospitals are unlikely to be a major point source. In addition, the use of factors such as pre-selection of compounds characteristic of a therapeutic class and more specifically analytically measurable are others limitations of the approach.

Table 17. Comparison of the prioritisation methodology developed by Mullot *et al.* (2011) and Le Corre *et al.* (2012).

	Mullot *et al.*, 2011	Le Corre *et al.*, 2012
Country	France	Australia
Aim of the Study	Model pharmaceutical loads in hospital wastewater and compare them to measured loads in both hospital and STP influent to evaluate the impact of hospital on pharmaceutical pollution in municipal wastewater.	Evaluate the contribution of hospitals to the loads of pharmaceuticals in municipal wastewater
Method	Analyse a list of 15,000 APIs used in three hospitals	Analyse audit data from six hospitals (1560 distinct APIs) and pharmaceutical consumption data by the general population (928 single and combined APIs).
	Select 350 APIs based on the annual consumption of each pharmaceutical in the three hospitals and the Maximum Recommended therapeutic Dose (MRTD). Additional parameters such as un-metabolised fraction (UF) and analytical capabilities were taken into account.	Exclude naturally occurring substances, drugs available over the counter resulting in a list of 589 individual APIs to evaluate.
	Calculate a ranking score derived from a cumulative consumption values at the three hospitals, the maximum recommended therapeutic dose and metabolised fraction.	Determine the contribution of each hospital based on consumption at the hospital and the total consumption in the catchment of the hospital.
	Select 100 APIs returning the highest scores	Select APIs for which the contribution of a hospital is in the range 97%-100% (*i.e.* compounds exclusively used in hospitals). This represented 153 distinct APIs.
	Predict concentrations of the 100 APIs in hospital effluents using daily drug and water consumptions. Select substances based on their concentrations (analytical feasibility) and to to cover a range of therapeutic class.	Predict the concentrations of these APIs in hospital effluent and influent of the corresponding STP. Assess potential effect of each API on human health by comparison of its predicted concentration with effect threshold values derived from therapeutic doses and acceptable daily intakes (ADI) as a margin of exposure MOE. An MOE > 100, indicates the concentration of an API is more than 100-fold below a "concentration of no concern".
Final List of APIs	13 substances covering 7 therapeutic classes were evaluated experimentally after screening.	12 hospital-specific APIs for which MOE were below 100 when predicted in STP influent were prioritised

Le Corre *et al.* (2012) and co-workers used a prioritisation approach based on consumption and pharmaceutical loads discharged by hospitals into municipal wastewater. This method was developed using consumption audit data from six hospitals and data on mass consumption by the general population. In a first step, the contribution of each of the six hospitals to the load of an API in municipal wastewater was used to screen a list of 589 APIs. The results showed that for 63 to 84% of the APIs evaluated hospitals were likely to contribute less than 15%. Therefore for these API, loads in municipal wastewater were mainly originating from domestic wastewater. An additional screening step, was therefore applied to the APIs which returned a hospital contribution >97%, that is to say for 153 compounds exclusively used in hospitals. This second screening step involved the predictions of concentrations for these API in both hospital effluent and influent of the corresponding STPs and an assessment of potential effects on human health (Table 17). Overall, this prediction revealed that 12 compounds would be present in the influent of STPs in concentrations less than 100 times below a concentration "of no concern". These included: one antineoplastic: vincristine sulphate; one mydriatic agent: tropicamide; one immunomodifier: infliximab; one neuromuscular blocking agent: pancuronium; three antibiotics: cefazolin, piperacillin and tazobactam; five anaesthetic agents (local and general): bupivacaine, levobupivacaine, ropivacaine, oxybuprocaine and suxamethonium.

Unlike the approach by Mullot *et al.* (2010), the method used by Le Corre *et al.* (2012) is not limited by a pre-selection of consumption data (i.e. most consumed pharmaceuticals) although dependent upon the availability and quality of audit data from hospitals. For instance, the method developed by Le Corre *et al.* (2012) excludes X-ray contrast agents. Indeed, in Queensland, Australia, a majority X-ray analysis are performed in private imaging centres, therefore these compounds are not

comprehensively accounted for in public hospitals consumption audit data. However, the method allows a rapid screening of pharmaceuticals used in hospitals and identifying potential compounds of concern that may require monitoring and specific treatment or disposal. One of the advantages of this consumption-based approach is that its first step does not require information on metabolism as this parameter has no impact on the estimation of a particular hospital's contribution to the total load of an API in municipal wastewater.

4.7.2. Recommendations to Prioritise Pharmaceuticals used in Hospitals

Based on the review of existing and recently developed prioritisation methodologies, we recommend approaches for the prioritisation of pharmaceuticals originating from hospitals includes the following aspects (Figure 20):

- As illustrated in sections 3.2 and 3.3, the degree of complexity of tools for the prioritisation of pharmaceuticals can vary significantly whether they are designed to rank compounds solely based on exposure assessment or based on risks (i.e. a combination of exposure and effects). Overall, a methodology exclusively based on exposure assessment is not sufficient as a whole to prioritise hospital-specific compounds that may require monitoring and specific treatment or disposal. For instance, a compound prioritised based on exposure may not be the most relevant in terms of potential effects on the environment/or human health.
- As a first step, a pre-screening of compounds by determining the contribution of hospitals to loads of pharmaceuticals in municipal wastewater is recommended. This step allows a rapid screening of pharmaceuticals based on the percentage contribution deemed relevant (for example in the range 50-100%). This pre-screening step requires comprehensive and good quality data on mass consumption by a hospital and pharmaceuticals consumption by the general population in the catchment of that hospital.
- As a second step, pharmaceuticals identified in step 1 should be further investigated. This implies the prediction of concentrations for each hospital-specific substance in the influent of the STP to which they discharge their effluents. Metabolism data are often not available for a large amount of compounds but could be used for a reduced number of pre-screened compounds at this stage. It should be noted however that not taking into account excretions at this stage results in conservative concentration estimations.
- As a third screening step, we recommend evaluating potential environmental and health risks resulting from exposure to pharmaceutical for which a hospital is identified as a major contributor. Potential risks to the aquatic environment can be predicted by calculating risk quotients (RQ) as a ratio of predicted concentrations to PNEC while potential risk to human health can be predicted by comparing predicted concentrations to effect thresholds derived from therapeutic doses as margin of exposure. At this stage, concentrations are predicted in raw wastewater, RQ and MOE results are therefore conservative, since concentrations of pharmaceuticals are expected to be significantly reduced after conventional wastewater treatment.
- As a result, compounds returning RQ >1 and MOE<1 for hospital specific-substances are the compounds that should require further investigations in terms of environmental and human toxicity, biodegradation and treatment or source control options, and eventually development of analytical method for detection in hospital effluent, raw and treated municipal wastewater. Parameters such as metabolism, removal in STPs and degradation of substances which are often not available for a large amount of compounds can also be applied in this final stage.
- Finally, prioritising pharmaceuticals of concerns used in hospitals is not the only aspect to consider when assessing the impact of hospital on environmental pollution, hence the implementation of decentralised treatment systems for hospital wastewater. Other substances such as disinfectants, detergents, solvents, heavy metals should be considered. As these compounds are not listed in hospital audit data, it is recommended to involve hospital experts and risk assessment specialists at this stage. In addition, the assessment of the contribution and survivability of antibiotic resistant bacteria from hospital to STPs is recommended to fully assess whether hospital contribute to the amount of antibiotic resistant bacteria in the aquatic environment.

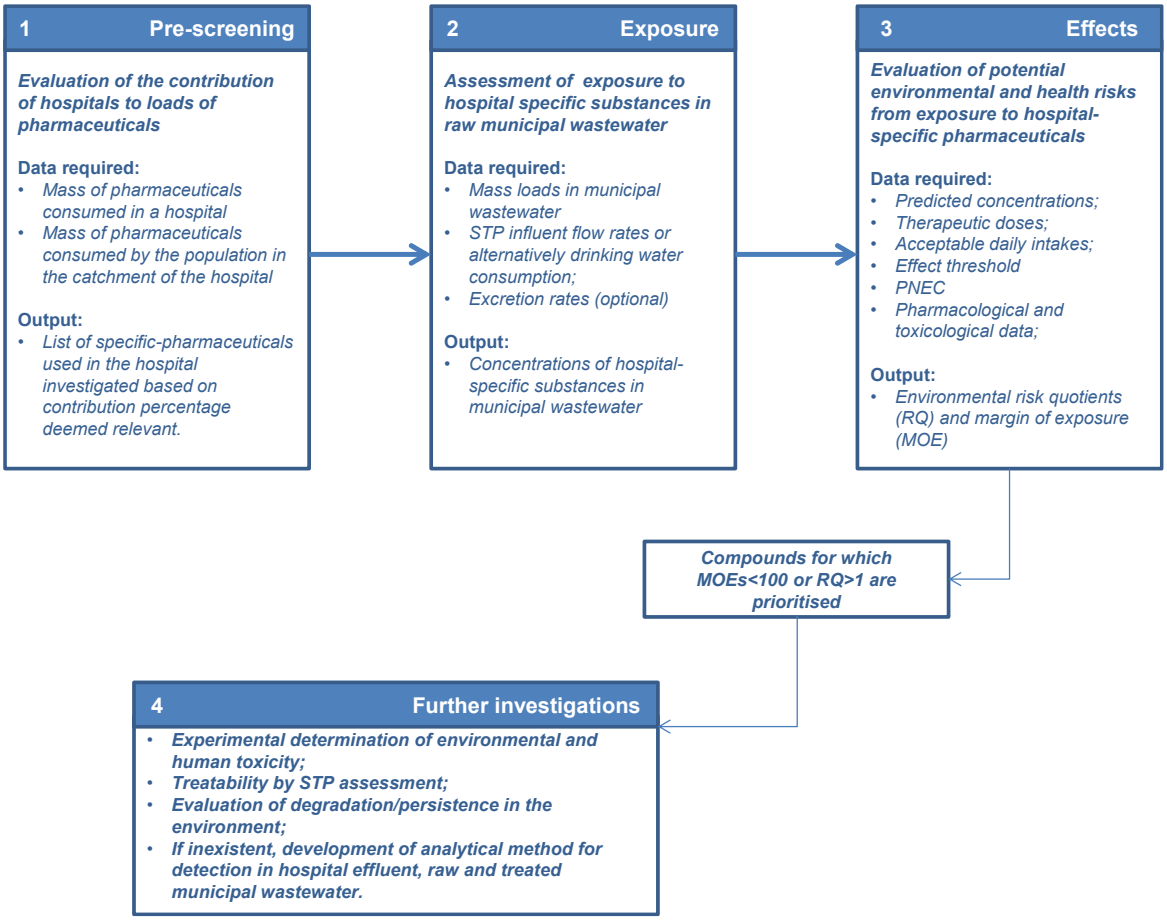


Figure 20. Suggested step for prioritisation of pharmaceuticals originating from hospitals.

4.8. Conclusions

This chapter has reviewed current approaches used to manage risks associated with the release of pharmaceuticals into the environment and prioritisation strategies that have been developed recently by the scientific community. Overall this review revealed that:

- The increasing consumption of pharmaceuticals has undoubtedly increased the potential for these compounds to reach the aquatic environment – mainly through the discharge of treated municipal wastewater to surface water - and eventually cause adverse effects on aquatic organisms and humans. However, despite the great development of analytical techniques, the detection of all pharmaceuticals consumed by patients in hospitals and other medical premises, and more broadly by the general population, is not conceivable. Therefore, it has become necessary to find ways of prioritising substances of potential concerns that should be more extensively studied and eventually be included in future monitoring programs.
- The review of recent studies on detection of pharmaceuticals in various water sources revealed that the most studied - and eventually detected - compounds are typically the most consumed ones. Indeed, compounds to be analysed are often selected by researchers based on criteria such as usage, prescription numbers, sales and/or production amounts. Other parameters used by researchers include known occurrence of substances in the environment, drug class and availability of analytical methods. However, prioritisation strategies to select the compounds under investigation are rarely mentioned in these studies. This suggests that the compounds analysed for may not necessarily be the most important ones in terms of toxicity or impact on the environment and human health.

- Tools to assess environmental risk of chemical substances, including pharmaceuticals, are in place in Europe (EMA) and the US (FDA). These are based on three-tier screening approaches including an exposure assessment phase (i.e. PEC determination), a risk screening phase (i.e. PNEC calculation and determination of risk quotients) and a risk refinement phase (i.e. chronic toxicity and micro-organisms specific tests). The use of these environmental risk assessment approaches for prioritising pharmaceuticals based on exposure are simple and PEC values correlate well with experimental measures. However, the lack of data for parameters such as consumption, metabolisms and treatability by STPs can alter the accuracy of the result. Furthermore, the lack of pharmacological and toxicological data is limiting the application of this method to screen a wide range of compounds according to their potential risk on the environment.
- Several methodologies have recently been developed in the literature in order to prioritise research on pharmaceutical residues in the aquatic environment. Most of these methodologies use consumption data as a starting point to screen pharmaceuticals based on exposure assessment. For a refinement of pharmaceuticals of potential concern, predictions of potential risks to the environment and/or human health are performed. Despite similarities in some of the methodologies, this review highlighted the need for a standardisation of prioritisation strategies to generate prioritisation tools transferrable from one country to another.
- In the case of pharmaceuticals originating from hospitals, prioritisation strategies need to be more specific as they should not only focus on the nationally or regionally most consumed compounds but also on consumptions by hospitals. This implies getting access to reliable data from hospitals.

Dissemination Outputs Related to Part A

Publications:

Le Corre, K.S., Kateley, D., Allen, B., Escher, B.I., Ort, C., Keller, J., 2012. 'Consumption-based approach for assessing the contribution of hospitals towards the load of pharmaceutical residues in municipal wastewater'. *Environ. Int.,* 45, 99-111.

Le Corre, K.S., Ort, C., Keller, J., 2012. 'Pharmaceutical residues in municipal wastewater: are hospitals a major point source? *Water, the Australian Journal of the Water Association*, 39 (4), 84-88.

Ort C., Lawrence M.G., Reungoat J., Eaglesham G., Carter S., Keller J., 2010. 'Determining the fraction of pharmaceutical residues in wastewater originating from a hospital'. *Water Res.*, 44(2):605-615.

Ort C., Lawrence M.G., Reungoat J., Mueller J.F., 2010. 'Sampling for PPCPs in wastewater systems: comparison of different sampling modes and optimisation strategies'. *Environ. Sci. Technol.*, 44(16), 6289-6296.

Ort C., Lawrence M.G., Rieckermann J., Joss A., 2010. 'Sampling for pharmaceuticals and personal care products (PPCPs) and illicit drugs in wastewater systems: are your conclusions valid? A critical review'. *Environ. Sci. Technol.*, 44(16), 6024-6035.

Conferences (platform presentations):

Le Corre, K., Ort, C., Keller, J. "To what extent do hospital discharges contribute to the pharmaceutical load in municipal wastewater*?"* SETAC Australasia, July 2012, Brisbane, Australia.

Le Corre, K., Kateley, D., Allen, B., Ort, C., Keller, J. 'Reduction of Pharmaceutical Loads in Municipal Wastewater: Would Onsite Treatment of Hospital Wastewater be Effective?' Science Forum and Stakeholder Engagement. Building linkages, Collaboration and Science Quality. June 2012, Brisbane, Australia.

Le Corre, K., Kateley, D., Allen, B., Ort, C., Keller, J. 'Determining key pharmaceuticals in wastewater primarily originating from hospital effluents: A consumption-based approach'. 8th IWA International Conference on Water Reclamation and Reuse, September 2011, Barcelona, Spain.

Le Corre, K., Kateley, D., Allen, B., Ort, C., Keller, J. 'Assessing the input of hospitals to the amount of pharmaceutical residues in municipal wastewater: a consumption-based approach'. Science Forum and Stakeholder Engagement. Building linkages, Collaboration and Science Quality. September 2011, Brisbane, Australia.

Ort, C., Lawrence, M.G., Reungoat, J., Keller, J. 'Are hospitals a major point source of pharmaceutical of pharmaceuticals in wastewater?' 7th IWA World Congress on Water Reclamation and Reuse, September 2009, Brisbane, Australia.

Ort C., Lawrence M.G., Reungoat J., Eaglesham G., Carter S., Keller J., 2009. ' Are Hospitals a Major Point Source of Pharmaceuticals in Wastewater?' Science Forum and Stakeholder Engagement. Building linkages, Collaboration and Science Quality. August 2009, Brisbane, Australia.

Conferences (poster presentations):

Le Corre, K., Ort, C. and Keller J. "Pharmaceutical audit data from hospital". Science Forum of the Urban Water Security Research Alliance, September 2010, Brisbane, Australia.

PART B: ANTIBIOTC RESISTANT BACTERIA IN HOSPITAL WASTEWATER

Authors

- Mohammad Katouli[*]
- Helen Stratton[**, #]

[*] Faculty of Science, Health and Education, University of the Sunshine Coast, QLD 4558, Queensland.
[**] School of Biomolecular and Physical Sciences, Griffith University, QLD 4111.
[#] Smartwater Research Centre, QLD 4222.

5. AN OVERVIEW OF ANTIBIOTIC RESISTANT BACTERIA IN HOSPITALS

5.1. Antibiotic Resistant Bacteria in Hospital Wastewater

Hospitals and healthcare settings are regarded as reservoirs for large numbers of pathogenic bacteria (Mulvey and Simor, 2009). Antibiotic resistance reported in hospitals is potentially associated with the fact that there is a high usage of antibiotics to treat infections in patients, which places bacteria under great selective pressure (Davison, 1999). An increase in resistance in many Gram-negative pathogens belonging to the *Enterobacteriaceae* family (Asensio *et al.*, 2011; Hu *et al.*, 2011; Wang *et al.*, 2010) as well as Gram-positive pathogens (Islam, 2011) has been noted in recent years in these clinical settings. Particular concerns surrounding antibiotic resistant bacteria (ARB) are their transmission and long-term survival in the environment. It is postulated that a highly possible route of dissemination of ARB from hospitals into the environment is through wastewater discharges (Pauwels and Verstraete, 2006).

Antibiotics and their metabolites end up in hospital wastewater via excretion of urine and faeces (Kümmerer, 2009). When considering therapeutical antibiotics, although subject to metabolic reactions, a significant amount of the original substance will leave the body un-metabolised and will therefore enter sewage (Baquero *et al.*, 2008). High levels of antibiotics present in hospital wastewater have also been well documented by others studies (Duong *et al.*, 2008).

Wastewaters from a hospital and pharmaceutical plant have been shown to increase resistance to one or more antibiotics among *Acinetobacter* species in the sewers (Guardabassi *et al.*, 1998). It is debated whether the amount of antibiotic compounds affect resistance by exerting selective pressure, or whether the antibiotic resistant isolates are a result of already resistant isolates entering the wastewater. Conversely, it is proposed that the transfer of resistance and the selection of resistant bacteria are not favoured at antibiotic concentrations found in hospital effluents or the aquatic environment (Ohlsen *et al.*, 2003).

5.2. Antibiotic Resistance in Environmental Waters

Pathogenic bacteria are constantly released via domestic wastewater into the surface waters (Baquero *et al.*, 2008). These pathogens may contain antibiotic resistance genes, which can be inserted into genetic mobile elements such as plasmids, transposons and integrons (Dahlberg *et al.*, 1998), thus enabling their spread among microbial communities in the water (Alonso *et al.*, 2001). Antibiotic resistant bacteria from these and other sources normally end up in sewage treatment plants (STPs) before they find their way into the environment. STPs or wastewater treatment plants (WWTPs) are designed to treat wastewater from a number of different sources such as municipal, agricultural, clinical and industrial waste (Yassi *et al.*, 2001). These wastes contain sewage from humans and animals, chemical compounds, pharmaceuticals, clinical waste and a variety of other materials.

Antibiotic resistant bacteria released from hospitals ultimately travel in the untreated wastes to the receiving STPs where they enter pre-treatment tanks and are joined by a vast number of bacteria from municipal wastewater. Although the number of ARB found in hospital effluents travelling to STPs is high (Wiethan *et al.*, 2001), it is possible that these bacteria may weaken in their resistance, as there is a decrease in selective pressure once they enter the STP. The concentration of antibiotics in municipal sewage and in STPs is typically 100 times lower compared to hospital effluent (Kümmerer, 2004). Despite this, there is still a large variety of antibiotic compounds present in STPs, which may affect the existing bacteria if their concentrations are high enough. It has been found that STPs that treat hospital waste as well as municipal sewage have higher resistant rates of certain strains of bacteria (Jury *et al.*, 2011). This finding however, is not conclusive as the ARB found in STP can originate from a number of different areas in the community, industries and the environment.

It has been proposed that wastewater treatment can actually select for the survival of resistant strains of bacteria (Bell *et al.*, 1983; Murray *et al.*, 1984). Some bacterial strains such as Enterococci have been shown to not only carry several antibiotic resistant genes but also survive the several stages of treatment in STPs (Da Silva *et al.*, 2006). Other studies have also shown an increase in the number of multidrug resistant bacteria following treatment of municipal sewage (Andersen, 1993; Morozzi *et al.*, 1988). These data however are not consistent in that some studies suggest that treatment processes have no or very little effect on selection for ABR present in STPs (Guardabassi *et al.*, 2002).

5.3. Transmission of Antibiotic Resistant Bacteria from STPs to Surface Waters

It is still debatable whether ARB that survive wastewater treatment are transmitted into the environment along with effluent and are able to survive and have a significant impact on the environment and public health (Baquero *et al.*, 2008). Some studies have indicated that rates of antibiotic resistance among isolates from sites adjacent to STP discharge points have been significantly higher compared to isolates from other sites (Watkinson *et al.*, 2007). Antibiotic resistant genes have also been identified in surface water such as the ampC gene coding ampicillin resistance in Enterococci. However it is not definitively known by what means these antibiotic resistant genes are finding their way into the environment (Schwartz *et al.*, 2003).

5.4. Aims of the Study

It is a well-established fact that some pathogenic bacteria may gain residency in hospitals and become resistant to several antimicrobial drugs. Such bacteria can be disseminated into the environment through routes such as hospital wastewater. It is postulated that these antibiotic resistant strains may then travel to STPs through the sewerage system, possibly survive the STP treatment process and find their way into the environment via the discharge of treated effluent from STPs. In view of the above, this study was undertaken to investigate the presence and prevalence of ARB in hospital wastewater (Case study 1) and also trace their transmission to a receiving STP and assess their survival through STP treatment processes (Case study 2).

6. EXPERIMENTAL APPROACH (I): DETERMINING THE PREVALENCE OF ANTIBIOTIC RESISTANT BACTERIA IN HOSPITAL WASTEWATER - CASE STUDY 1

6.1. Material and Methods

6.1.1. Case Study 1

Hospital 1 with more than 370 beds acts as the major medical facility for the region. The hospital has a history of providing more than 35,000 emergency procedures and admitting more than 42,000 patients in 2010. Untreated wastewater samples were collected for 14 consecutive weeks from the main outlet pipe of the hospital between January and April 2012. Wastewater samples were collected in 500ml sterile microbiological containers mounted onto a handle of appropriate length using "grab-sampling" technique. They were transported to the laboratory on ice and processed within 4 hours of collection.

At the same time and in an attempt to provide evidence of the presence/absence of selected antibiotic resistant bacteria in a domestic wastewater treatment plant without a hospital or other health care facilities in the corresponding catchment, untreated samples were also collected from the inlet of two local STPs for 4 consecutive weeks. Both STPs service an equivalent population of 130,000 and they both have a 12-13 day sludge-treatment cycle. Neither of the STPs received samples from the hospital included in this study. All samples were collected between 7.30am and 8.30am of the same day and processed as described above. Selected bacterial strains for this study were *Escherichia coli* and enterococci.

6.1.2. Isolation and Identification of *E. coli*

Wastewater samples were processed using serial dilutions and cultured on two mFC Agar plates (Oxoid) with and without 32μg mL^{-1} of ceftazidime and 16μg mL^{-1} of aztreonam. After an overnight incubation at 44°C, up to 24 suspected *E. coli* colonies (where possible) were picked up from each sample and tested for the presence of the highly specific *E. coli* universal stress protein (*usp*A) gene. For each bacterial strain, chromosomal DNA was extracted using Real Biotech Corporation (RBC) Genomic DNA Extraction Kit (Blood/Bacterial/Cultured Cells) and the *usp*A gene was amplified as described by Chen and Griffiths (1998). Confirmed *E. coli* strains were further tested for their resistance to 11 antimicrobial agents.

6.1.3. Antimicrobial Resistance Testing

Antimicrobial susceptibility tests were performed using the disc diffusion assay on Muller-Hinton agar (MHA) according to the Calibration Dichotomous Susceptibility (CDS) method (Bell *et al.*, 2006). Antimicrobial agents included were: cefoxitin (FOX 30μg), imipenem (IPM 10μg), gentamicin (GEN 10μg), amikacin (AMK 30μg), tetracycline (TET 30μg), sulphamethoxazole (RL 100μg), ciprofloxacin (CIP 5μg), norfloxacin (NOR 10μg), nalixidic acid (NAL 30μg), nitrofurantoin (NIT 300μg), and chloramphenicol (CHL 30μg). The CDS method reports antimicrobial drug susceptibilities as either "susceptible" or "resistant" and therefore the results were not expressed as minimum inhibitory concentration (MIC) values, and interpreted as susceptible (S) or resistant (R).

6.1.4. Search for Extended Spectrum Beta-Lactamase (ESBL) Producing Strains

E. coli strains showing resistance to ceftazidime and aztreonam were confirmed for the production of ESBL according to the method described by Bell *et al.* (2006). The synergy between the beta-lactam antibiotic disc and clavulanate was monitored by placing a disk of beta-lactam inhibitor/beta-lactam antibiotic combination drug and a disk of beta-lactam antibiotic on an inoculated MHA plate 25mm apart (centre to centre). These included; amoxicillin-clavulanic acid (AMC 60μg), ticarcillin-clavulanate (TIM 85μg), cefotaxime (CTX 5μg), ceftazidime (CAZ 10μg) cefepime (FEP 10μg) and

aztreonam (ATM 30 µg). The presence of a clear extension of the edge of the beta-lactam antibiotic inhibition zone toward the disk containing beta-lactam inhibitor was regarded as an ESBL producer. The test was repeated placing the antibiotic discs 20mm and 15mm apart (centre to centre) if resistance zones were small making it difficult to identify clear zone between discs. Two ESBL-producing *E. coli* strains i.e. K6 and EC10 and a susceptible *E. coli* strain ACM5185 (kindly provided by S. M. Bell, The Prince of Wales Hospital in Sydney, Australia) were included in all tests as the positive and negative controls respectively.

6.1.5. Typing of Isolates

Phenotypically confirmed ESBL-producing *E. coli* isolates were typed using multi-locus variable number tandem repeat analysis (MLVA), PhP fingerprinting method and phylogenetic grouping. MLVA was performed as described previously by Lindstedt *et al.* (2007) targeting seven tandem repeats (CVN001, CVN002, CVN003, CVN004, CVN007, CVN014, and CVN015) using two multiplexes and one uniplex PCR. However, the fluorescent labels used for the forward primers were changed as follows; CVN001-VIC, CVN002-VIC, CVN-003-NED, CVN004-NED, CVN007-FAM, CVN014-FAM, CVN015-PET. Polymerase chain reaction (PCR) products were sized on a 16 capillary Applied Biosystems 3130xl Genetic Analyser with a 50cm capillary and POP-7 was used as the separation matrix. Fragment sizing was performed using Gene Mapper V4.0 software (Applied Biosystems) using LIZ600 as the size standard. Each peak was identified according to fluorescent label and size and the allele number was assigned based on fragment sizes, described by Lindstedt *et al.* (2007). Alleles for which amplicons were absent were designated an allele number of "0".

PhP fingerprinting was done using high resolution biochemical fingerprinting plates i.e. PhP-RE plate (PhPlate AB, Stockholm, Sweden), which are specifically developed for typing of *E. coli* strains (Landgren *et al.*, 2005). Inoculation of the plates was done according to the manufacturer's instruction and the plates were incubated at 37°C. The rate of each reaction was evaluated by measuring the absorbance value in each well after 7, 24 and 48 h of incubation using a digital scanner (HP Scanjet 4890 scanner). After the final scan, the PhPlate software was used to create absorbance (A620) data from the scanned plate images and the mean absorbance in each well over the three reading was calculated, yielding the biochemical fingerprint for each isolate. The biochemical fingerprints of the isolates were compared pair wise and the similarity among the isolates was calculated as the correlation (similarity) coefficient and clustered according to the unweighted pair-group method with arithmetic averages (UPGMA) (Saeedi *et al.*, 2005; Sneath and Sokal, 1973). Isolates showing a similarity coefficient to each other above the default identity level (0.975) of software were regarded as identical and assigned to the same biochemical phenotype (BPT). All data handling, including calculations of correlations and coefficients as well as clustering were performed using the PhPlate software version 4002 (PhPlate AB, Stockholm, Sweden).

Phylogenetic grouping was done for these selected strains using multiplex PCR with primers coding for *chu*A and *yja*A genes and the DNA fragment TSPE4.C2 according to Clermont *et al.* (2000). Strains belonging to the same BPTs, with identical MLVA profiles and phylogenetic group were considered as members of the same cluster and were regarded as common (C) types and those differing by one or two of the three methods were regarded as single (S) types.

6.1.6. Isolation and Identification of Enterococci

Samples were diluted 5-fold with phosphate-buffered saline (PBS) before filtration on 0.45 µm membrane (Millipore Corporation, Bedford, MA, USA) as described before (Iversen *et al.*, 2002). The membranes were then transferred onto mEnterococcus agar (Becton Dickinson and Co., Sparks, MD, USA) and incubated for 48 h at 37°C. The membrane filters with well-isolated colonies were then transferred to bile esculin agar plates and incubated for 2 h at 44°C. Black colonies were selected for Gram staining and the presumed *Enterococcus* spp. were defined as isolates that grew at 44°C, in 6.5% NaCl, were esculin and PYR positive, and catalase negative (Iversen *et al.*, 2002). Those isolates were identified to the species level using the following biochemical tests: acid production of L-arabinose, lactose, D-sorbitol, D-mannitol, L-sorbose, glucose, methyl-α-D-glucopyranoside, arginine dihydrolase, motility, hippurate hydrolysis, haemolysis, pigmentation, tetrazolium 0.01% and

tellurite 0.04% reduction (Facklam and Collins, 1998). Identification of species was also confirmed by polymerase chain reaction (PCR) using species-specific primers listed in the Table 18.

6.1.7. DNA Extraction and PCR

DNA for PCR was extracted by the boiling method as described before (Yost and Nattress, 2000). PCR assays were performed with specific primers (Table 18) in a total volume of 25 µL containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM each dNTPs, 0.5U of Taq DNA polymerase (HT Biotechnology, Cambridge, United Kingdom) and each primer (0.5 µM). Each PCR cycle was done as follow: an initial denaturation at 95°C for 4 min, with 30 cycles of denaturation at 95°C for 30 S, annealing at 54°C (for *E. faecium*) and 50°C (for *E. gallinarum* and *E. casseliflavus*) for 1 min, elongation at 72°C for 1 min and final extension at 72°C for 7 min (35). The PCR products were analysed on a 1.5% agarose gel with 0.5x Tris-borate-EDTA buffer (TBE). A 100-bp DNA ladder molecular size marker was used and the gels were stained with ethidium bromide and photographed under UV light.

Table 18. Primers used for identification of enterococci species.

Primer Specificity	Size of PCR Product (bp)	Primer Pair Sequences	Reference
E. faecium	359	5'-CGAATTTAAATTCAGCAATTGA-3' 5'-CTTTCCTTCCATCAATGGAG-3'	This study
E. faecalis	347	5'-ATGTGACTAACTTAAACGCAG-3' 5'-AATCTTGGTTTGGTGTTGAA-3'	This study
E. hirae	189	5'-TAAATTCTTCCTTAAATGTTG-3' 5'-CTTTCTGATATGGATGCTGT-3'	Jackson *et al.*, 2004
E. mundtii	301	5'-CAGACATGGATGCTATTCCATCT-3' 5'-AGGTTTCTTGCCTTCCATCAAT-3'	Jackson *et al.*, 2004
E. gallinarum	173	5'-TTACTTGCTGATTTTGATTCG-3' 5'-TGAATTCTTCTTTGAAATCAG-3'	Jackson *et al.*, 2004
E. casseliflavus	253	5'-GCTAGTTTACCGTCTTTAACG-3' 5'-TTAGCAGACTTTTCTTCTGTAC-3'	Jackson *et al.*, 2004

6.1.8. Antibiotic Susceptibility Test for Enterococci

The antibiotic susceptibility tests were performed using disk diffusion method and interpreted according to the guidelines from the Clinical and Laboratory Standards Institute (CLSI) (National Committee for Clinical Laboratory Standards, 2001). The antibiotics used for susceptibility tests (Becton Dickinson and Company, Sparks, MD, USA) were: ampicillin (AMP, 10 µg), erythromycin (30 µg), vancomycin (VAN, 30 µg), tetracycline (TET, 30 µg), sulfamethoxazole (RL, 100 µg), gentamicin (GEN, 120 µg), chloramphenicol (CHL, 30 µg), nitrofurantoin (NIT, 300 µg) and ciprofloxacin (CIP, 5 µg). Minimal inhibitory concentration (MIC) of vancomycin resistant enterococci was determined using E-test (AB Biodisk, Solna, Sweden*). E. faecalis* ATCC 29212 and *E. faecium* ATCC 51299 were used as quality control strains.

6.2. Results

6.2.1. Common Types and Resistance among Non-ESBL Producing Strains

In all, 497 *E. coli* strains were isolated in the hospital wastewater and untreated influents from both STPs of which 252 were ESBL-producing strains. During the same time, 101 strains of enterococci were also collected from the same sources (Table 19).

Table 19. Number of *E. coli* and enterococci strains isolated from hospital 1 wastewater and incoming influent sites of two local STPs.

Bacterial Strains	Number of Isolates from:			Total n°.
	HWW	STP1	STP2	
Non-ESBL producing *E. coli*	160	62	23	245
ESBL producing *E. coli*	198	29	25	252
Enterococci	65	18	18	101

Typing of the *E. coli* isolates from hospital showed that non-ESBL producing strains belonged to different clonal groups than those producing ESBL enzymes. These strains belonged to five C-types comprising 128 (65%) isolates and 70 S-types. The C-type 2, with 48 isolates (38%), was the most dominant type and was found in 11 out of the 14 sampling occasions indicating the persistence and dominance of this type in hospital 1 (Table 20).

The other C-types were also presents in different quantities and were present in four to 10 samples (Table 20).

Table 20. Presence of different clones of Non-ESBL producing strains of *E. coli* in samples collected from hospital 1 wastewater over 14 weeks of samplings. Red dots indicate the presence of the same bacterial strain in wastewater sample in each week (W1-W14). C-type: Common type (strains that were found in more than one sampling occasion). The number of isolates for C-type is given in brackets.

C-types	Weeks where *E. coli* strains were found													
	W1	W2	W3	W4	W5	W6	W7	W8	W9	W10	W11	W12	W13	W14
C1 (18)	•		•				•	•	•		•			•
C2 (48)	•	•	•	•	•	•		•	•		•	•	•	
C3 (14)	•		•		•		•							
C4 (28)	•	•				•	•		•	•	•			•
C5 (20)	•	•	•			•	•	•		•	•		•	•

Resistance of strains belonging to C-types was measured against 11 antibiotics (see 6.1.3). The highest resistance was observed against sulphafurazole and gentamicin (94% each), tetracycline (93%) and imipenem (92%), with more than 81% of the isolates being resistant to more than five antibiotics (Table 21). In contrast, more than 82% of the isolates from STP 1 and more than 91% of the isolates from STP2 were resistant to less than five antibiotics (Table 21).

Table 21. Distribution of the isolates found in wastewater of hospital 1 (HWW) and two sewage treatment plants (STPs) based on the number of antibiotics to which they were resistant. Samples collected from the inlet of two STPs that did not receive waste from hospital 1.

No. of antibiotics to which bacteri were resistant	No. of strains		
	HWW	STP-I	STP-2
None	0	2	1
1	0	13	2
2	4	15	5
3	8	15	7
4	12	6	5
5	20	1	1
6	23	3	1
7	22	2	0
8	23	1	1
9	8	2	0
10	5	2	0
11	3	0	0

The mean number of antibiotics to which hospitals strains were resistant (i.e. 6.3 ± 2.1) was significantly ($p<0.001$) higher than that of STP-1 (3.2 ± 2.7) and STP-2 (3.1 ± 1.7) ($p<0.001$) (Figure 21). Fisher's exact test was used for comparing significance of difference between two compared samples. p -value of <0.05 was considered statistically significant.

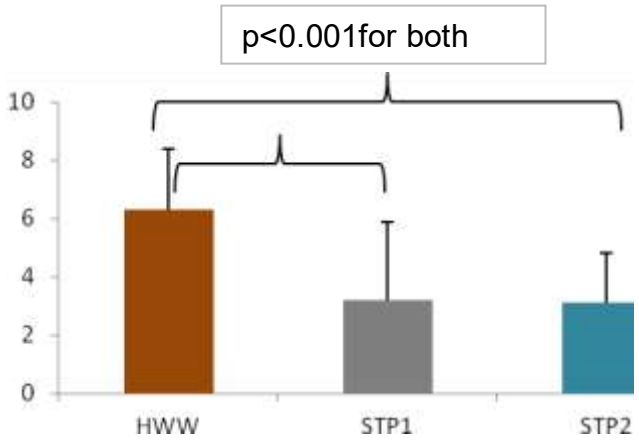


Figure 21. Mean number of antibiotics to which strains from hospital wastewater (HWW) and sewage treatment plants (STP) 1 and 2 were resistant.

6.2.2. Common Types and Resistance among ESBL Producing Strains

The ESBL producing *E. coli* strains consisted of seven C-types (ranging from 6 to 88 isolates each) and 27 S-types. Strains belonging to C-type 2 (CT2) were found in all 14 samples collected from hospital wastewater (Table 22). Twenty seven (14%) of ESBL producing isolates were found in only one of the 14 weekly samples and were regarded as S-types.

Table 22. Presence of different clones of ESBL producing strains of *E. coli* in samples collected from hospital 1 wastewater over 14 weeks of samplings. Red dots indicate the presence of the same bacterial strain in wastewater sample in each week (W1-W14). C-type: Common type (strains that were found in more than one sampling occasion). The number of isolates for C-type is given in brackets.

Common types (no. of isolates)	Number of strains in each C-type over 14 weeks (W)													
	W1	W2	W3	W4	W5	W6	W7	W8	W9	W10	W11	W12	W13	W14
CT1 (10)	● (1)	● (4)					● (3)					● (2)		
CT2 (88)	● (3)	● (5)	● (3)	● (2)	● (7)	● (7)	● (1)	● (10)	● (8)	● (20)	● (7)	● (3)	● (11)	● (1)
CT3 (6)				● (1)	● (2)			● (3)						
CT4 (12)	● (1)			● (3)					● (3)	● (2)	● (2)		● (1)	
CT5 (22)	● (3)		● (7)	● (8)	● (2)		● (2)							
CT6 (20)	● (3)					● (3)		● (5)	● (3)				● (2)	● (4)
CT7 (13)	● (1)	● (3)			● (1)					● (2)		● (4)	● (2)	
S1 to S27*	● (4)	● (2)	● (2)	● (2)	● (1)	● (3)	● (2)	● (1)	● (2)		● (1)	● (1)	● (2)	● (4)
Total (198)	16	14	12	16	13	13	8	19	16	24	10	10	18	9

* Strains belonging to single (S) types (n=27) were found only in one sampling occasion out of the 14 weeks sampling.

Typing of the isolates from STPs showed the presence of four C-types (n=26) and three S-types in STP1 and three C-types (n=20) and five S-types in STP2 (Table 23). Again, some strains were found in more than one occasion in samples collected from STPs with strains belonging to CT1 (in STP1) found on all four occasions (Table 23).

Table 23. Presence of different clones of ESBL producing strains of *E. coli* in samples collected from STP1 and STP2 over 4 weeks of samplings. Red dots indicate the presence of the same bacterial strain in wastewater sample in each week (W1-W4). C-type: Common type (strains that were found in more than one sampling occasion). The number of isolates for C-type is given in brackets.

	Types	No. of Strains in Each C-type over 4 weeks (W)			
Source	(no. of isolates)	W1	W2	W3	W4
STP1	CT1 (11)	●(3)	●(3)	●(3)	●(2)
STP1	CT2 (6)	●(5)	●(1)		
STP1	CT3 (4)	●(2)		●(2)	
STP1	CT4 (5)		●(2)		●(3)
STP1	S1 to S3*	●(1)		●(2)	
STP2	CT1 (4)		●(3)	●(1)	
STP2	CT2 (8)	●(7)			●(1)
STP2	CT3 (8)	●(1)	●(4)	●(3)	
STP2	S1 to S5*		●(2)	●(2)	●(1)
Total (54)		19	15	13	7

* Strains belonging to single types (n=3 in STP1 and 5 in STP2) were found only in one sampling occasion out of the 4 weeks sam pling with the numbers of types found in each week

The mean number of ESBL-producing *E. coli* found at each sampling occasion from HWW (14.1±1.2) was significantly higher than those of STP isolates (7.3±2.3 for STP1 and 6.3±2.7 for STP2) ($p<0.0001$).

6.2.3. Antibiotic Resistance among HWW and STP Isolates

Strains of each C-type isolated in different weeks (44 isolates from HWW samples and 25 isolates from both STPs samples) were tested for their resistance against 17 antimicrobial drugs (see section 6.1.3 and 6.1.4).

Amongst the HWW isolates, apart from cephalosporins and monobactam antibiotics, the highest resistance was observed against sulphafurazole and gentamicin (100% each), tetracycline (99%) and imipenem (92%) (Table 24). Amongst the STPs isolates, apart from the cephalosporins and monobactam antibiotics, the highest resistance was observed against sulphafurazole (100%), tetracycline (78%), nalixidic acid (46%) and norfloxacin (41%) (Table 24). Strains isolated from HWW were significantly ($p<0.0001$) more resistant to amoxicillin/ clavulanic acid, cefotetan, imipenem, gentamicin, amikacin and tetracycline than the STPs strains (Table 24). In contrast, strains isolated from the STPs were significantly ($p<0.0001$) more resistant to ciproflaoxacin, norfloxacin and nalidixic acid than UHWW strains (Table 24).

Table 24. Percentage of antibiotic-resistant ESBL producing strains in samples from HWW and STPs. Only antibiotics that showed significant differences between two sources are shown.

	Resistant to (%)								
Sources	AMC	CTT	IPM	GEN	AMK	TET	CIP	NOR	NAL
HWW	91%[a]	94%[a]	92%[a]	100%[a]	51%[a]	99%[a]	0%	0%	3%
STPs	33%	13%	9%	17%	11%	78%	24%[b]	41%[b]	46%[b]

AMC, amoxicillin-clavulanic acid; CTT, cefotetan; IPM, imipenem; GEN, gentamicin; AMK, amikacin; TET, tetracycline; CIP, ciprofloxacin; NOR, norfloxacin, NAL, nalixidic acid;
[a]$p<0.0001$ for hospital versus STP strains
[b]$p<0.0001$ for STPs versus hospital strains

Generally, strains belonging to each C-type showed the same pattern of resistance over 14 weeks. However, there were occasional differences in the resistance of the strains in some samples with the highest difference observed against amikacin (AMK), chloramphenicol (CHL) and nitrofurantoin (NIT) (Table 25). These changes have been highlighted in Table 25 where resistance of the strains to these antibiotics in each week was compared to those observed for the same strains found in week 1 (Table 25).

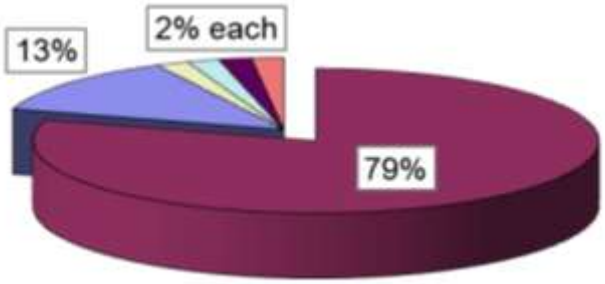
Table 25. Phylogenetic groups (PGG) and antibiotic resistance pattern of ESBL-producing *E. coli* strains belonging to seven common types (CT), found in hospital 1 wastewater over 14 weeks (W1-W14) of sampling. Green colour boxes show sensitivity (S) to antibiotics of the same strains after the first week of sampling and orange boxes show resistance (R) to antibiotics of the same strains after the first week of sampling.

C-types-Week (no of isolates)	PGG	Antimicrobial agents																
		AMC	CAZ	CTX	ATM	FEP	CTT	FOX	IPM	GEN	AMK	TET	RL	CIP	NOR	NAL	NIT	CHL
CT1-W1 (1)	B2	S	R	R	R	R	S	S	S	R	R	R	R	S	S	R	R	S
CT1-W2 (4)	B2	S	S	R	R	S	S	S	S	R	R	R	R	S	S	R	R	S
CT1-W7 (3)	B2	S	R	R	R	R	S	S	S	R	S	R	R	S	S	S	S	S
CT1-W12 (2)	B2	S	R	R	R	R	S	S	S	R	S	R	R	S	S	S	S	S
CT2-W1 (3)	A	R	R	R	R	R	R	R	R	R	R	R	R	S	S	S	R	S
CT2-W2 (5)	A	R	R	R	R	R	R	R	R	R	R	R	R	S	S	S	R	S
CT2-W3 (3)	A	R	R	R	R	R	R	R	R	R	R	R	R	S	S	S	S	S
CT2-W4 (2)	A	R	R	R	R	R	R	R	R	R	R	R	R	S	S	S	S	S
CT2-W5 (7)	A	R	R	R	R	R	R	R	R	R	R	R	R	S	S	S	S	R
CT2-W6 (7)	A	R	R	R	R	R	R	R	R	R	R	R	R	S	S	S	S	R
CT2-W7 (1)	A	R	R	R	R	R	R	R	R	R	R	R	R	S	S	S	S	R
CT2-W8 (10)	A	R	R	R	R	R	R	R	R	R	R	R	R	S	S	S	S	S
CT2-W9 (8)	A	R	R	R	R	R	R	R	R	R	R	R	R	S	S	S	S	S
CT2-W10 (20)	A	R	R	R	R	R	R	R	R	R	S	R	R	S	S	S	S	R
CT2-W11 (7)	A	R	R	R	R	R	R	R	R	R	S	R	R	S	S	S	S	S
CT2-W12 (3)	A	R	R	R	R	R	R	R	R	R	S	R	R	S	S	S	S	S
CT2-W13 (11)	A	R	R	R	R	R	R	R	R	R	S	R	R	S	S	S	S	S
CT2-W14 (1)	A	R	R	R	R	R	R	R	R	R	S	R	R	S	S	S	S	S
CT3-W4 (2)	A	R	R	R	R	R	R	R	R	R	R	R	R	S	S	S	S	R
CT3-W5 (2)	A	R	R	R	R	R	R	R	R	R	R	R	R	S	S	S	S	S
CT3-W8 (3)	A	R	R	R	R	R	R	R	R	R	S	R	R	S	S	S	S	S
CT4-W1 (1)	A	S	R	R	R	R	S	S	S	R	S	S	R	S	S	S	S	S
CT4-W4 (3)	A	R	R	R	R	R	R	R	R	R	S	R	R	S	S	S	S	R
CT4-W9 (3)	A	R	R	R	R	R	R	R	R	R	S	R	R	S	S	S	S	R
CT4-W10 (2)	A	R	R	R	R	R	R	R	R	R	R	R	R	S	S	S	S	S
CT4-W11 (2)	A	S	R	R	R	R	R	R	S	R	S	R	R	S	S	S	S	R
CT4-W13 (1)	A	R	R	R	R	R	R	R	S	R	S	R	R	S	S	S	S	R
CT5-W1 (3)	A	R	R	R	R	R	R	R	R	R	R	R	R	S	S	S	S	S
CT5-W3 (7)	A	R	R	R	R	R	R	R	R	R	R	R	R	S	S	S	S	S
CT5-W4 (8)	A	R	R	R	R	R	R	R	R	R	R	R	R	S	S	S	S	S
CT5-W5 (2)	A	R	R	R	R	R	R	R	R	R	S	R	R	S	S	S	S	R
CT5-W7 (2)	A	R	R	R	R	R	R	R	R	R	S	R	R	S	S	S	S	S
CT6-W1 (3)	A	R	R	R	R	R	R	R	R	R	R	R	R	S	S	S	S	S
CT6-W6 (3)	A	R	R	R	R	R	R	R	R	R	R	R	R	S	S	S	S	S
CT6-W8 (5)	A	R	R	R	R	R	R	R	R	R	R	R	R	S	S	S	S	S
CT6-W9 (3)	A	R	R	R	R	R	R	R	R	R	S	R	R	S	S	S	S	S
CT6-W13 (2)	A	R	R	R	R	R	R	R	R	R	S	R	R	S	S	S	S	S
CT6-W14 (4)	A	R	R	R	R	R	R	R	R	R	S	R	R	S	S	S	S	S
CT7-W1 (1)	A	R	R	R	R	R	R	R	R	R	R	R	R	S	S	S	R	S
CT7-W2 (3)	A	R	R	R	R	R	R	R	R	R	S	R	R	S	S	S	S	S
CT7-W5 (1)	A	R	R	R	R	R	R	R	R	R	R	R	R	S	S	S	R	R
CT7-W10 (2)	A	R	R	R	R	R	R	R	R	R	S	R	R	S	S	S	S	S
CT7-W12 (4)	A	R	R	R	R	R	R	R	R	R	S	R	R	S	S	S	S	S
CT7-W13 (2)	A	R	R	R	R	R	R	R	R	R	S	R	R	S	S	S	S	S

AMC, amoxicillin-clavulanic acid; CAZ, ceftazidime; CTX, cefotaxime; ATM, aztreonam; FEP, cefepime; CTT, cefotetan; FOX, cefoxitin; IPM, imipenem; GEN, gentamicin; AMK, amikacin; TET, tetracycline; RL, sulphamethoxazole; CIP, ciprofloxacin; NOR, norfloxacin, NAL , nalixidic acid; NIT, nitrofurantoin; CHL,chloramphenicol.

6.2.4. Antibiotic Resistance among Enterococci in HWW

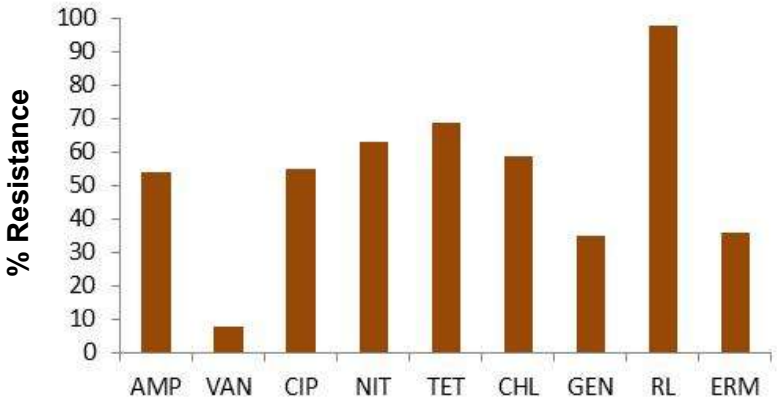
In all, 101 strains of enterococci were isolated during the 14 rounds of sampling from wastewater of this hospital. These strains were identified to the species level using PCR with species specific primers. More than 79% of the isolates were *E. faecalis* and 13% were identified as *E. faecium*. The remaining strains were in small quantities and distributed evenly among the samples (Figure 22). Samples from STPs were not tested for the presence of enterococci.



E. faecalis ■, *E. hirae* ■, *E. faecium* ■, *E. gallinarum* ■, *E. mundtii* ■, *E. caseliflavus* ■

Figure 22. Distribution of enterococci species among samples collected from wastewater of hospital 1.

Among the nine antimicrobial drugs tested for these strains, the highest resistance (98%) was observed against sulfamethoxazole (RL) and the lowest one (6%) against vancomycin. Resistance to other antimicrobial drugs varied and ranged from 32% against gentamycin to 67% against tetracycline (Figure 23).



AMP: ampicillin, VAN: vancomycin, CIP: ciprofloxacin, NIT: nitrofurantoin, TET: tetracycline, GEN: gentamycin, RL: Sulfamethoxazole, ERM: erythromycin.

Figure 23. Percentage of antibiotic resistance among enterococci isolated from HWW.

6.3. Discussion

The presence of antibiotic resistant bacteria in the community and the environment, as well as their ecotoxic effects, continues to be a vexing problem for public health authorities. Over the past decade, the prevalence of *E. coli* strains with or without the ability to produce ESBL enzymes has attracted the attention of health authorities as reports indicate their presence not only in patients (Bradford, 2001; Rodrigues-Bano *et al.*, 2006) but in healthy individuals (Valverde *et al.*, 2004), animals (Mesa *et al.*,

2006) and the environment (Kümmerer, 2009a, Diwan *et al.*, 2010). In the present study, we postulated that hospital wastewaters and STPs can serve as sources of resistant *E. coli* strains capable of producing ESBL enzymes and therefore, characterisation of these strains will provide a better understanding of the spread of antibiotic resistant genes and their sources outside hospitals.

Resistance to several antimicrobial agents in *E. coli* isolated from hospital wastewaters and the STPs has been reported elsewhere (Kümmerer, 2009a, Diwan *et al.*, 2010). However little data are available on ESBL-producing *E. coli* in HWW and community STPs. Our results indicate the presence of different clonal groups of ESBL and non-ESBL producing *E. coli* strains in both HWW and STP influents, some of which were resistant to many of the tested antimicrobial agents including imipenem. In this study, we showed much higher percentages of ESBL-producing *E. coli* in hospital wastewaters than in STPs influent. Whilst it is uncertain whether the presence of antibiotics in the aquatic environment would result in development of resistance in bacteria, it has been shown that antibiotic resistant strains might be selected or favoured by the presence of high level of antibiotics residues in hospital wastewater (Baquero *et al.*, 2008). Despite the fact that beta-lactam antibiotics are one of the major groups of antibiotics prescribed in hospitals, they have only been detected in a limited number of studies and to a lesser extent in hospital wastewater (Watkinson *et al.*, 2009). This is probably due to easy degradation of the beta-lactam ring, its high metabolic rate and the process of decarbocxylation (Kümmerer, 2009).

In our study, the presence of high level of ESBL-producing *E. coli* in the hospital wastewater could mainly be due to the persistence of these strains in the hospital environment and their regular discharge in hospital wastewater. This view is supported by the findings of Römling *et al*. (1994) who demonstrated the predominance of a clone of *Pseudomonas aeruginosa* in a hospital and the aquatic environment and postulated that this might be due to the selective advantage of the clone in that hospital and its overspread in soil and aquatic environment. Our results also suggest that resistant strains can become dominant in a hospital as they constituted a high percentage of multidrug resistant *E. coli* strains in hospital wastewater. Using a combination of different typing methods to identify clonal groups of ESBL-producing strains in both sources we found that a majority (86%) of the isolates in HWW belonged to a few C-types based on the typing methods used. Strains of these C-types were frequently found in weekly samples with the most common clonal type, H-CT2, constituting 44% (88 out of 198 isolates tested) of the hospital strains.

In this study, we also looked for the presence and prevalence of ESBL-producing *E. coli* in STPs that did not receive wastewater from hospitals in order to obtain an estimation of the prevalence of ESBL producing strains in municipal STPs. Due to logistical problems we limited our sampling to only four weeks but extended our search to two STPs. We noticed diverse types of *E. coli* strains with or without the ability to produce ESBL enzymes, most of which were found only in one sampling occasion (S-types). This was somehow expected as municipal STPs normally serve a high population ranging from hundreds of thousands to millions of residents (Payment *et al.*, 2001) and as such the population of *E. coli* strains in these treatment plants should be quite diverse (Escobar-Paramo *et al.*, 2004).

Despite this, we found the presence of the same clonal types (four in STP1 and three in STP2) on more than one occasion, indicating that either these strains are commonly introduced into the STP or they have a better ability to survive the STP or a combination of both. This finding also indicates that a community can also serve as a source of antibiotic resistant bacteria *E. coli,* with some clonal type, being more common or persistent in the community and/or STPs. More studies however, are required to identify the extent to which communities contribute to the load of ARB found in STPs.

ESBL producing strains are known to be increasingly associated with resistance to non-beta lactam antimicrobials due to different mechanisms such as expression of the related genes on plasmid (Poirel *et al.*, 2006), or loss of an outer membrane porin protein (Elliott *et al.*, 2006). Strains isolated in our study were also highly resistant against unrelated antimicrobial drugs including imipenem (carbapenems), cefepime and beta-lactam/beta-lactam inhibitor combination drugs. To our knowledge, this is the highest resistance against imipenem found among ESBL-producing *E. coli* isolated from hospital wastewater and STPs.

One major concern about the high level of ESBL-producing *E. coli* in hospital wastewater is the presence and transfer of conjugative plasmids in these strains, which also carry genes for resistance to aminoglucosides and sulphonamides (Paterson, 2006). In our study, the highest resistance was observed against sulphonamide in both the HWW and the STPs with resistance to aminoglicosides also found frequently among strains from hospital wastewater. Watkinson *et al.* (2007) have found that ciprofloxacin was one of the dominant antibiotics detected in wastewater influents in Queensland (Watkinson *et al.*, 2007). In our study we did not find any resistance to ciprofloxacin among our isolates from hospital wastewater and except for one common type in STP1 (i.e. STP1-CT2), none of the STP isolates were resistant to this antibiotic either. Considering the long duration of our sampling from hospital wastewater (14 weeks) it is possible that some changes might have occurred in bacterial population in the hospital and therefore in our samples.

6.3.1. Antibiotic Resistance among Enterococci

Enterococci have emerged as important nosocomial pathogens in many countries mainly because they have intrinsic resistance to several antimicrobial agents (Huycke *et al.*, 1998). Unlike acquired resistance and virulence (traits which are usually transposon or plasmid encoded), intrinsic resistance is based in chromosomal genes, which typically are non-transferrable (Mundy *et al.*, 2000). Several species of this genus are now found as a causative agent of nosocomial infection, with *E. faecalis* being the most commonly found species. It has been postulated that these strains can survive not only in the hospital environment where antibiotics are commonly used but they may also disseminate to the environment. Despite this, very little information is available on their presence and prevalence in hospital wastewater. In our study, *E. faecalis* dominated (79%) HWW isolates and were consistently isolated over the 14 weeks of sampling. Some of the strains belonging to this species were multi-resistant to seven of the nine antibiotics tested. Although we did not type these strains to identify their persistence in HWW, the identical pattern of antibiotic resistance among certain strains suggested that they might belong to the same clonal group. The highest resistance among these strains were found against sulfamethoxazole and up to 65% resistant to other antibiotics. Resistance to vancomycin was very low and was found in only four *E. faecalis* and two *E. faecium* strains which was the second largest group of these strains.

6.3.2. Conclusion

Our data demonstrated the presence of certain clonal groups of *E. coli* in both HWW and STP influents. These strains were also resistant to several non-beta-lactam antibiotics. In general, strains found in HWW were significantly more resistant to beta-lactam inhibitor combination drugs such as cephamycin and carbapenem than STP strains which might be the result of high usage of these antibiotics in hospitals. In fact, these antimicrobial agents are among the most commonly used drugs in human medicine and according to the most recent report from the Joint Expert Advisory Committee on Antibiotic Resistance between 1992 and 1997 (JETACAR, 1998) the average use of beta-lactam inhibitors, carbapenems and cephalosporins in Australia was 40.000 kg per annum. More than 90% resistance was found against amoxicillin-clavulanic acid, cefotetan, imipenem, gentamicin, and tetracycline among the *E. coli* strains isolated from HWW. In contrast, there was a lower resistance ranging from 9% to 78% against these antibiotics among STP isolates. We also found that there was very little change in the pattern of antibiotic resistance of *E. coli* strains in HWW over time. Resistance to antibiotics among enterococci isolates was far less than those found among *E. coli.*

7. EXPERIMENTAL APPROACH (II): CASE STUDY 2

7.1. Description of Sites and Sampling

Hospital 2 was selected to further extend our earlier observation on the presence of antibiotic resistant bacteria (ARB) in hospital wastewater and to investigate their survival during transition to the receiving STP and after biological treatment and chlorination. The STP was an activated sludge plant with N and P reduction and services an equivalent population of 130,000 and has a 12-13 day sludge age. Weekly grab samples were collected from a sewer exclusively collecting wastewater from hospital 2 for 8 weeks at 10.30a.m and at 11.00am of the same day from its receiving STP. Grab samples were collected from the incoming raw sewage and treated effluent after the activated sludge treatment and chlorination. The final effluent is discharged to a nearby waterway.

All hospital wastewater and STP samples were processed in accordance with the Australian and New Zealand Standards for Water Microbiology and Water Quality Sampling (Australian and New Zealand Standards, 2007; Australian and New Zealand Standards 1998). In brief, wastewaters were collected in 500ml sterile microbiological containers mounted onto a handle of appropriate length using "grab-sampling" technique. They were transported to the laboratory on ice and processed within 4 hours of collection.

7.2. Isolation and Identification of Bacterial Strains

Hospital wastewater and STP incoming influent (SI) samples were processed using serial dilutions and the STP outgoing effluent (SO) was processed by membrane filtration. For isolation of *Staphylococcus aureus* (SA) and methicillin resistant *S. aureus* (MRSA), direct and filtered samples were cultured on Vogel-Johnson agar with and without 6 μg mL^{-1} of cefoxitin, as a surrogate for methicillin (Broekema *et al.*, 2009). After 24-48 hours incubation at 37°C, suspected *S. aureus* colonies that showed a positive catalase reaction were transferred to nutrient broth containing 20% glycerol and stored at -80°C for further analysis.

Identification of *S. aureus* strains was done after DNA extraction of the isolates using species-specific primers for the *nuc*A gene, which codes for thermo-stable nuclease specific to *S. aureus* as described before (Pinto *et al.*, 2005).

Isolation and identification of Gram-negative strains was done by cultivating the same samples on three MacConkey agar no. 3 plates (Oxoid). From each plate, up to 30 morphologically distinct colonies (where possible) were picked and identified to the species level using API-20E tests. The 9 digit profile obtained from API system was used to identify bacterial strains using identification software of the API. Suspected strains were further tested using species –specific primers.

7.3. Antibiotic Resistance Testing

7.3.1. *S. aureus* and MRSA

Using the method of Clinical Laboratory Standard Institute (CLSI) (Clinical Laboratory Standards Institute, 2011), all *S. aureus* strains isolated from hospital wastewaters, SI and SO were tested for the their resistance against eight antimicrobial agents using the following antimicrobial impregnated disks (Oxoid): tetracycline (30μg), amoxycillin-clavulonic acid (20/10μg), ampicillin (10μg), gentamicin (10μg), ciprofloxacin (5μg), chloramphenicol (30μg), amikacin (30μg), cefoxitin (30μg) (a surrogate for methicillin). All isolates were also tested for their resistance against vancomycin using the agar dilution method recommended by CLSI standards (Clinical Laboratory Standards Institute, 2011) with 8 μg mL^{-1} and 32 μg mL^{-1} vancomycin. For isolates which exhibited resistance against cefoxitin and vancomycin (32 μg/ml), minimum inhibitory concentration (MIC) was determined using the E-test strips (Oxoid M.I.C.E. test) (Brown and Brown, 1991) for the antibiotics oxacillin (representing methicillin resistance) and vancomycin as per manufacturer instructions. All *S. aureus* strains were tested for their resistance to methicillin based on the presence of the *mec*A gene that codes for

production of penicillin binding protein 2 according to the method described before (Yadgar *et al.*, 2009).

7.3.2. PCR Confirmation of Methicillin Resistance Gene

In view of the discrepancies found in literature re-presence or absence of *mec*A gene in MRSA isolates all *S. aureus* strains were tested for the presence of *mec*A gene. The primer sequences used were F 5'-CCTAGTAAAGCTCCGGAA-3' and R 5'-CTAGTCCATTCGGTCCA-3' (Yadgar *et al.*, 2009) which generates a 314 base pair fragment. PCR amplification was performed as described by Yadgar *et al.* (2009) using a reaction mixture containing a master mix of 10.75µL of filter-sterilised Milli-Q water, 2.5µL of 10x PCR buffer (Bioline), 0.25µL dNTP (10mM)(Fisher Biotech), 1.5µL MgCl2 (50mM)(Bioline), 0.25µL of forward and reverse *mec*A primers (10µM) (Invitrogen), 0.2µL Taq Polymerase (5U/uL) (Bioline) and 2µL of purified DNA. The PCR reaction cycle consisted of; denaturation for 5 minutes at 95°C; 35 cycles of 2 minutes at 95°C, one minute at 58°C and one minute at 72°C; and a final extension step of 10 minutes at 72°C. Amplified PCR product was electrophoresed and bands were visualised as described above.

7.3.3. Antibiotic Susceptibility of Gram-Negative Strains

All isolates were tested for their resistance against 14 commonly used antimicrobial drugs according to the CDS method (Bell *et al.*, 2006). These included: ampicillin (AMP 32 µg), ceftazidime (CAZ 16 µg), imipenem (IPM 4 µg), aztreonam (ATM 16 µg), piperacillin-tazobactam (TZP 128 µg), gentamicin (GEN 16 µg), nalidixic acid (NAL 32 µg), trimethoprim-sulphafurazole (TSF 4/76 µg), tetracycline (TET 16 µg), cefoxitin (CXT 32 µg), amikacin 64 µg), streptomycin (64 µg), ciprofloxacin (4 µg) and chloramphenicol (32 µg). All susceptibility tests were calibrated for the chosen antimicrobial drugs using *E. coli* strain ATCC 25922 for quality control. The CDS method reports antimicrobial drug susceptibilities as either "susceptible" or "resistant" and therefore the results were not expressed as minimum inhibitory concentration (MIC) values, and interpreted as susceptible (S) or resistant (R). Isolates with resistance to two or more antimicrobial drugs were regarded as multidrug resistant (MDR) (Bartoloni *et al.*, 2004).

7.3.4. Typing of Isolates

All isolates were typed using a high-resolution biochemical fingerprinting method specifically developed for typing of staphylococci (PhP-CS plate), *E. coli* (PhP-RE plates), *Klebsiella* and *Enterobacter* (PhP-KL plates) according to the manufacturer instruction (PhPlate microplate techniques AB, Sweden) (see section 7.1. 5) and Random Amplified Polymorphism DNA (RAPD)-PCR as described before (Naffa *et al.*, 2006). The random sequence primers used were chosen for each bacterial species (e.g. S 5'-TCACGATGCA-3' for *S. aureus*; or 5'-ACACGCACACGGAAGAA-3' for *E. coli*). RAPD-PCR bands were scored with data coded as a factor of 1 or 0, representing presence or absence of each band. Using the PhP software (version 4.2), the banding patterns obtained were compared pair wise and clustered as described. Isolates belonging to the same PhP-RAPD type were considered as members of the same clonal group and classified as common (C) types.

7.4. Results

7.4.1. Antibiotic Resistance among *S. aureus* and MRSA Strains

Overall, 167 *S. aureus* strains were isolated from hospital 2 wastewater (HWW) (n=85) and its receiving STP inlet (SI) (n=74) and outlet (SO) (n=8). Typing of these isolates showed the presence of 10 common (C) (n=61) and 106 single (S) PhP-RAPD types altogether (Table 26). Strains from HWW and its receiving STP consisted of 10 C-types and 106 S-types. These S-types included strains that were present in hospital wastewater but never found in STP samples. Although strains belonging to the common type 1 (i.e. C1) were found in several sampling occasions in HWW, they were never found in STP samples and were regarded as those unable to survive transition to STP. Similarly strains belonging to C-types 2-6 and 9 and 10, were found in HWW and STP inlet samples, however these

strains disappeared during the STP treatment process (including chlorination) and were never found in samples collected from the STP outlet. Only two C-types (i.e. C7 and C8) were found not only in HWW but also in the STP inlet as well as the outlet and were regarded as persistent C-types (Table 26). All isolates belonging to these C-types were resistant to a number of antibiotics and were all resistant to methicillin (i.e. *mec*A positive). Among the isolates belonging to C-types, 9 isolates were also vancomycin resistant (VRSA; n=2) or intermediate resistant VRSA (n=7) and one of them belonging to common type 8 (C8) was also found in STP outlet samples.

Table 26. Common (C) and persistent types of *S. aureus* and MRSA strains found in hospital 2 wastewater and its receiving sewage treatment plant (STP). W1-8: Weeks 1-8; STP-I : STP influent, STP-O: STP outlet.

C-type	Sources	W1	W2	W3	W4	W5	W6	W7	W8
C1	HWW	●	●	●		●			
C2	HWW and STP-I	●							●
C3	HWW and STP-I		●	●		●	●		
C4	HWW and STP-I			●			●		
C5	HWW and STP-I		●		●	●	●	●	
C6	HWW and STP-I			●		●	●	●	
C7	HWW and STP-I and STP-O				●	●	●	●	
C8	HWW and STP-I and STP-O			●		●	●	●	
C9	HWW and STP-I	●		●		●	●	●	
C10	HWW and STP-I			●		●	●	●	

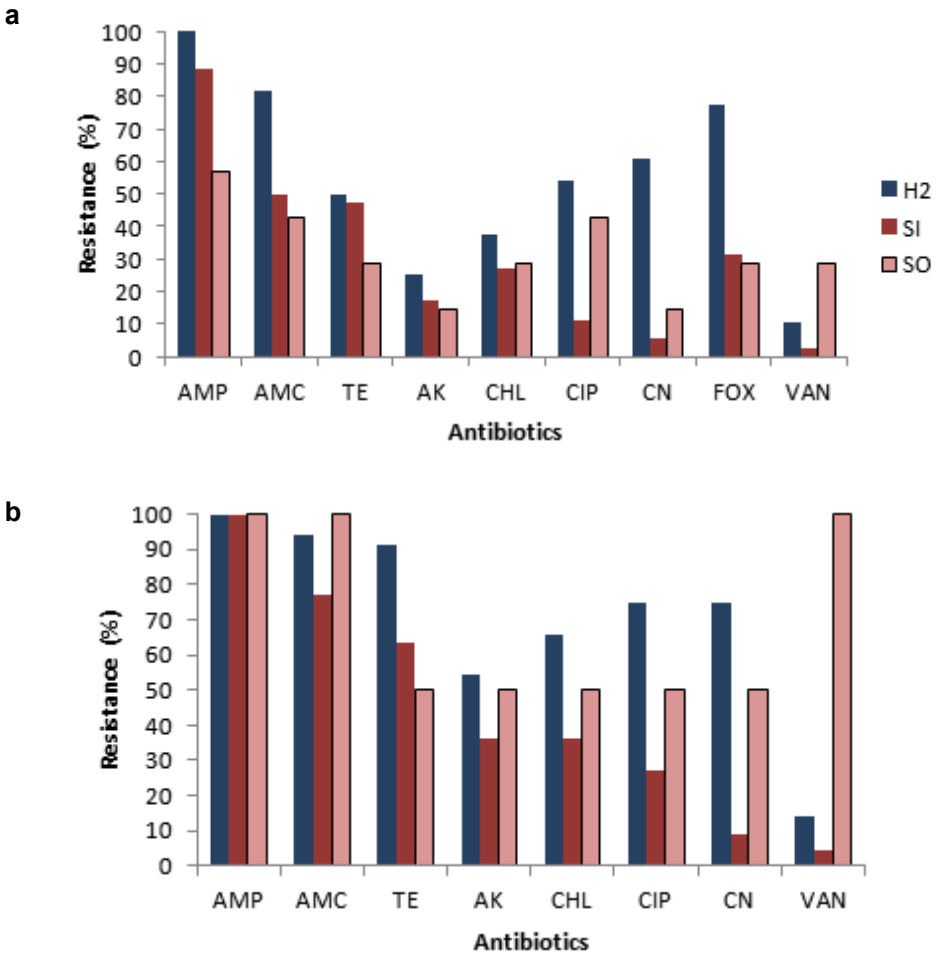
7.4.2. Antibiotic Resistant Patterns of *S. aureus* from Hospital Wastewater and STP

The pattern of antibiotic resistance among the *S.aureus* strains varied in samples collected from different sites. In all, 137 (82%) out of the 167 *S.aureus* strains tested from wastewater of this hospital and its receiving STP were resistant to between two to nine antibiotics (multidrug resistant) of which 97 (71%) strains were MRSA as shown by their phenotypic resistance against cefoxitin and the presence of *mec*A gene (Table 27) with 24 being also VRSA (data not shown).

Within the strains isolated from wastewaters the highest resistance was observed towards ampicillin (100) followed by amoxicillin/clavulonic acid (82%), gentamicin (76%) and cefoxitin (78%) (Figure 24a). Almost similar pattern of resistance was observed when only MRSA positive strains were considered (Figure 24b). Strains from hospital wastewater consisted of the highest number of MRSA as indicated by their resistance to cefoxitin (78%) compared to those from STP-I (31%) and STP-O (29%) (Figure 24a).

Table 27. Antimicrobial resistance patterns for *S. aureus* (including MRSA strains) isolated from hospital 2 wastewater and its receiving STP. STP-I : STP inlet influent, STP-O : STP outlet.

Resistance to Antibiotics	Total no. of Isolates	Source and no. of Isolates		
		HWW	STP-I	STP-O
None	6	0	6	0
1	24	7	16	1
2	23	7	11	5
3	12	4	8	0
4	25	2	22	1
5	32	27	5	0
6	30	29	1	0
7	11	6	5	0
8	2	2	0	0
9	2	1	0	1
Total	167	85	74	8



AMC=amoxicillin/clavulonic acid, AMP=ampicillin, AK=amikacin, CN=gentamicin, CIP=ciprofloxacin, CHL=chloramphenicol, FOX=cefoxitin, VAN=vancomycin, TE=tetracycline.

Figure 24. Prevalence of antibiotic resistant *S. aureus* strains (A) and antibiotic resistance among MRSA (methicillin resistant *S. aureus*) strains (B) isolated from hospital 2 wastewater (H2) and its receiving STP inlet (SI) and outlet (SO).

7.4.3. Antibiotic Resistance among Gram-Negative Strains

In all, 364 strains of Gram-negative bacteria belonging to different species were isolated from hospital wastewater and its receiving STP (inlet and outlet). Samples from HWW were dominated by four species of Gram-negative strains. These included *Pseudomonas* spp. (35%), *E. coli* (25%), *Klebsiella* spp. (15%) and *Enterobacter* spp. (12%) (Figure 25a). In contrast, samples collected from STP were mainly dominated by *E. coli* (38%) and Pseudomonas spp. (24%) (Figure 25b), suggesting that these strains have better ability to reach the STP once they are released in HWW.

These strains were also found in samples collected from the STP-outlet indicating that they also have a better ability to survive the treatment process of a STP. The type and the number of Gram-negative bacteria strains collected from HWW and STP samples are provided in Table 28.

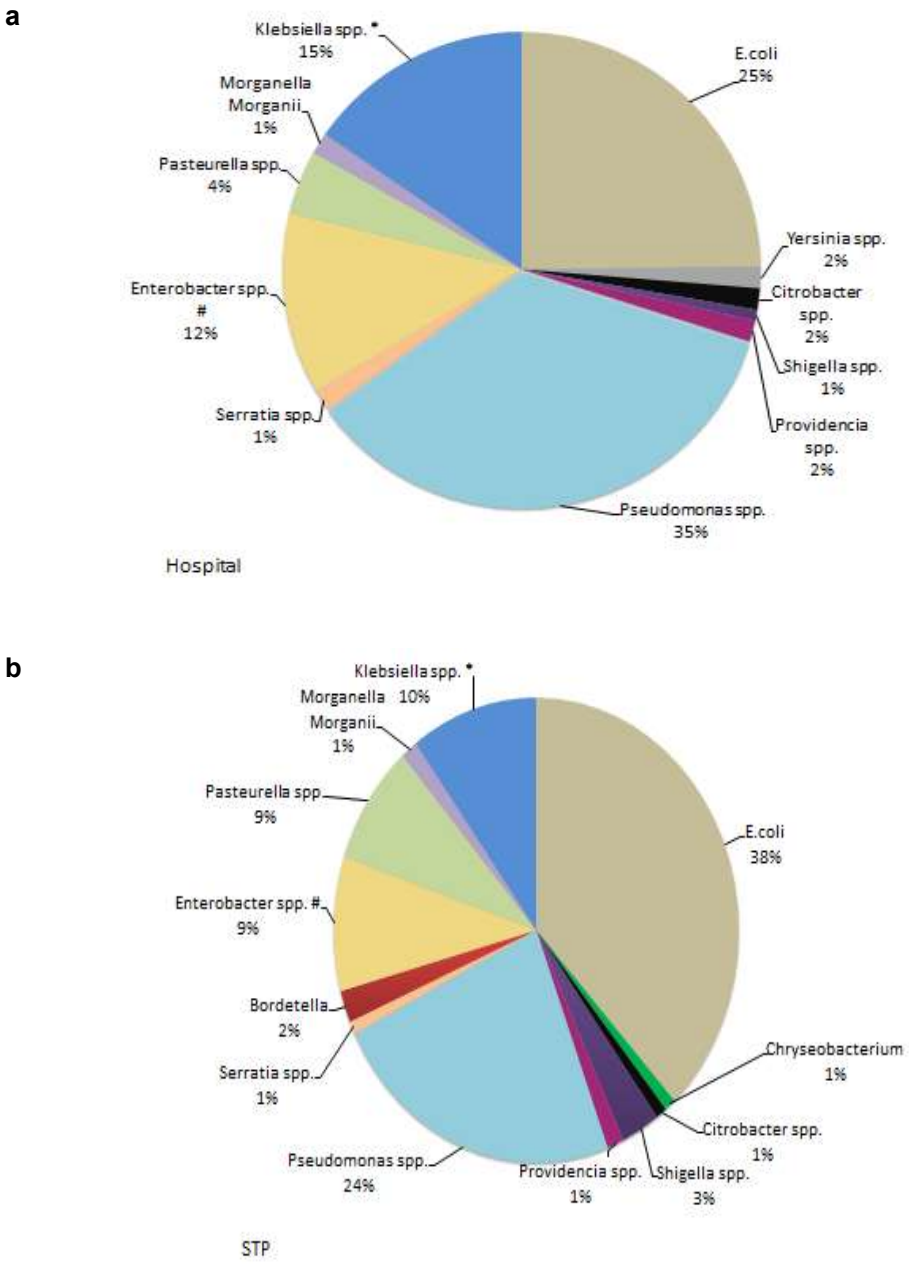


Figure 25 Distribution of Gram-negative bacterial species found in hospital 2 wastewater (a) and STP inlet and outlet effluent (b).

Table 28. The number of Gram-negative bacterial strains found in HWW and it STP (inlet and outlet).

Bacterial species	Site where they were isolated (no.)			Total number
	HWW	STP-I	STP-O	
E. coli	34	55	31	120
Klebiella spp.	21	19	4	44
Pseudomonas spp.	49	42	11	102
Enterobacter spp.	17	18	3	38
Pasteurella spp.	6	18	1	25
Other minor species	11	16	8	35

A high level of resistance was found against the 14 antibiotics tested among all Gram-negative strains however, strains isolated from HWW showed to be resistant to more number of antibiotics than those isolated from STP (Table 29 to Table 31). All *E. coli* strains from HWW were resistant to more than 7 out of the 14 antibiotics tested. This figure for STP isolates was 76% (Table 29). Almost similar data were obtained for *Klebsiella* strains (100% for HWW isolates versus 74% for STP isolates) or *Enterobacter* spp. (100% vs 67%) (Table 30). Of these however, only *Klebsiella* and *Enterobacter* strains from HWW showed to be resistant to significantly more number of antibiotics than those found in STP.

Table 29. Distribution of the *E. coli* and *Klebsiella* strains isolated from HWW and STP-I and STP-O based on the number of antibiotics to which they were resistant.

Resistance (no. of antibiotics)	*E. coli*			Klebsiella spp.		
	HWW	STP-I	STP-O	HWW	STP-I	STP-O
None	0	0	0	0	0	0
1	0	0	0	0	0	0
2	0	0	0	0	0	0
3	0	1	0	0	1	0
4	1	1	2	0	3	0
5	0	5	4	0	0	0
6	0	6	8	0	1	2
7	4	14	3	0	3	1
8	7	5	4	1	2	0
9	12	4	5	3	4	0
10	8	9	3	1	0	0
11	1	4	2	6	2	0
12	1	4	0	9	0	1
13	0	1	0	1	2	0
14	0	1	0	0	1	0

As shown in Figure 25, the strains isolated from STP were more diverse than the ones found in HWW with strains being resistant to a wider number of antibiotics. Interestingly, whilst some of the strains found in STP were resistant to only two or three antibiotics, some were resistant to a much higher number of antibiotics than HWW strains. This was seen with *E. coli*, *Klebsiella* spp. and *Pseudomonas* spp. (Table 29 and Table 30). It is possible that these strains were either sourced from the community or escaped our detection in HWW.

One interesting finding was that almost all Gram-negative strains tested for were not only found in STP inlet samples but were also found in samples collected from STP outlet. In most cases, these strains carried the same pattern of antibiotic resistance and were resistant to the same number of

antibiotics although some strains that were resistant to lesser number of antibiotics (community strains) were also found in samples from STP outlet (Table 29 to Table 31). This may indicate that not only Gram-negative strains from HWW can survive the STP treatment process but community strains may also have the ability to survive sewage treatment plants to be released into the environment. The number of such strains however was much lower than highly resistant ones (Table 29 to Table 31).

Table 30. Distribution of the *Pseudomonas* and *Enterobacter* strains isolated from HWW and STP-I and STP-O based on the number of antibiotics to which they were resistant.

Resistance (no. of antibiotics)	Pseudomonas spp.			Enterobacter spp.		
	HWW	STP-I	STP-O	HWW	STP-I	STP-O
None	0	0	0	0	0	0
1	0	0	0	0	0	0
2	0	1	0	0	0	0
3	0	0	0	0	1	0
4	0	0	0	0	2	1
5	0	0	0	0	3	0
6	1	2	2	0	0	0
7	2	7	3	1	3	0
8	2	8	3	3	2	0
9	14	9	2	2	2	2
10	9	6	0	3	3	0
11	9	5	1	2	0	0
12	8	3	0	5	2	0
13	3	0	0	1	0	0
14	0	1	0	0	0	0

Table 31. Distribution of the *Pasteurella* and other minor groups of Gram-negative strains isolated from HWW and STP-I and STP-O based on the number of antibiotics to which they were resistant

Resistance (no. of antibiotics)	Pasteurella spp.			Other Gram-negative spp.		
	HWW	STP-I	STP-O	HWW	STP-I	STP-O
None	0	0	0	0	0	0
1	0	0	0	0	0	0
2	0	0	0	0	0	0
3	1	0	0	0	0	1
4	0	1	0	0	0	2
5	0	1	0	0	0	1
6	0	0	0	1	3	0
7	0	5	1	0	3	0
8	0	0	0	1	2	0
9	2	5	0	2	3	2
10	0	2	0	4	1	1
11	2	2	0	2	1	2
12	0	2	0	1	2	0
13	1	0	0	0	1	0
14	0	0	0	0	0	0

7.5. Discussion and Conclusion

To our knowledge, this is the first study that traces the movement and survival of bacteria from hospital wastewater to STP and its discharged effluent. Typing of the isolates using a combination of a high resolution PhP typing and RAPD-PCR confirmed that certain clonal groups of these bacteria were commonly found in wastewaters from this hospital. These strains were regarded as common types. The total number of strains belonging to C-types was always higher than the number of strains belonging to single (S) types. However, the prevalence and thus the diversity of S-types were always high in samples collected from hospital wastewaters and STP samples. We postulated that the C-types were resident strains in hospitals and therefore, likely to be constantly found in hospital wastewaters.

Two important factors to be considered in studies such as this one would be the transition time of the wastewater from hospital to the STP, and the high dilution of bacteria while in the sewer system and before they reach the STP. Based on these factors as well as the logistical problem of collecting STP samples after initial hospital sampling, we decided to extend our sampling number for eight weeks in order to increase the chance of detecting hospital strains in the incoming effluent at the STP. Using this sampling protocol we were able to isolate some of these hospital clones from the inlet of the STP and showed that they belonged to the same PhP-RAPD types. Interestingly, most of these strains had an identical or very similar antibiotic resistance pattern. Whilst the prevalence of *S. aureus* and MRSA strains in hospital wastewaters was much higher than in the STP, we found that certain strains such as *E. coli*, *Pseudomonas*, *Klebsiella* and *Enterobacter* were not only dominated in samples collected from the HWW but they also formed the dominant population of Gram-negative strains in the STP. These strains were also resistant to a high number of antibiotics in both HWW and the STP indicating that Gram-negative bacteria in HWW have a much better ability to transit to the STP and survive than Gram-positive strains such as *S. aureus*.

Of the different groups of resistant *S. aureus* strains (including those resistant to MRSA and VRSA) found in the hospital wastewaters and the incoming samples of the STP, only eight resistant strains were recovered from STP outlet samples. This finding suggests that there was a notable reduction in the number of resistant strains of *S. aureus* during their transition to the receiving STP and throughout the STP treatment processes, thus proving treatment to be quite effective in this pathogen's removal. However, this was not the case with Gram-negative strains as almost all Gram-negative strains tested in our study were recovered from the STP outlet.

Hospitals present an environment for a concentrated source of resistant bacteria, which may be released into the sewer system. It is therefore important that any study investigating the prevalence of antibiotic resistant bacteria in hospital wastewater consider factors that impact the level of antibiotic resistant bacteria in such wastewaters. For instance, it is likely that some of these antibiotic resistant bacteria are sourced from community effluents upstream of the hospital since the bulk of antibiotic treatment in the community would occur at home. In this study, we found several Gram-negative strains having a much a higher level of antibiotic resistance than those found in HWW suggesting that they might have originated from community sources.

In conclusion, we found common types of Gram-positive and Gram-negative strains in untreated hospital wastewater and the same types at the STP inlet. This suggests that these common type strains were able to survive transition to the inlet of the STP. However, Gram-negative strains were found to survive the sewage treatment plant process far better than Gram-positive strains. These strains were resistant to high number of antibiotics and in our study the mean number of antibiotics to which they were resistant was 8.9 antibiotics for Gram-negative and 5.1 for Gram-positive bacteria. These strains were frequently present in hospital wastewater suggesting their persistence in the hospital environment. Our study also indicated that resistant strains are unlikely to lose their resistance once they are released into the wastewater and after their transition to STP. The significance of this for public health is not clear and requires further work to characterise and quantify the input of multidrug resistant bacteria from hospitals compared with those originating from the general community or other wastewater related sources.

APPENDICES

A. Supporting Information for Chapter 1

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Determining the fraction of pharmaceutical residues in wastewater originating from a hospital

Christoph Ort [a,*], *Michael G. Lawrence* [a], *Julien Reungoat* [a], *Geoff Eaglesham* [b], *Steve Carter* [b], *Jurg Keller* [a]

[a] *The University of Queensland, Advanced Water Management Centre (AWMC), Brisbane, QLD 4072, Australia*
[b] *Queensland Health Forensic and Scientific Services, Organics Laboratory, QLD 4108, Australia*

Supporting information A1:

Table SI 1. Services Provided in Caboolture Hospital

Service	Description
Emergency	accident & emergency medicine
Medical	general, critical care, respiratory, thoracic and cardiac
Surgical	general, gastroenterology, gynaecology, anaesthetics, day surgery
Obstetrics	maternity services
Paediatric	paediatrics, neonatology
Specialist outpatient clinics	oncology, ante-natal, diabetic, colposcopy, cardiac, orthopaedics, general medical and surgical, gynaecology
Medical imaging and nuclear medicine	-
Pharmacy	-
Allied health	allied health services include dietetics and nutrition, occupational therapy, physiotherapy, podiatry, speech pathology, psychology, and social work

Supporting information A2:

Pharmaceuticals and Personal Care Products, Brief Method, API4000Q (provided byQHFSS, Queensland Health Forensic and Scientific Services).

Samples were filtered using a 0.45 micron syringe filter (Phenex-RC, 25mm, (Phenomenex, Torrance, CA)). Two 5 mL aliquots samples were then diluted to 50 mL with deionised water. To one of the duplicates 1 mL of formic acid 98 % - 100 % purity was added for acidic extraction. Forty mL of both duplicates were extracted through Waters Oasis HLB 60 mg (3 mL) cartridges, which were preconditioned with 5 mL each of dichloromethane, methanol and deionised water. The analytes of interest were eluted from the cartridge using 1mL Acetonitrile (2% ammonium hydroxide / 98% acetonitrile for acidic extraction) and 2mL dichloromethane.

The eluent was evaporated gently to dryness under a nitrogen gas flow and then redissolved in 0.4 mL of 15 % acetonitrile/water prior to LC-MSMS analysis, concentrating the samples 10 times. As some analytes can be present at high levels in these samples, each sample was also directly analysed by LC-MSMS without extraction. All extracted samples were spiked with a mixture of five chemicals prior to extraction to monitor the efficiency of the extraction. Table SI 2.1 shows the long-term mean recovery and standard deviation of these surrogate chemicals from all water samples analysed, ranging from pure drinking water to raw sewerage influent.

Table SI 2. Long term (> 1 year) mean recovery and standard deviation for the five surrogate chemicals.

Surrogate Chemicals	Mean % Recovery	Standard Deviation (%)
Diclofenac D_4	82	15
Carbamazepine D_{10}	98	15
Caffeine D_3	98	20
Atrazine D_5	89	16
$^{13}C_6$ 2,4-Dichlorophenoxy Acetic Acid	91	13

Prior to LC-MSMS analysis, all standards and samples were additionally spiked with four chemicals to act as internal standards in the quantitation (acetyl sulphamethoxazole D5; fluoxetine D5; 2,4 dichlorophenyl acetic acid; simazine D10). The sample extracts were analysed by HPLC/tandem Mass Spectrometry using an AB/Sciex API4000 QTrap mass spectrometer equipped with an electrospray (TurboV) interface (MDS Sciex, Concord, Ont., Canada) with each sample extract analysed separately in both positive and negative ion multiple reaction monitoring mode (mrm). All samples were also run without extraction and analysed using positive mrm using the same HPLC conditions as for the extracted samples. Ranitidine, atenolol and gabapentin give poor recovery in the solid phase extraction and were determined more accurately using direct injection of the sample. The direct injection sample was also used for quantitation where concentrations in the extracted samples exceeded the linear range of quantitation. Separation was achieved using a Shimadzu Prominence HPLC system (Shimadzu Corp., Kyoto, Japan) with a 3 micron 150 X 2 mm Luna C18(2) column (Phenomenex, Torrance, CA) run at 45oC, and a flow rate of 0.35 mL min-1with a linear gradient starting at 15% B for 0.3 minutes, ramped to 100% B in 10 minutes, held for 4 minute and then to 15% B in 0.2 minutes and equilibrated for 4 minutes. (A = 1% acetonitrile/99% HPLC grade water, B = 95% acetonitrile/5% HPLC grade water both containing 0.1% formic acid). Using an 8 microlitre injection volume the limit of detection for this method is typically less than 2 nanograms L-1 (in sample as received assuming 100x concentration factor), with a reporting limit of 10 nanograms L-1 for most analytes. Response is linear to at least 500 nanograms L-1 for all analytes.

Table SI 3. Details (alphabetic) of all compounds analysed for in this study using positive electrospray. Abbreviations are explained below Table SI 4.

Name	Classification	U %	Rec. %	DP	EP	Q1	Q3 (quant)	CE	CXP	Q3 (conf)	CE	CXP
Atenolol	Beta-blocker	16	44	71	10	267.2	190.1	27	10	145	39	8
Atorvastatin	Hypolipidemic agent	5	34	70	10	559.5	440.3	31	10	250.2	62	10
Caffeine	-	70	83	61	10	195.1	138.1	29	6	110.1	33	4
Carbamazepine	Anticonvulsant	24	90	96	10	237.2	194	31	16	193	47	12
Cephalexin	Antibiotic (cephalosporin)	ND	ND	45	10	348.3	158.1	13	8	174.1	21	10
Chlortetracycline	Antibiotic (tetracycline)	11	16	50	10	479.3	444.3	32	6	154.1	42	12
Ciprofloxacin	Antibiotic (quinolone)	43	32	61	10	332.3	231.1	54	12	288.2	24	16
Citalopram	Antidepressant	25	86	70	10	325.3	109	38	4	262.2	28	4
Codeine	Analgesic	45	58	86	10	300.2	215.2	35	12	152.1	89	6
Cyclophosphamide	Cancer chemotherapy agent	41	104	70	10	261.1	106	28	10	120	33	10
Dapsone	Antituberculotic and antileprotic	38	45	60	10	249.2	156	22	7	92	34	14
DEET	Insect repellent	43	79	86	10	192.1	119	26	10	91	44	6
Desmethyl Citalopram	Citalopram metabolite	17	70	60	10	311.3	109	35	8	262.2	25	15
Desmethyl Diazepam	Anxiolytic/diazepam metabolite	27	92	70	10	271.2	140.1	41	15	165.1	41	15
Diazepam	Anxiolytic	25	84	76	10	285.2	154.1	36	12	193.2	42	14
Diclofenac	Non-steroidal anti-inflammatory	21	52	40	10	296.2	214	50	10	250.1	21	10
Doxylamine	Sedative	90	20	40	10	271.2	182	24	8	167.1	45	7
Enrofloxacin	Veterinary antibiotic (quinolone)	52	58	28	10	360.3	316.2	30	15	245.2	40	15
Erythromycin	Antibiotic (macrolide)	34	42	50	10	734.7	576.4	27	18	158.1	45	8
Fluoxetine	Antidepressant	43	44	51	10	310.1	44	37	8	148	13	7
Gabapentin	Anticonvulsant	ND	ND	66	10	172.1	154	19	8	137	23	6
Ifosfamide	Cancer therapy drug	38	95	70	10	261.1	92	37	10	63	64	10
Indomethacin	Nonsteroidal anti-inflammatory agent	25	48	66	10	358.3	138.9	31	10	75	107	12
Iopromide	X-Ray contrast media	64	74	100	10	791.7	573.1	35	14	559.1	41	14
Lincomycin	Antibiotic (lincosamide)	55	51	60	10	407.3	126.1	44	8	359.3	28	20
Metoprolol	Beta-blocker	55	78	70	10	268.2	116.1	28	7	191.1	27	10
Naproxen	Nonsteroidal anti-inflammatory agents	34	100	61	10	231.2	185.1	19	10	170.1	37	8
Norfloxacin	Antibiotic (quinolone)	22	22	70	10	320.3	276.2	26	14	233.2	35	14
Oxazepam	Anxiolytic	33	96	60	10	287.2	241.2	32	10	104	52	10
Oxycodone	Narcotic analgesic	64	73	65	10	316.2	298.2	26	16	241.2	42	16
Oxytetracycline	Antibiotic (tetracycline)	43	30	30	10	461.3	426.3	28	6	443.3	17	6
Paracetamol	Analgesic, antipyretic	52	38	61	10	152.1	110	23	6	65.1	42	6
Phenytoin	Anticonvulsant	39	98	66	10	253.2	182	29	18	104	48	14
Praziquantel	Anthelmintic	21	70	70	10	313.3	203.2	25	10	55	72	8
Propranolol	Beta-blocker	21	80	70	10	260.2	116.1	28	8	183.1	28	8
Ranitidine	Histamine-blocker	38	50	56	10	315.2	176.1	25	8	130.1	35	6
Roxithromycin	Antibiotic (macrolide)	21	21	10	10	837.6	679.5	32	9	158	52	5
Sertraline	Antidepressants	21	57	35	10	306.3	159.1	35	12	275.2	18	12
Simvastatin	Hypolipidemic agent	ND	ND	62	10	419.3	285.2	16	15	199.1	18	15
Sulfasalazine	Anti-inflammatory	14	21	30	10	399.3	223.1	43	11	119.1	63	7
Sulphadiazine	Antibiotic (sulfonamide)	33	40	71	10	251.2	92	37	14	65	61	10
Sulphamethoxazole	Antibiotic (sulfonamide)	35	65	51	10	254.2	156	23	8	92.1	38	8
Sulphathiazole	Antibiotic (sulfonamide)	48	69	51	10	256.2	156.1	22	10	92.1	40	8
Temazepam	Sedative	30	96	55	10	301.2	255.1	32	8	283.1	21	8
Tetracycline	Antibiotic (tetracycline)	32	19	50	10	445.3	410.2	28	24	154.1	40	7
Tramadol	Narcotic analgesic	51	87	45	10	264.2	58	44	8	42	125	3
Trimethoprim	Antibiotic	60	85	85	10	291.2	230.1	35	14	123.1	35	8
Tylosin	Antibiotic (macrolide)	13	12	10	10	916.7	174.1	58	5	101.1	72	12
Venlafaxine	Antidepressant	42	79	45	10	278.2	58	50	7	121	40	10
D3 Caffeine	Surrogate	50	100	60	10	198.1	138	27	8	110	35	8
D10 Carbamazepine	Surrogate	28	97	65	10	247.2	204.1	30	8	202.1	51	8
D5 Atrazine	Surrogate	30	88	60	10	221.1	179	27	6	101	36	6
D4 Diclofenac	Surrogate	30	80	45	10	300.1	219.1	30	8	218.1	46	8
D5 Acetyl Sulfamethoxazole	INTD STD1	-	-	60	10	302.3	202.1	26	9	138.1	36	9
D5 Fluoxetine	INTD STD2	-	-	45	10	315.2	44	42	5	153.1	14	10
D10Simazine	INTD STD3	-	-	60	10	212	137	40	10	134	38	10

Table SI 4. Details (alphabetic) of all compounds analysed for in this study using negative electrospray.

Name	Classification	U %	Rec. %	DP	EP	Q1	Q3 (quant)	CE	CXP	Q3 (conf)	CE	CXP
Acetylsalicylic acid	Analgesic, antipyretic, anti-inflammatory	42	67	-35	-10	178.9	136.9	-9	-11	92.9	-30	-5
Atorvastatin	Hypolipidemic agent	5	34	-70	-10	557.4	278.1	-60	-13	397.2	-39	-16
Chloramphenicol	Antibiotic	18	89	-70	-10	321	152	-25	-13	257	-16	-13
Diclofenac	Non-steroidal anti-inflammatory	21	52	-53	-10	294.1	250.1	-17	-12	214	-28	-12
Fluvastatin	Hypolipidemic agent	17	30	-10	-10	410.3	348.1	-22	-26	210.1	-42	-11
Furosemide	Diuretic	9	72	-57	-10	329	285	-21	-13	205	-33	-13
Gemfibrozol	Hypolipidemic agent	11	44	-60	-10	249.1	121	-18	-8	127	-15	-9
Hydrochlorthiazide	Diuretic	15	91	-55	-10	296	205	-34	-12	269	-28	-20
Ibuprofen	Nonsteroidal anti-inflammatory agent	74	101	-52	-10	205.1	161	-11.5	-10	159	-11	-10
Iopromide	X-Ray contrast media	64	74	-80	-10	790.0	127	-55	-7	NA		
Naproxen	Nonsteroidal anti-inflammatory agents	34	100	-53	-10	229.2	185.1	-11	-10	170.1	-22	-10
Salicylic acid	Acetylsalicylic acid metabolite	8	54	-45	-10	137	93	-24	-6	65	-40	-6
Triclosan	Biocide	9	12	-50	-10	287	35	-30	-3	**35 (289)**	**-30**	**-3**
Warfarin	Anticoagulant	27	89	-170	-10	307	161	-28	-11	250	-30	-9
13C6 24D	Surrogate	25	90	-36	-10	225	167	-21	-10	**169 (227)**	**-21**	**-10**
D4 Diclofenac	Surrogate	30	80	-50	-10	302.1	258	-16	-8	**256 (300.)**	**-16**	**-8**
Di Chloro Phenyl Acetic Acid	INTD STD	-	-	-25	-10	205	161	-10	-10	**159 (203)**	**-10**	**-7**

Key to Tables SI 3 and SI 4:

U = expanded uncertainty (per cent), at 95% confidence level at a concentration in sample of 1 microgram L-1, determined from long term spike data; Rec. % = recovery (per cent) at a concentration in the sample of 1 microgram L-1 (ND indicates insufficient data to determine); DP = declustering potential; EP = entrance potential; Q1 = parent ion; Q3(quant) = fragment ion used for quantitation; Q3(conf) = fragment ion used for confirmation; CE = collision energy; CXP = collision cell exit potential. Q3 data in bold represents fragments from an isotope of Q1 of different mass. For example Q3(conf) for Triclosan is the fragment from the isotope of mass 289 (Q1 = 289). INTD STD = internal standard, three internal standards are used in the positive run to cover different retention time intervals.

Supporting information A3:

Table SI 5. Consumption data for Australia (population approximately 20 million), estimated consumption by the population in the South Caboolture STP (inhabitants and employees 45,000), delivery from the hospital's pharmacy to the wards of Caboolture Hospital and predicted contribution of the Caboolture public hospital to the total influent of the STP (see main paper for more details).

Compound	Annual consumption in Australia	Average consumption calculated for the population in Caboolture	Consumption in Caboolture public hospital	Percentage of pharmaceuticals originating from the Caboolture Hospital in the influent of the STP
	$Cons_{AUS}$ [kg a^{-1}]	$Cons_{Cab.Pop.}$ [g a^{-1}] *	$Cons_{Cab.Hosp.}$ [g a^{-1}]	$P_{Cab.Hosp.}$ [%] **
ASPIRIN	19,793	44534	5335	10.7
ATENOLOL	7,138	16061	96	0.6
ATORVASTATIN	11,177	25148	239	0.9
CARBAMAZEPINE	14,047	31606	613	1.9
CEFALEXIN	42,875	96469	5989	5.8
CHLORAMPHENICOL	88	198	0.09	0.05
CIPROFLOXACIN	2,256	5076	569	10.1
CITALOPRAM	1,277	2873	48	1.6
CODEINE	5,959	13408	946	6.6
DIAZEPAM	508	1143	140	10.9
DICLOFENAC	3,107	6991	128	1.8
ERYTHROMYCIN	8,069	18155	817	4.3
FLUOXETINE HYDROCHLORIDE	1,020	2295	18	0.8
FUROSEMIDE	5,942	13370	836	5.9
GABAPENTIN	6,414	14432	699	4.6
GEMFIBROZIL	8,797	19793	77	0.4
HYDROCHLOROTHIAZIDE	3,676	8271	38	0.5
IBUPROFEN	11,292	25407	24619	49.2
INDOMETHACIN	994	2237	249	10.0
METOPROLOL SUCCINATE	9,148	20583	494	2.3
NAPROXEN	16131	36295	103	0.3
NORFLOXACIN	1705	3836	128	3.2
OXAZEPAM	955	2149	33	1.5
OXYCODONE	1,285	2891	150	4.9
PARACETAMOL	537,979	1210453	136305	10.1
PHENYTOIN	3,465	7796	339	4.2
PROPRANOLOL HYDROCHLORIDE	1,687	3796	30	0.8
RANITIDINE HYDROCHLORIDE	9,519	21418	1289	5.7
ROXITHROMYCIN	3,916	8811	2065	19.0
SERTRALINE	7,735	17404	218	1.2
SIMVASTATIN	6,708	15093	82	0.5
SULPHASALAZINE	22,541	50717	367	0.7
TEMAZEPAM	709	1595	71	4.3
TRAMADOL	7,545	16976	1215	6.7
TRIMETHOPRIM	1,779	4003	960	19.3
VENLAFAXINE	10,679	24028	496	2.0
WARFARIN	366	824	16	1.9

$$*P_{Cab.Hosp.} = \frac{Cons_{Cab.Hosp.}}{Cons_{Cab.Pop.} + Cons_{Cab.Hosp.}}, \quad **Cons_{Cab.Pop.} = \frac{Cons_{AUS}}{20{,}000{,}000} \cdot 45{,}000$$

B. Supporting Information for Chapter 2

Environment International 45 (2012) 99–111

Contents lists available at SciVerse ScienceDirect

Environment International

journal homepage: www.elsevier.com/locate/envint

Consumption-based approach for assessing the contribution of hospitals towards the load of pharmaceutical residues in municipal wastewater

Kristell S. Le Corre [a], Christoph Ort [a,b,*], Diana Kateley [c], Belinda Allen [c], Beate I. Escher [d], Jurg Keller [a]

[a] The University of Queensland, Advanced Water Management Centre (AWMC), Brisbane, QLD 4072, Australia
[b] Swiss Federal Institute of Aquatic Science and Technology (Eawag), CH-8600 Duebendorf, Switzerland
[c] Medication Services Queensland, Clinical and Statewide Services Division, Queensland Health, Herston, QLD 4029, Australia
[d] The University of Queensland, National Research Centre for Environmental Toxicology (Entox), Brisbane, QLD 4108, Australia

Supporting information B1:

Table SI 6. List of compounds evaluated.

Generic	Class Acronym	Therapeutic Class	Pharmacological Class	Mode of Administration
Abacavir	AV	Antiviral	Nucleoside reverse transcriptase inhibitor (NRTI)	Oral
Abciximab	Acog	Anticoagulant / Antithrombotic	Platelet aggregation inhibitor	Injection
Acamprosate	DxA	Detoxifying agent	Gamma aminobutyric acid inhibitor (GABA) analog	Oral
Acetazolamide	AGl	Antiglaucoma agent	Carbonic anhydrase inhibitor	Oral / injection
Acetylcholine Chloride	MY	Mydriatic	Cholinergic neurotransmitter agent	Ophthalmic
Acetylcysteine	DxA	Detoxifying agent	N-acetyl derivative of naturally occurring amino acid	Injection
Acitretin	Apso	Antipsoriatic	Second generation retinoid	Oral
Adalimumab	ARh	Antirheumatic, immunomodulator	Tumor necrosis factor (TNF) Alfa blocker	Injection
Adapalene	-	Acne treatment	Retinoid	Topical
Adefovir Dipivoxil	AV	Antiviral	Nucleotide analog reverse transcriptase inhibitor (ntRTI).	Oral
Albendazole	AH	Anthelminthic	Benzimidazole antihelmintic	Oral
Alendronate Sodium	BP	Bone Resorption inhibitor	Biphosphonate	Oral
Alfentanil	AG	Analgesic	Synthetic opioid analgesic	Injection
Allopurinol	EI	Antigout drug	Xanthine oxidase inhibitor	Oral
Alprazolam	AL	Anxiolytic	Benzodiazepine	Oral
Alprostadil	VA	Vasodilator	Prostaglandin	Injection / Infusion
Amantadine Hydrochloride	AP	Antiparkinsonian, Antiviral	Anticholinergic-like agent	Oral
Amethocaine	AA	Anaesthetic agent	Anaesthetic agent *(local)*	Ophthalmic
Amikacin	AB	Antibiotic	Aminoglycoside	Injection
Amiloride Hydrochloride	DI	Diuretic	Potassium-sparing diuretic	Oral
Aminophylline	BD	Bronchodilator	Xanthine	Injection
Amiodarone Hydrochloride	AR	Antiarrhythmic agents	Potassium channel blocker	Tablets / Injection
Amisulpride	APsy	Antipsychotic	D_2 and D_3 receptor antagonist	Oral
Amitriptyline Hydrochloride	AD	Antidepressant	Serotonin-norepinephrine reuptake inhibitor,	Oral

Generic	Class Acronym	Therapeutic Class	Pharmacological Class	Mode of Administration
Amlodipine	Ahyp	Antihypertensive / vasodilator	Calcium channel blocker	Oral
Amoxycillin	AB	Antibiotic	Beta-Lactam antibiotic	Oral / injection / topical
Amphotericin	AF	Antifungal	Antifungal	Infusion
Ampicillin	AB	Antibiotic	Beta-Lactam antibiotic	Injection
Anagrelide	AN	Antineoplastic	Platelet aggregation inhibitor / phosphodiesterase inhibitor	Oral
Anastrozole	AN	antineoplastic agent	Aromatase inhibitor	Oral
Apomorphine	AP	Antiparkinsonian agent	Dopaminergic, dopamine-receptor agonist	Injection / Infusion
Apraclonidine	AGl	Antiglaucoma agent	a_2-adrenergic agonists	Ophthalmic
Aripiprazole	Apsy	Antipsychotic / antidepressant	D2 partial agonist, 5-HT_1A Partial Agonist, 5-HT_2A Antagonist…	Oral
Arsenic Trioxide	AN	Antineoplastic agent	Amphoteric oxide	Injection
Artemether	AM	Antimalarial	Antimalarial	Oral
Artesunate	AM	Antimalarial	Semi-synthetic derivative of artemisinin	Oral
Atazanavir	AV	Antiviral	HIV-1 protease inhibitor.	Oral
Atenolol	βB	Beta-Blocker	β1 receptor antagonist	Oral
Atomoxetine	CNS	Central nervous system agent	Selective norepinephrine reuptake inhibitor	Oral
Atorvastatin	HL	Hypolipidemic agent	HMG-CoA reductase inhibitor	Oral
Atovaquone	AM	Antimalarial	Antimalarial	Oral
Atracurium	NB	Neuromuscular blocking agent	Non depolarizing curare	Injection
Auranofin	ARh	Antirheumatic agent	Organogold compound inducing heme oxygenase 1 (HO-1) mRNA	Oral
Azathioprine	IM	Immunosuppressant	Purine antagonist	Oral
Azithromycin	AB	Antibiotic	Macrolide	Injection
Aztreonam	AB	Antibiotic	Monobactam	Intraveneous / Intramuscular
Bacitracin	AB	Antibiotic	Antibacterial polypeptide	Topical
Baclofen	NB	Neuromuscular blocker / Muscle relaxant / antispasmodic	Skeletal muscle relaxant	Oral
Balsalazide	AI	Anti-inflammatory	Prodrug of the anti-inflammatory mesalamine	Oral
Benserazide	AP	Antiparkinsonian agent	Levodopade carboxylation inhibitor	Oral
Benzathine Penicillin	AB	Antibiotic	Penicillin	Oral / injection
Benzathine Phenoxymethylpenicillin	AB	Antibiotic	Penicillin	Oral / injection
Benzhexol Hydrochloride	AP	Antiparkinsonian agent	Cholinergic muscarinic antagonist	Oral
Benztropine Mesylate	AP	Antiparkinsonian agent	Anticholinergic	Oral / injection
Benzylpenicillin	AB	Antibiotic	Penicllin	Injection
Betahistine	VA	Vasodilator	-	Oral
Betamethasone	AI	Antiasthmatic, anti-inflammatory	Glucocorticoid	Topical
Betaxolol Hydrochloride	βB	Beta-Blocker	β1-selective adrenergic receptor blocking agent	Drops
Bethanechol Chloride	ChS	Urinary and GI tract stimulant	cholinergic stimulant	Oral
Bevacizumab	AN	Antineoplastic	Vascular endothelial growth factor monoclonal Ab	Infusion
Bicalutamide	AN	Antineoplastic agent	Nonsteroidal antiandrogen	Oral
Bimatoprost	AGl	Antiglaucoma agent	Prostaglandin agonist	Ophtalmic
Biperiden Hydrochloride	AP	Antiparkinsonian agent	Anticholinergic	Oral
Bisoprolol	βB	Beta-Blocker	β1 receptor antagonist	Oral
Bivalirudin	Acog	Anticoagulant	Thrombin inhibitor	Injection

Generic	Class Acronym	Therapeutic Class	Pharmacological Class	Mode of Administration
Bleomycin	AN	Antineoplastic antibiotic	Antitumor antibiotic	Injection
Bortezomib	AN	Antineoplastic agent	Proteasome inhibitor	Injection
Bosentan	VA	Antihypertensive, vasodilator	Endothelin-receptor antagonist, vasodilator	Oral
Brimonidine	AGl	Antiglaucoma agent	α-adrenergic receptor agonist	Ophtalmic
Brinzolamide	AGl	Antiglaucoma agent	carbonic anhydrase inhibitor	Ophtalmic
Bromazepam	AX	Anxiolytic	Benzodiazepine	Oral
Bromhexine Hydrochloride	Mu	Expectorant / mucolytic agent	-	Oral
Bromocriptine Mesylate	AP	Antiparkinsonian agent	Ergot-derivative dopamine agonist	Oral
Bumetanide	DI	Diuretic / antihypertensive	Loop diuretic	Oral
Bupivacaine	AA	Anaesthetic Agent	amide local anaesthetic	Injection
Buprenorphine	AG	Analgesic	Opioid agonist-antagonist	Oral /patch
Bupropion	AD	Antidepressant	Aminoketone	Oral
Buspirone Hydrochloride	AX	Anxiolytic	Azaspirodecanedione	Oral
Busulfan	AN	Antineoplastic	Alkylating agent	Oral
Butylscopolamine	ASp	Abdominal antispasmodic	Anticholinergic	Injection
Cabergoline	AP	Antiparkinsonian agent	Dopamine-receptor agonist	Oral
Calcium Folinate	DxA	Detoxifying agent	Antidote to folic acid antagonist	Injection
Candesartan	Ahyp	Antihypertensive	Angiotensin II inhibitors	Oral
Capecitabine	AN	Antineoplastic	Fluoropyrimidine, antimetabolite (pyrimidine analog)	Oral
Captopril	Ahyp	Antihypertensive	Angiotensin-converting enzyme (ACE) inhibitor	Oral
Carbamazepine	AC	Anticonvulsant	Iminostilbene derivative	Oral
Carbidopa	AP	Antiparkinsonian agent	Dopamine agonist	Oral
Carbimazole	AT	Antithyroid agent	Thioamide derivative	Oral
Carboplatin	AN	Antineoplastic	Alkylating agent	Infusion
Carmellose Sodium	MP/LU	Mucoprotectant / Lubricant	-	Ophtalmic / Topical
Carmustine	AN	Antineoplastic	Alkylating agent	Injection
Carvedilol	βB	Beta-Blocker	Beta-adrenergic blocker	Oral
Caspofungin Acetate	AF	Antifungal Agent	Glucan synthesis inhibitor	Infusion
Cefaclor	AB	Antibiotic	Second-generation cephalosporin	Oral
Cefalexin	AB	Antibiotic	First-generation cephalosporin	Oral
Cefalotin	AB	Antibiotic	First-generation cephalosporin	Injection
Cefazolin	AB	Antibiotic	First-generation cephalosporin	Injection
Cefepime	AB	Antibiotic	Fourth-generation cephalosporin	Injection
Cefotaxime	AB	Antibiotic	Third-generation cephalosporin	Injection
Cefoxitin	AB	Antibiotic	Second-generation cephalosporin	Injection
Ceftazidime	AB	Antibiotic	Third-generation cephalosporin	Injection
Ceftriaxone	AB	Antibiotic	Third-generation cephalosporin	Injection
Cefuroxime	AB	Antibiotic	Second-generation cephalosporin	Oral
Celecoxib	AI	Anti-inflammatory	Nonsteroidal cyclooxygenase-2 (COX-2) inhibitor, non-steroidal anti-inflammatory drug (NSAID)	Oral
Cetuximab	AN	Antineoplastic agent	Epidermal growth factor receptor (EGFR) inhibitor	Infusion
Chloral Hydrate	SE	Sedative-hypnotic	Central Nervous system agent	Oral
Chlorambucil	AN	Antineoplastic	Alkylating agent	Oral
Chloroquine	AM	Antimalarial	Antimalarial	Oral
Chlorpromazine Hydrochloride	APsy	Antipsychotic, Anxiolytic	Phenothiazine	Injection / Oral
Chlorthalidone	DI	Diuretic	Thiazide-like diuretic	Oral
Ciclesonide	AI	Anti-inflammatory	Glucocorticoid	Inhalation

Generic	Class Acronym	Therapeutic Class	Pharmacological Class	Mode of Administration
Cidofovir	AV	Antiviral	DNA synthesis inhibitor	Infusion
Cilastatin	-	*(Combined with the AB imipenem)*	Dehydropeptidase inhibitor	Injection
Cimetidine	AU	Antiulcer drug	Histamine2-receptor antagonist	Oral
Cinacalcet	EA	Endocrine and metabolic agent	Calcimimetic	Oral
Ciprofloxacin	AB	Antibiotic	Fluoroquinolone	Topical application
Cisapride	GP	Gastrointestinal stimulant	Gastroprokinetic agent	Oral
Cisatracurium	NB	Neuromuscular blocking agent	benzyl-isoquinolinium agent	Injection
Cisplatin	AN	Antineoplastic	Alkylating agent	Injection
Citalopram	AD	Antidepressant	Selective serotonin reuptake inhibitor	Oral
Cladribine	AN	Antineoplastic	antimetabolite / halogenated / ribonucleotide reductase inhibitor	Injection / Oral
Clarithromycin	AU	Antiulcer drug	Macrolide	Oral
Clavulanic Acid	BLI	Beta-Lactamase Inhibitors *(used in combination with amoxycillin)*	Beta-lactamase Inhibitor	Oral / Infusion
Clindamycin	AB	Antibiotic	Lincosamide	Oral / injection
Clioquinol	AF	Antifungal agent	-	Topical
Clobazam	AL	Anxiolytic / Anticonvulsant	Benzodiazepine	Oral
Clofazimine	AB	Antibiotic	-	Oral
Clomiphene Citrate	OS	Ovulation stimulant	Chlorotrianisene derivative	Oral
Clomipramine Hydrochloride	AD	Antidepressant	Dibenzazepine	Oral
Clonazepam	AL	Anxiolytic	Benzodiazepine	Injection
Clonidine	Ahyp	Antihypertensive	Centrally acting sympatholytic	Oral
Clopidogrel	Acog	Anticoagulant / Antithrombotic	Platelet inhibitor	Oral
Clozapine	APsy	Antipsychotic	Dibenzodiazepine derivative	Oral
Cocaine	AA	Anaesthetic Agent	Local-ophtalmologic	Ophtalmic
Colchicine	-	Antigout drug	Colchicum alkaloid	Oral
Colestyramine	HL	Hypolipidemic agent	Bile acid sequestrant	Oral
Colistimethate Sodium	AB	Antibiotic	Colistine derivative	Injection
Cyclizine	AE	Antiemetic	Anticholinergic	Oral
Cyclizine Lactate	AE	Antiemetic	Anticholinergic	Oral
Cyclopentolate	CM	cycloplegic and mydriatic agent	Antimuscarinic	Ophtalmic
Cyclophosphamide	AN	Antineoplastic	Alkylating agent	Injection / oral
Cycloserine	AB	Antibiotic	Septomyces derivative	Oral
Cyclosporin	IM	Immunosuppressant	Macrolide	Oral
Cyproterone Acetate	Aan	Anti-androgen	Anti-androgenic progestin	Oral
Cytarabine	AN	Antineoplastic	Antimetabolite / halogenated/ ribonucleotide reductase inhibitor	Injection
Dabigatran	Acog	Anticoagulant	Direct thrombin inhibitor	Oral
Dacarbazine	AN	Antineoplastic	Alkylating agent	Injection
Dactinomycin	AN	Antineoplastic	Polypeptide Antibiotic	Injection
Dalfopristin	AB	Antibiotic	Streptogramin	Infusion
Dalteparin	Acog	anticoagulant	Low-molecular-weight heparin	Injection
Danaparoid	Acog	anticoagulant	Factor Xa inhibitor	Injection
Danazol	HM	Gonadal hormones	Androgen	Oral
Dantrolene Sodium	sMR	Skeletal muscle relaxant	Hydantoin derivative	Oral / Injection
Dapsone	ALP	Antileprotic, antimalarial	Synthetic sulfone	Oral
Daptomycin	AB	Antibiotic	-	Infusion
Darunavir	AV	Antiviral	Protease inhibitor	Oral

Generic	Class Acronym	Therapeutic Class	Pharmacological Class	Mode of Administration
Dasatinib	AN	Antineoplastic agent	Protein-tyrosine kinase inhibitors	Oral
Daunorubicin	AN	Antineoplastic antibiotic	Anthracycline glycoside	Injection
Deferasirox	DxA	Detoxifying agent	Iron-chelating agent	Oral
Deferiprone	DxA	Detoxifying agent	Iron-chelating agent	Oral
Defibrotide	Acog	Anticoagulant	Deoxyribonucleic acid derivative	Oral
Demeclocycline	AB	Antibiotic	Tetracycline	Oral
Desferrioxamine	DxA	Detoxifying agent	Iron-chelating agent	Injection
Desmopressin	aDI	Antidiuretic	Posterior pituitary hormone	Injection / oral
Desvenlafaxine	AD	Antidepressant	Serotonin-norepinephrine reuptake inhibitor	Oral
Dexamethasone	AI	Anti-inflammatory	Glucocorticoid	Oral / injection / Topical (ear drops)
Dexamphetamine Sulphate	CNS	CNS stimulant	Amphetamine	Oral
Dexmedetomidine Hydrochloride	S	Sedative	a_2 adrenoceptor agonist sedative	Infusion
Dextropropoxyphene	AG	Analgesic	μ-opioid receptor agonist	Oral
Diazepam	AL	Anxiolytic	Benzodiazepine	Oral
Diazoxide	VA	Antihypertensive	Vasodilator	Injection
Dicloxacillin	AB	Antibiotic	Penicillinase-resistant penicillin	Oral /injection
Dicobalt edetate	DxA	Detoxifying agent	-	Injection
Didanosine	AV	Antiviral	Nucleoside reverse transcriptase inhibitor	Oral
Digoxin	AR	Antiarrhythmic	Cardiac glycoside	Infusion /injection
Dihydroergotamine	VP	Vasopressor	α adrenergic blocker	Injection
Diloxanide	APZ	Antiprotozoal agent	-	
Diltiazem Hydrochloride	AR	Antiarrhythmic	Calcium channel blocker	Oral
Diphenoxylate Hydrochloride	ADy	Antidiarrheal	Anticholinergic	Oral
Dipivefrine	AGl	Antiglaucoma agent	Prodrug of epinephrine	Topical
Dipyridamole	Acog	Anticoagulant	Platelet adhesion inhibitor	Injection / oral
Disodium Pamidronate	BP	Bone resorption inhibitor	Bisphosphonate, hypocalcemic	Infusion
Disopyramide	AR	Antiarrhythmic agents	Pyridine derivative	Oral
Disulfiram	-	Antioxidant	-	Oral
Dobutamine	VP	Vasopressor	β-adrenergic stimulating agent	Injection / infusion
Docetaxel	AN	Antineoplastic	Mitosis inhibitor	Infusion
Dofetilide	AR	Antiarrhythmic	Potassium channel blocker	Oral
Domperidone	AP	Antiparkinsonian agent	Dopamine antagonist	Oral
Donepezil	AZ	Anti-Alzheimer's agent	Acetylcholinesterase inhibitor	Oral
Dopamine	VP	Vasopressor	Catecholamine, adrenergic	Oral
Dorzolamide	AGl	Antiglaucoma agent	carbonic acid anhydrase inhibitor	Topical
Dothiepin Hydrochloride	AD	Antidepressant	Serotonin-norepinephrine reuptake inhibitor	Oral
Doxepin Hydrochloride	AD	Antidepressant	Serotonin-norepinephrine reuptake inhibitor	Oral
Doxorubicin Hydrochloride	AN	Antineoplastic	Anthracycline	Injection
Doxycycline	AB	Antibiotic	Tetracycline	Oral
Droperidol	Apsy	Antipsychotic agent	Butyrophenone	Injection
Duloxetine	AD	Antidepressant	Selective serotonin and norepinephrine reuptake inhibitor	Oral
Edrophonium	DxA	Detoxifying agent, diagnosis	Anticholinesterase	Injection

Generic	Class Acronym	Therapeutic Class	Pharmacological Class	Mode of Administration
Efavirenz	AV	Antiviral	Non-nucleoside reverse transcriptase inhibitor	Oral
Eformoterol	BD	Bronchodilator	β2-agonist	Inhalation
Emtricitabine	AV	Antiviral	Non-nucleoside reverse transcriptase inhibitor	Oral
Enalapril Maleate	Ahyp	Antihypertensive	Angiotensin-converting enzyme (ACE) inhibitor	Oral
Enalaprilat	Ahyp	Antihypertensive	Angiotensin-converting enzyme (ACE) inhibitor	Oral
Enfuvirtide	AV	Antiviral agent	Human immunodeficiency-1 (HIV-1) fusion inhibitor	Injection
Enoxaparin	Acog	Anticoagulant	Low-molecular-weight heparin	Injection
Entacapone	AP	Antiparkinsonian	COMT inhibitor	Oral
Entecavir	AV	Antiviral	Guanosine nucleoside analogue	Oral
Ephedrine	VP	Vasopressor	Adrenergic	Injection / Nasal
Epirubicin Hydrochloride	AN	Antineoplastic	Anthracycline	Injection
Eplerenone	Ahyp	Antihypertensive	Aldosterone receptor blocker	Oral
Epoprostenol	VA	Vasodilator	Platelet aggregation inhibitor	Infusion
Eprosartan Mesylate	Ahyp	Antihypertensive	Angiotensin II receptor antagonist	Oral
Eptifibatide	Acog	Anticoagulant	Platelet aggregation inhibitor	Injection
Ergometrine	OA	Oxytocic agent	Amine ergot alkaloid	Injection
Ergotamine	Amig	Antimigraine agent	α-adrenergic blocker	Oral / Rectal
Erlotinib	AN	Antineoplastic agent	Epidermal growth factor receptor (EGFR) inhibitor	Oral
Ertapenem	AB	Antibiotic	Carbapenem	Injection
Erythromycin	AB	Antibiotic	Macrolide	Oral
Escitalopram	AD	Antidepressant	Selective serotonin reuptake inhibitor	Oral
Esmolol	βB	Cardio selective beta 1 -blocker	Beta-adrenergic blocker	Infusion
Esomeprazole	AU	Antiulcer agent	proton pump inhibitors	Oral /injection
Etanercept	TNF	Antiarthritic	Immunomodulator / Tumor necrosis factor inhibitor	Injection
Ethacrynic Acid	DI	Diuretic	-	Oral
Ethambutol	AB	Antibiotic	Synthetic antitubercular	Oral
Ethosuximide	AC	Anticonvulsant	-	Oral
Etoposide	AN	Antineoplastic agent	Podophyllotoxin derivative	Injection / infusion
Etravirine	AV	Antiviral	Non-nucleoside reverse transcriptase inhibitor	Oral
Everolimus	AN	Antineoplastic agent	$mTORC_1$ inhibitor	Oral
Exemestane	AN	Antineoplastic agent	Aromatase inhibitor	Oral
Ezetimibe	HL	Hypolipidaemic agent	Cholesterol absorption inhibitor	Oral
Famciclovir	AV	Antiviral	Synthetic nucleoside	Oral
Felodipine	Ahyp	Antihypertensive	Calcium Channel blockers	Oral
Fenofibrate	HL	Hypolipideamic agent	Fibric acid derivative	Oral
Fentanyl	AG	Analgesic	Opioid agonist	Injection
Filgrastim	HP	Hematopoietic stimulator	Granulocyte colony-stimulating factor	Injection
Finasteride	SA	Synthetic antiandrogen	Type II 5-alpha reductase inhibitor	Oral
Flecainide Acetate	AR	Antiarrhythmic agent	Cardiac benzamide local anaesthetic	Oral / Injection
Flucloxacillin	AB	Antibiotic	Penicillinase-resistant penicillin	Oral
Flucytosine	AF	Anti-fungal agent	Fluorinated pyrimidine analog	Oral (also injectable)
Fludarabine Phosphate	AN	Antineoplastic	Purine antimetabolite	Injection / Oral
Fludrocortisone Acetate	MC	Synthetic mineralocorticoid	Adrenocorticoid	Oral
Flumazenil	DxA	Detoxifying agent	benzodiazepine receptor antagonist	Injection
Flumethasone Pivalate	AI	Anti-inflammatory	Glucocorticoid	Topical / Ophthalmic
Flunitrazepam	S	Sedative	Benzodiazepine derivative	Oral

Generic	Class Acronym	Therapeutic Class	Pharmacological Class	Mode of Administration
Fluorometholone	AI	Anti-inflammatory	Glucocorticoid	Topical / Ophthalmic
Fluorouracil	AN	Antineoplastic	Antimetabolite	Injection / Topical
Fluoxetine Hydrochloride	AD	Antidepressant	Selective serotonin reuptake inhibitor	Oral
Flupenthixol	APsy	Antipsychotic agent	Thioxanthene	Injection
Fluphenazine Decanoate	APsy	Antipsychotic agent	Phenothiazine, dopaminergic blocker	Injection
Flutamide	AN	Antineoplastic agent	Anti-androgen	Oral
Fluvastatin	HL	Hypolipidaemic agent	HMG-CoA reductase inhibitor	Oral
Fluvoxamine	AD	Antidepressant	Selective serotonin reuptake inhibitor (SSRI)	Oral
Fondaparinux Sodium	Acog	Anticoagulant	Selective factor Xa inhibitor	Injection
Foscarnet	AV	Antiviral	Pyrophosphate analog	Injection
Fosfomycin	AB	Antibiotic	UDP-N-acetylglucosamine-3-enolpyruvyltransferase (MurA) inhibitor	Oral
Fosinopril	Ahyp	Antihypertensive	Angiotensin-converting enzyme (ACE) inhibitor	Oral
Fotemustine	AN	Antineoplastic	Alkylating agent	Injection
Framycetin Sulphate	AB	Antibiotic	Aminoglycoside	Topical / ophthalmic
Frusemide	DI	Diuretic	Na-K-2Cl symporter inhibitor	Injection / Oral
Fulvestrant	AN	Antineoplastic	Selective Estrogen Receptor Down-Regulator (SERD).	Injection
Gabapentin	AC	Anticonvulsant	GABA analogue	Oral
Galantamine	AZ	Anti-Alzheimer's agent	Cholinesterase inhibitor	Oral
Ganciclovir	AV	Antiviral	Acyclic purine nucleoside analogue of 2'-deoxyguanosine	Infusion / implant
Gemcitabine	AN	Antineoplastic	Antimetabolite	Injection / infusion
Gemfibrozil	HL	Hypolipidemic agent	Fibric acid derivative	Oral
Gentamicin Sulphate	AB	Antibiotic	Aminoglycoside	Injection
Glibenclamide	HA	Hypoglycaemic agent	Sulfonylurea	Oral
Gliclazide	HA	Hypoglycaemic agent	Sulfonylurea	Oral
Glimepiride	HA	Hypoglycaemic agents	Sulfonylurea	Oral
Glipizide	HA	Hypoglycaemic agents	Sulfonylurea	Oral
Glucagon Hydrochloride	-	Insulin antagonist	Antihypoglycemic	Injection
Glycopyrrolate	ASp	Antispasmodic	Anticholinergic	Injection
Gramicidin	AB	Antibiotic	Corticosteroid	Topical
Granisetron	AE	Antiemetic	5HT3 receptor antagonists	Injection / oral
Griseofulvin	AF	Antifungal		Oral
Guanethidine	Ahyp	Antihypertensive	Adrenergic-blocking agent	Oral
Haloperidol	APsy	Antipsychotic	Butyrophenone	Injection / Oral
Homatropine Hydrobromide	MY	Mydriatic	Anticholinergic agent	Ophtalmic
Hydralazine Hydrochloride	Ahyp	Antihypertensive	Vasodilator	Oral
Hydrochlorothiazide	DI	Diuretic	Thiazide diuretic	Oral
Hydromorphone	AG	Analgesic	Opioid agonist	Injection / oral
Hydroxychloroquine Sulphate	AM	Anti-malarial	4-aminoquinolone	Oral
Hydroxyurea	AN	Antineoplastic	Antimetabolite	Oral
Ibandronic Acid	BP	Calcium regulator	Biphosphonate	Oral
Idarubicin Hydrochloride	AN	Antineoplastic	Anthracycline antibiotic	Oral / Injection
Ifosfamide	AN	Antineoplastic	Alkylating agent	Injection (hospital only)
Imatinib	AN	Antineoplastic agent	Protein-tyrosine kinase inhibitor	Oral
Imipenem	AB	Antibiotic	Carbapenem	Injection
Imipramine Hydrochloride	AD	Antidepressant	Dibenzazepine derivative	Oral

Generic	Class Acronym	Therapeutic Class	Pharmacological Class	Mode of Administration
Imiquimod	AV	Antiviral	Immune response modifier	Topical
Indapamide	DI	Diuretic	Thiazide-like diuretic	Oral
Indinavir	AV	Antiviral	Protease inhibitor	Oral
Infliximab	ARh	Antirheumatic agent	Tumor necrosis factor (TNF) Alfa blocker	Infusion
Irbesartan	Ahyp	Antihypertensive	Angiotensin II inhibitors	Oral
Irinotecan	AN	Antineoplastic agent	Topoisomerase inhibitor	Injection / Infusion
Isoniazid	TB	Antitubercular agent	Isonicotinic acid hydrazide	Oral
Isoprenaline	BD	Bronchodilator	beta1-adrenergic and beta2-adrenergic agonist	Injection
Isosorbide Mononitrate	VA	Vasodilator	Nitrate	Oral
Itraconazole	AF	Antifungal Agent	Synthetic triazole	Oral
Ivabradine	VA	Vasodilator	-	Oral
Ivermectin	ATh	Antihelmintic	-	Oral
Ketamine	AA	Anaesthetic Agent	NMDA receptor antagonists	Injection
Ketorolac	AI	Anti-inflammatory	Nonsteroidal Anti-inflammatory drug (NSAID)	Injection / ophtalmic
Labetalol Hydrochloride	βB	Beta-Blocker	Beta-adrenergic blocker (nonselective), alpha-adrenergic blocker (selective)	Oral
Lamivudine	AV	Antiviral	Nucleoside reverse transcriptase inhibitor	Oral
Lamotrigine	AC	Anticonvulsant	Phenyltriazine	Oral
Lansoprazole	AU	Antiulcer	Gastric acid pump inhibitor	Oral
Lapatinib	AN	Antineoplastic agent	Receptor tyrosine kinases inhibitor	Oral
Latanoprost	AGl	Antiglaucoma agent	-	Ophtalmic
Leflunomide	ARh	Antirheumatic agent	Pyrimidine synthesis inhibitor	Oral
Lenalidomide	IM	Immunosuppressant	-	Oral
Lenograstim	HP	Hematopoietic stimulator	Colony stimulating factor	Injection
Lepirudin	Acog	Anticoagulant	Thrombin inhibitor	Injection
Lercanidipine	Ahyp	Antihypertensive agent	Calcium Channel blockers	Oral
Letrozole	AN	Antineoplastic	Aromatase inhibitor	Oral
Levetiracetam	AC	Anticonvulsant / antiepileptic	Pyrrolidine derivative	Oral / Infusion
Levobunolol	βB	Beta-Blocker	-	Topical
Levobupivacaine	AA	Anaesthetic Agent	amide local anaesthetic	Injection / infusion
Levodopa	AP	Antiparkinsonian agent	Dopaminergic agent	Oral
Levomepromazine	Apsy	Antipsychotic	-	Oral
Levosimendan	CaS	Calcium sensitizers	-	Injection
Lincomycin	AB	Antibiotic	-	Injection
Linezolid	AB	Antibiotic	Oxazolidinone	Injection / Oral
Lisinopril	Ahyp	Antihypertensive	Angiotensin-converting enzyme (ACE) inhibitor	Oral
Lomustine	AN	Antineoplastic	Alkylating agent	Oral
Lopinavir	AV	Antiviral	Protease inhibitor	Oral
Lorazepam	AX	Anxiolytic	Benzodiazepine	Oral
Losartan	Ahyp	Antihypertensive	Angiotensin-converting enzyme (ACE) inhibitor	Oral
Lumefantrine	AM	Antimalarial	Antimalarial	Oral
Mebeverine Hydrochloride	ASp	Antispasmodic	-	Oral
Mefloquine	AM	Antimalarial	Antimalarial	Oral
Meloxicam	AI	Anti-inflammatory	Nonsteroidal anti-inflammatory drug (NSAID)	Oral
Melphalan	AN	Antineoplastic	Alkylating agent	Oral / Injection
Memantine Hydrochloride	AZ	Anti-Alzheimer's agent	NMDA Receptor Antagonist	Topical / Oral

Generic	Class Acronym	Therapeutic Class	Pharmacological Class	Mode of Administration
Mercaptopurine	AN	Antineoplastic	Antimetabolite	Oral
Meropenem	AB	Antibiotic	Carbapenem	Injection
Mesalazine	AI	Anti-inflammatory	5-amino-2-hydroxybenzoic acid	Oral
Metaraminol tartrate	VP	Vasopressor	α-adrenergic agonist	Injection
Metformin Hydrochloride	AdB	Ant diabetic	Biguanide	Oral
Methadone Hydrochloride	AG	Analgesic	Opioid agonist	Oral
Methotrexate	AN	Antineoplastic	Antimetabolite (folic acid analog)	Oral
Methyldopa	Ahyp	Antihypertensive	Centrally acting antiadrenergic	Oral
Methylphenidate	CNS	CNS Stimulant - Psychostimulant	Piperidine derivative	Oral
Methylprednisolone	AI	Anti-inflammatory	Glucocorticoid	Injection
Methysergide	Amig	Anti-migraine agent	Serotonin antagonist	Oral
Metolazone	DI	Diuretic	Thiazide-like diuretic	Oral
Metoprolol	βB	Beta-Blocker	Beta-adrenergic blocker	Oral
Metronidazole	AB	Antibiotic	Nitroimidazole derivative	Infusion / oral
Metyrapone	Diag	Dianostic agent	adrenocortical 11β-dehydroxylase inhibitor	Oral
Mexiletine Hydrochloride	AR	Antiarrhythmic agents	Lidocaine-like agent	Oral
Mianserin Hydrochloride	AD	Antidepressant	-	Oral
Midazolam	AX	Anxyolitic	Benzodiazepine	Injection
Midodrine	VP	Vasopressor	$Alpha_1$-adrenergic agonist	Oral
Milrinone	VA	Vasodilator	Bipyridine phosphodiesterase inhibitor	Injection
Miltefosine	APZ	Antiprotozoal agent	-	Oral
Minocycline	AB	Antibiotic	Tetracycline	Oral
Mirtazapine	AD	Antidepressant	Piperazinoazepine derivative	Oral
Misoprostol	AI	Anti-inflammatory	Prostaglandin E_1 analog	Oral
Mitomycin	AN	Antineoplastic	Antitumor antibiotic	Injection
Mitotane	AN	Antineoplastic	-	Injection
Mitozantrone	AN	Antineoplastic	Antineoplastic antibiotic	Injection
Mivacurium	NB	Neuromuscular blocking agent	-	Injection
Moclobemide	AD	Antidepressant	Mono-amine oxidase inhibitor	Oral
Modafinil	-	Analeptic / Stimulant	Nonamphetamine CNS stimulant	Oral
Montelukast	-	Anti asthmatic	Leukotriene receptor antagonist	Oral
Morphine	AG	Analgesic	Opioid agonist	Injection / Oral
Moxifloxacin	AB	Antibiotic	Fluroquinolone	Infusion / Oral
Moxonidine	Ahyp	Anti hypertensive	Selective agonist the imidazoline receptor subtype 1 (I1).	Oral
Mupirocin	AB	Antibiotic	Dermatologic agent	Topical
Muromonab-CD3	IM	Immunosuppressant		Injection
Mycophenolate Mofetil	IM	Immunosuppressant	Mycophenolic acid derivative	Oral / Infusion
Mycophenolate Sodium	IM	Immunosuppressant	Mycophenolic acid derivative	Oral / Infusion
Naloxone Hydrochloride	DxA	Detoxifying agent	Opioid antagonist	Injection
Naltrexone	DxA	Detoxifying agent	Opioid antagonist	Oral
Nandrolone Decanoate	AS	Anabolic steroid	-	Injection
Natamycin	AF	Antifungal Agent	Antibiotic	Ophtalmic
Neomycin	AB	Antibiotic	Aminoglycoside	Topical / Oral
Neostigmine	MS	Muscular stimulant	Anticholinesterase inhibitor	Injection
Nevirapine	AV	Antiviral	nonnucleoside inhibitor of HIV-1reverse transcriptase	Oral
Nicorandil	VA	Vasodilator	Antianginal Agents	Oral
Nifedipine	Ahyp	Antihypertensive antianginal	Calcium channel blocker	Oral
Nilotinib	AN	Antineoplastic agent	Tyrosine kinase inhibitor	Oral
Nimodipine	VA	Vasodilator	Calcium Channel blockers	Oral
Nitazoxanide	AB	Antibiotic	Antiprotozoal	Oral
Nitrazepam	AX	Anxiolytic	Benzodiazepine	Oral

Generic	Class Acronym	Therapeutic Class	Pharmacological Class	Mode of Administration
Nitrofurantoin	AB	Antibiotic	5-nitrofuran derivative	Oral
Norfloxacin	AB	Antibiotic	Fluroquinolone	Oral
Nortriptyline Hydrochloride	AD	Antidepressant	Tricyclic compound	Oral
Ofloxacin	AB	Antibiotic	Fluroquinolone	Topical
Olanzapine	Apsy	Antipsychotic	Thienobenzodiazepine	Injection
Olmesartan Medoxomil	Ahyp	Antihypertensive	Angiotensin II type 1-receptor antagonist	Oral
Olopatadine	AHt	Antihistamine	Histamine H_1 receptor antagonist	Ophtalmic
Olsalazine Sodium	AI	Anti-inflammatory	Salicylate	Oral
Omeprazole	AU	Antiulcer agent	Proton pump inhibitor	Oral
Ondansetron	AE	Antiemetic	Serotonin type 3 (5-HT_3) antagonist	Injection
Orphenadrine Citrate	MR	Muscle relaxant	-	Oral
Oseltamivir	AV	Antiviral	Viral neuroaminidase inhibitor	Oral
Oxaliplatin	AN	Antineoplastic	Alkylating agent	Infusion
Oxazepam	AX	Anxiolytic	Benzodiazepine	Oral
Oxcarbazepine	AC	Anticonvulsant	Carboxamide derivative	Oral
Oxpentifylline	HmT	Hematologic agent	Xanthine derivative	Oral
Oxybuprocaine	AA	Anaesthetic agent	-	Ophtalmic
Oxybutynin	ASp	Antispasmodic	Anticholinergic	Oral
Oxycodone	AG	Analgesic	Opioid agonist	Oral
Oxytetracycline	AB	Antibiotic	Tetracycline	Oral
Paclitaxel	AN	Antineoplastic	Antimicrotubule agent	Injection / infusion
Paliperidone	APsy	Antipsychotic agent	Benzisoxazole derivative	Oral
Pancuronium	NB	Neuromuscular blocking agent	-	Injection
Papaverine	VA	Vasodilator	cyclic nucleotide phosphodiesterase inhibitor	Injection
Paraldehyde	S	Sedative	-	Injection
Parecoxib Sodium	AI	Anti-inflammatory	COX2 selective inhibitor	Injection
Paromomycin	AB	Antibiotic	-	Injection
Paroxetine	AD	Antidepressant	Selective serotonin reuptake inhibitor	Oral
Pegfilgrastim	HP	Hematopoietic stimulator	Granulocytic colony stimulating factor	Injection
Pemetrexed	AN	Antineoplastic	Folate antimetabolite	Infusion
Pentamidine Isethionate	AB	Antibiotic	Antiprotozoal	Injection
Pentobarbitone	S	Sedative	Barbiturate	Injection
Perfluorooctane	AA	Anaesthetic agent (breathable liquid)	-	Inhalation
Pergolide	AP	Antiparkinsonian agent	Dopaminergic agent	Oral
Perhexiline Maleate	VA	Vasodilators	Calcium Channel blockers	Oral
Pericyazine	APsy	Antipsychotic	Phenothiazine	Oral
Perindopril	Ahyp	Antihypertensive	Angiotensin converting enzyme (ACE) inhibitors	Oral
Pethidine Hydrochloride	AG	Analgesic	Opioid agonist	Injection
Phenelzine Sulphate	AD	Antidepressant	Monoamine oxidase inhibitor	Oral
Phenindione	Acog	Anticoagulant	Vitamin K inhibitor	Oral
Phenobarbitone	AC	Anticonvulsant	Barbiturate	Injection / Oral
Phenoxybenzamine Hydrochloride	VA	Vasodilator	a_1-adrenergic receptor blocker	Oral
Phenoxymethylpenicillin	AB	Antibiotic	Penicillin	Oral / injection
Phentolamine Mesylate	Ahyp	Antihypertensive	a-adrenergic blocker	Injection
Phenytoin	AC	Anticonvulsant	Hydantoin derivative	Oral
Pilocarpine	AGl	Antiglaucoma agent	Cholinergic alkaloid	Ophtalmic
Pimozide	Apsy	Antipsychotic	Diphenylbutylpiperidine	Oral
Pindolol	βB	Beta-Blocker	Beta-adrenergic blocker (nonselective)	Oral
Pioglitazone Hydrochloride	HA	Hypoglycaemic agents	Thiazolidinedione	Oral

Generic	Class Acronym	Therapeutic Class	Pharmacological Class	Mode of Administration
Piperacillin	AB	Antibiotic	Penicillin	Injection / infusion
Piroxicam	AI	Anti-inflammatory	Oxicam derivative / nonsteroidal anti-inflammatory drug (NSAID)	Topical
Podophyllotoxin	AV	Antiviral	Non-alkaloid toxin lignan	Topical
Posaconazole	AF	Antifungal	Triazole	Oral
Pralidoxime	DxA	Detoxifying agent	Oxime	Injection
Pramipexole	AP	Antiparkinsonian agent	Non-ergot dopamine agonist	Oral
Prasugrel	Acog	Anticoagulant	Platelet aggregation inhibitor	Oral
Pravastatin	HL	Hypolipidemic agent	HMG-CoA reductase inhibitor	Oral
Praziquantel	AH	Anthelminthic	-	Oral
Prazosin Hydrochloride	Ahyp	Antihypertensive	Alpha1-adrenergic blocker	Oral
Pregabalin	AC	Anticonvulsant	GABA analogue	Oral
Primaquine	AM	Antimalarial	Aminoquinolone	Oral
Primidone	AC	Anticonvulsant	Barbiturate	Oral
Pristinamycin	AB	Antibiotic	Streptogramin	Oral
Probenecid	-	Antigout drug	Sulphonamide-derived uricosuric	Oral
Procainamide	AR	Antiarrhythmic agents	Membrane stabilizer	Injection / Oral
Procaine	AA	Anaesthetic agent	Aminobenzoic acid	Injection
Procaine Penicillin	AB	Antibiotic	Penicillin	Oral / injection
Procarbazine	AN	Antineoplastic	Alkylating agent	Oral
Prochlorperazine	AE	Antiemetic	Phenothiazine	Oral / Injection / Rectal
Proguanil	AM	Antimalarial	Antimalarial	Oral
Propantheline	ASp	Antispasmodic	Anticholinergic agent	Oral
Propofol	AA	Anaesthetic Agent	-	Intraveneous
Propranolol Hydrochloride	βB	Beta-Blocker *(antianginal)*	Beta-adrenergic blocker (nonselective)	Oral
Propylthiouracil	AT	Antithyroid agent	Thioamide derivative	Oral
Prothionamide	TB	Tuberculosis treatment	-	Oral
Pyrazinamide	TB	Antitubercular agent	Niacinamide derivative	Oral
Pyridostigmine Bromide	MS	Muscular stimulant	Anticholinesterase	Oral
Pyrimethamine	AM	Antimalarial	Folic acid antagonist	Oral
Quetiapine	Apsy	Antipsychotic	Dibenzothiazepine derivative	Oral
Quinapril	Ahyp	Antihypertensive	Angiotensin-converting enzyme (ACE) inhibitor	Oral
Quinupristin	AB	Antibiotic	-	Infusion
Raloxifene	BP	Bone resorption inhibitor	Nonsteroidal benzothiophene derivative	Oral
Raltegravir	AV	Antiviral	Integrase inhibitors	Oral
Ramipril	Ahyp	Antihypertensive	ACE inhibitors - angiotensin converting enzyme	Oral
Reboxetine	AD	Antidepressant	Noradrenaline reuptake inhibitor	Oral
Remifentanil	AA	Anaesthetic agent	Opioid anaesthetic	Injection
Ribavirin	AV	Antiviral	Synthetic nucleoside analog	Inhalation
Rifabutin	AB	Antibiotic	Rifamycin derivative	Oral
Rifampicin	TB	Antitubercular agent	Rifamycin derivative	Oral
Riluzole	CNS	Central nervous system agent	Glutamate antagonist	Oral
Risedronate Sodium	BP	Bone resorption inhibitor	Bisphosphonate	Oral
Risperidone	APsy	Antipsychotic	Benzisoxazole derivative	Oral
Ritonavir	AV	Antiviral	Protease inhibitor	Oral
Rituximab	AN	Antineoplastic agent	Murine/human monoclonal antibody	Infusion
Rivaroxaban	Acog	Anticoagulant	Factor Xa inhibitor	Oral
Rivastigmine	AZ	Anti Alzheimer's agent	Cholinesterase inhibitor	Oral / Topical

Generic	Class Acronym	Therapeutic Class	Pharmacological Class	Mode of Administration
Rocuronium	NB	Neuromuscular blocking agent	nondepolarizing neuromuscular blocking agent	Injection
Romiplostim	HS	Haemostatic Agent	Platelet aggregation inhibitors	Injection
Ropivacaine	AA	Anaesthetic agent	-	Injection
Rosiglitazone	HA	Hypoglycaemic agents	Thiazolidinedione	Oral
Rosuvastatin	HL	Hypolipidaemic agent	HMG-CoA reductase inhibitor	Oral
Rotigotine	AP	Antiparkinsonian agent	Dopamine agonist	Topical (patch)
Roxithromycin	AB	Antibiotic	Macrolide	Oral
Salmeterol	BD	Bronchodilator	Beta2-adrenergic receptor agonist	Inhalation
Saquinavir	AV	Antiviral	Protease inhibitor	Oral
Selegiline Hydrochloride	AP	Antiparkinsonian agent	MAO inhibitor	Oral
Sertraline	AD	Antidepressant	Selective serotonin reuptake inhibitor	Oral
Sevelamer	DxA	Detoxifying agent	Phosphate binder	Oral
Sildenafil	IA	Impotence agent	PDE5 inhibitor Bottom of Form	Oral
Silver Sulfadiazine	AB	Antibiotic	Sulphonamide	Topica
Simvastatin	HL	Hypolipidemic agent	HMG-CoA reductase inhibitor	Oral
Sirolimus	IM	Immunosuppressant	Macrocyclic lactone	Oral
Sitagliptin	HA	Hypoglycaemic agents	Dipeptidyl peptidase 4 (DPP-4) inhibitor	Oral
Sodium Clodronate	BP	Bone resorption inhibitor	Bisphosphonate	Oral
Sodium Fusidate	AB	Antibiotic	-	Oral / Topical
Sodium Nitroprusside	Ahyp	Antihypertensive	Vasodilator	Injection
Sodium Phenylbutyrate	-	Orphan drug	-	Oral
Sodium Tetradecyl sulphate	SC	Sclerosing agents	-	Injection
Sodium Thiosulphate	DxA	Detoxifying agent	-	Injection
Sodium Valproate	AC	Anticonvulsant	Carboxylic acid derivative	Oral / injection
Solifenacin Succinate	ASp	(urinary) antispasmodic	Anticholinergic	Oral
Sotalol Hydrochloride	βB	Beta-Blocker	Beta-adrenergic blocker	Oral
Spironolactone	DI	Diuretic	Aldosterone inhibitor	Oral
Stavudine	AV	Antiviral	Nucleoside reverse transcriptase inhibitor	Oral
Streptomycin Sulfate	AB	Antibiotic	Aminoglycoside	Oral
Strontium Ranelate	-	Anti-osteoporotic agent	-	Oral
Succimer	ChA	Chelating agent	-	Oral
Sufentanyl	AG	Analgesic	Opioid analgesic	Injection
Sugammadex	DxA	Detoxifying agent	Selective relaxant binding agent (SRBA)	Injection
Sulfadiazine	AB	Antibiotic	Sulphonamide	Injection
Sulfadoxine	AM	Antimalarial	Sulphonamide	Oral
Sulindac	AI	Anti-inflammatory	Cyclooxygenase-1 (COX-1) enzyme inhibitor	Oral
Sulphamethoxazole	AB	Antibiotic	Sulphonamide	Oral
Sulphasalazine	AB	Antibiotic	Sulphonamide	Oral
Sulthiame	AC	Anticonvulsant	Sulphonamide	Oral
Sumatriptan	Amig	Antimigraine	Selective 5-hydroxytryptamine1 (5-HT1) agonist	Injection / oral /spray
Sunitinib	AN	Antineoplastic	Receptor tyrosine kinase inhibitor	Oral
Suxamethonium	NB	Neuromuscular blocking agent	Depolarising neuromuscular blocker Bottom of Form	Injection
Tacrolimus	IM	Immunosuppressant	Macrolide	Oral / Infusion
Tamoxifen	AN	Hormonal antineoplastic agent	Estrogen receptor antagonist	Oral

Generic	Class Acronym	Therapeutic Class	Pharmacological Class	Mode of Administration
Tamsulosin	-	Anti adrenergic	α adrenergic blocker	Oral
Tazarotene	APSo	Antipsioratic	Retinoid prodrug	Topical cream
Tazobactam	AB	Antibiotic	Beta-lactamase inhibitor	Oral
Teicoplanin	AB	Antibiotic	Glycopeptide antibiotic	Injection
Telmisartan	Ahyp	Antihypertensive	Angiotensin II receptor antagonists	Oral
Temazepam	SE	Sedative	Benzodiazepine	Oral
Temocillin	AB	Antibiotic	Penicillin	Oral
Temozolomide	AN	Antineoplastic	Alkylating agent	Oral
Tenecteplase	FB	Fibronolytic agent	Tissue plasminogen activator	Injection
Tenofovir	AV	Antiviral	Reverse transcriptase inhibitor	Oral
Terbutaline Sulphate	BD	Bronchodilator	Selective beta$_2$-adrenergic receptor agonist	Injection / inhalation
Teriparatide	PH	Parathyroid hormone	Biosynthetic fragment of human parathyroid hormone	Injection
Terlipressin	VP	Vasopressor	-	Injection
Tetrabenazine	CNS	Central nervous system agent	Neurotransmitter uptake inhibitor	Oral
Tetracycline	AB	Antibiotic	-	Oral
Thalidomide	AN	miscellaneous anti neoplastic	TNF-α inhibitor	Oral
Thioguanine	AN	Antineoplastic	Antimetabolite	Oral
Thiopentone	AA	Anaesthetic Agent *(General)*	Barbiturate	Injection
Thioridazine Hydrochloride	APsy	Antipsychotic	Phenothiazine	Oral
Thiotepa	AN	Antineoplastic	Alkylating agent	Injection
Tiagabine	AC	Anticonvulsant	-	Oral
Ticarcillin	AB	Antibiotic	Penicillin	Injection
Tigecycline	AB	Antibiotic	Protein synthesis inhibitor	Infusion
Timolol	AGl	Antiglaucoma agent	carbonic anhydrase inhibitor (glaucoma preparation)	Ophtalmic drops
Tinidazole	AB	Antibiotic	Nitro- imidazole derivative	Oral
Tiotropium	BD	Bronchodilator	Anticholinergic / muscarinic antagonist	Inhalation
Tipranavir	AV	Antiviral	Nonpeptidic protease inhibitor of human immunodeficiency virus type 1 (HIV-1)	Oral
Tirofiban	Acog	Anticoagulant	Glycoprotein (GP IIb/IIIa)-receptor inhibitor	Infusion
Tobramycin	AB	Antibiotic	Aminoglycoside	Injection / ophtalmic drops/ ointment
Topiramate	AC	Anticonvulsant	Sulfamate-substituted monosaccharide derivative	Oral
Topotecan	AN	Antineoplastic	DNA topoisomerase inhibitor	Infusion
Tramadol	AG	Analgesic	Opioid partial μ agonist	Oral
Trandolapril	Ahyp	Antihypertensive	Angiotensin-converting enzyme (ACE) inhibitor	Oral
Tranexamic Acid	HS	Haemostatic Agent	-	Oral
Tranylcypromine	AD	Antidepressant	Monoamine oxidase inhibitor	Oral
Trastuzumab	AN	Antineoplastic agent	Human epidermal growth factor receptor 2 (HER2) monoclonal Ab	Infusion
Triamterene	DI	Diuretic	Potassium-sparing diuretic	Oral
Trifluoperazine Hydrochloride	APsy	Antipsychotic	Piperazine phenothiazine	Oral
Trimethoprim	AB	Antibiotic	-	Oral
Trimipramine	AD	Antidepressant	Dibenzazepine	Oral
Trometamol	AI	Anti-inflammatory	Nonsteroidal anti-inflammatory analgesic (NSAID)	Injection / ophtalmic drops
Tropicamide	MY	Mydriatic	Anticholinergic	Ophtalmic drops
Tropisetron	AE	Antiemetic	serotonin 5-HT3 receptor antagonist	Oral / Injection
Ursodeoxycholic Acid	BT	Bile therapy	-	Oral
Valaciclovir	AV	Antiviral	Purine analog	Oral

Generic	Class Acronym	Therapeutic Class	Pharmacological Class	Mode of Administration
Valganciclovir	AV	Antiviral	Prodrug for ganciclovir	Oral
Vancomycin	AB	Antibiotic	Glycopeptide antibiotic	Injection /oral
Varenicline	NA	Nicotinic agonist	Partial α4β2 agonist	Oral
Vecuronium	NB	Neuromuscular blocking agent	Depolarising neuromuscular blocking agents	Injection
Venlafaxine	AD	Antidepressant	Serotonin-norepinephrine reuptake inhibitor	Oral
Verapamil Hydrochloride	Ahyp	Anihypertensive	Calcium Channel blockers	Oral
Vigabatrin	AC	Anti convulsing	GABA transaminase inhibitor	Oral
Vinblastine Sulphate	AN	Antineoplastic	Mitotic inhibitor	Injection
Vincristine Sulphate	AN	Antineoplastic	Mitotic inhibitor	Injection
Vinorelbine	AN	Antineoplastic	Mitotic inhibitor	Injection
Voriconazole	AF	Antifungal Agent	Triazole	Oral
Warfarin	Acog	Anticoagulant	Vitamin K antagonist	Oral
Zanamivir	AV	Antiviral	Neuraminidase inhibitor	Inhalation
Zidovudine	AV	Antiviral	Nucleoside analog reverse transcriptase inhibitor (NRTI),	Oral
Ziprasidone	APsy	Antipsychotic	Multi receptors aganosit and antagonist	Oral / injection
Zoledronic Acid	BP	Bone resorption inhibitor	Bisphosphonate	Infusion
Zolpidem	S	Sedative	Non-benzodiazepine hypnotic	Oral
Zopiclone	S	Sedative	Non-benzodiazepine hypnotic	Oral
Zuclopenthixol	APsy	Antipsychotic	Thioxanthene neuroleptic	Injection

Supporting information B2: Percentage contributions of hospitals to the load of pharmaceuticals in influent of the corresponding treatment plant – year 2008

Table SI 7. Compounds for which the contribution of hospitals is 100%

Contributions =100%											
QEII		CAB		IPS		PC		PA		RBWH	
Abacavir	100	Alfentanil	100	Abacavir	100	Alfentanil	100	Abacavir	100	Abacavir	100
Adapalene	100	Amethocaine	100	Alfentanil	100	Amethocaine	100	Alfentanil	100	Alfentanil	100
Alfentanil	100	Amikacin	100	Amethocaine	100	Amikacin	100	Amethocaine	100	Amethocaine	100
Aminophylline	100	Aminophylline	100	Amikacin	100	Aminophylline	100	Amikacin	100	Amikacin	100
Artemether	100	Bupivacaine	100	Aminophylline	100	Anagrelide	100	Aminophylline	100	Aminophylline	100
Atracurium	100	Capecitabine	100	Artemether	100	Artemether	100	Artemether	100	Anagrelide	100
Bupivacaine	100	Cilastatin	100	Atracurium	100	Atracurium	100	Artesunate	100	Artemether	100
Cisatracurium	100	Cisatracurium	100	Aztreonam	100	Aztreonam	100	Atracurium	100	Artesunate	100
Cyclopentolate	100	Cyclopentolate	100	Bupivacaine	100	Bupivacaine	100	Aztreonam	100	Atracurium	100
Dexmedetomidine Hydrochloride	100	Dexmedetomidine Hydrochloride	100	Capecitabine	100	Busulfan	100	Bupivacaine	100	Aztreonam	100
Diazoxide	100	Diazoxide	100	Cidofovir	100	Capecitabine	100	Capecitabine	100	Bupivacaine	100
Dicobalt edetate	100	Dicobalt edetate	100	Cisatracurium	100	Caspofungin Acetate	100	Caspofungin Acetate	100	Busulfan	100
Dobutamine	100	Dobutamine	100	Cocaine	100	Cilastatin	100	Cidofovir	100	Capecitabine	100
Dopamine	100	Dopamine	100	Cyclopentolate	100	Cisatracurium	100	Cisatracurium	100	Caspofungin Acetate	100
Droperidol	100	Droperidol	100	Dexmedetomidine Hydrochloride	100	Colistimethate Sodium	100	Cocaine	100	Cidofovir	100
Emtricitabine	100	Ergometrine	100	Diazoxide	100	Danaparoid	100	Colistimethate Sodium	100	Cisatracurium	100
Ergometrine	100	Ergotamine	100	Dicobalt edetate	100	Demeclocycline	100	Cyclopentolate	100	Cocaine	100
Ergotamine	100	Esmolol	100	Disulfiram	100	Dexmedetomidine Hydrochloride	100	Dacarbazine	100	Colistimethate Sodium	100
Ertapenem	100	Ethambutol	100	Dobutamine	100	Diazoxide	100	Dactinomycin	100	Cyclopentolate	100
Ethambutol	100	Flumazenil	100	Dopamine	100	Disulfiram	100	Dalfopristin	100	Dacarbazine	100
Flumazenil	100	Glycopyrrolate	100	Droperidol	100	Dobutamine	100	Danaparoid	100	Dactinomycin	100
Glycopyrrolate	100	Imipenem	100	Ergometrine	100	Dopamine	100	Daptomycin	100	Danaparoid	100
Isoprenaline	100	Isoprenaline	100	Ergotamine	100	Droperidol	100	Daunorubicin	100	Darunavir	100
Ketamine	100	Ketamine	100	Ertapenem	100	Emtricitabine	100	Dexmedetomidine Hydrochloride	100	Daunorubicin	100
Lenograstim	100	Levobupivacaine	100	Esmolol	100	Ertapenem	100	Disulfiram	100	Defibrotide	100
Levobupivacaine	100	Meropenem	100	Ethambutol	100	Esmolol	100	Dobutamine	100	Demeclocycline	100

Contributions =100%											
QEII		CAB		IPS		PC		PA		RBWH	
Levomepromazine	100	Metaraminol tartrate	100	Flumazenil	100	Ethambutol	100	Dofetilide	100	Dexmedetomidine Hydrochloride	100
Lumefantrine	100	Mivacurium	100	Glycopyrrolate	100	Flucytosine	100	Dopamine	100	Diazoxide	100
Meropenem	100	Mycophenolate Mofetil	100	Isoprenaline	100	Flumazenil	100	Droperidol	100	Dicobalt edetate	100
Metaraminol tartrate	100	Neostigmine	100	Ketamine	100	Foscarnet	100	Edrophonium	100	Disulfiram	100
Mitomycin	100	Nimodipine	100	Lenograstim	100	Fosfomycin	100	Emtricitabine	100	Dobutamine	100
Mycophenolate Mofetil	100	Oxybuprocaine	100	Levobupivacaine	100	Glycopyrrolate	100	Enalaprilat	100	Dofetilide	100
Neostigmine	100	Parecoxib Sodium	100	Lumefantrine	100	Imipenem	100	Ergotamine	100	Dopamine	100
Nimodipine	100	Piperacillin	100	Meropenem	100	Isoprenaline	100	Ertapenem	100	Droperidol	100
Oxybuprocaine	100	Pralidoxime	100	Metaraminol tartrate	100	Ketamine	100	Esmolol	100	Edrophonium	100
Pancuronium	100	Propofol	100	Metolazone	100	Lepirudin	100	Ethambutol	100	Emtricitabine	100
Piperacillin	100	Remifentanil	100	Mitomycin	100	Levomepromazine	100	Flucytosine	100	Enfuvirtide	100
Pralidoxime	100	Rocuronium	100	Mivacurium	100	Levosimendan	100	Flumazenil	100	Ergometrine	100
Propofol	100	Ropivacaine	100	Neostigmine	100	Linezolid	100	Foscarnet	100	Ergotamine	100
Pyrazinamide	100	Sodium Fusidate	100	Nimodipine	100	Lopinavir	100	Glycopyrrolate	100	Ertapenem	100
Ropivacaine	100	Sodium Nitroprusside	100	Oxybuprocaine	100	Lumefantrine	100	Isoprenaline	100	Esmolol	100
Sodium Fusidate	100	Sodium Thiosulphate	100	Pancuronium	100	Meropenem	100	Ketamine	100	Ethambutol	100
Sodium Nitroprusside	100	Tazobactam	100	Paraldehyde	100	Metaraminol tartrate	100	Lenalidomide	100	Etravirine	100
Tazobactam	100	Teicoplanin	100	Parecoxib Sodium	100	Metolazone	100	Lenograstim	100	Flucytosine	100
Tenofovir	100	Thiopentone	100	Piperacillin	100	Metyrapone	100	Lepirudin	100	Flumazenil	100
Thiopentone	100	Tropicamide	100	Pralidoxime	100	Midodrine	100	Levomepromazine	100	Foscarnet	100
Trimipramine	100	Vecuronium	100	Primaquine	100	Milrinone	100	Levosimendan	100	Glycopyrrolate	100
Tropicamide	100	Voriconazole	100	Propofol	100	Mitomycin	100	Linezolid	100	Guanethidine	100
Vecuronium	100	Zidovudine	100	Pyrazinamide	100	Mivacurium	100	Lopinavir	100	Isoprenaline	100
		Suxamethonium	100	Remifentanil	100	Mycophenolate Mofetil	100	Lumefantrine	100	Ketamine	100
		Ephedrine	100	Rocuronium	100	Mycophenolate Sodium	100	Meropenem	100	Lenalidomide	100
		Cefoxitin	100	Ropivacaine	100	Neostigmine	100	Metaraminol tartrate	100	Lenograstim	100
		Ceftazidime	100	Sodium Clodronate	100	Nimodipine	100	Metolazone	100	Lepirudin	100
				Sodium Fusidate	100	Olopatadine	100	Metyrapone	100	Levobupivacaine	100
				Sodium Nitroprusside	100	Oxybuprocaine	100	Midodrine	100	Levomepromazine	100
				Sufentanyl	100	Oxytetracycline	100	Mitomycin	100	Levosimendan	100
				Tazobactam	100	Pancuronium	100	Mitotane	100	Linezolid	100
				Teicoplanin	100	Parecoxib Sodium	100	Mivacurium	100	Lomustine	100

Contributions =100%											
QEII		CAB		IPS		PC		PA		RBWH	
				Thiopentone	100	Pentamidine Isethionate	100	Muromonab-CD3	100	Lopinavir	100
				Tigecycline	100	Piperacillin	100	Mycophenolate Mofetil	100	Lumefantrine	100
				Tropicamide	100	Posaconazole	100	Mycophenolate Sodium	100	Meropenem	100
				Vecuronium	100	Pralidoxime	100	Natamycin	100	Metaraminol tartrate	100
				Voriconazole	100	Primaquine	100	Neostigmine	100	Metolazone	100
				Zidovudine	100	Pristinamycin	100	Nimodipine	100	Midodrine	100
				Suxamethonium	100	Propofol	100	Olopatadine	100	Mitomycin	100
				Ephedrine	100	Pyrazinamide	100	Oxybuprocaine	100	Mivacurium	100
				Ceftazidime	100	Remifentanil	100	Pancuronium	100	Mycophenolate Mofetil	100
						Ribavirin	100	Parecoxib Sodium	100	Mycophenolate Sodium	100
						Rocuronium	100	Pentamidine Isethionate	100	Natamycin	100
						Ropivacaine	100	Piperacillin	100	Neostigmine	100
						Saquinavir	100	Posaconazole	100	Nimodipine	100
						Sodium Clodronate	100	Pralidoxime	100	Nitazoxanide	100
						Sodium Fusidate	100	Primaquine	100	Oxybuprocaine	100
						Sodium Nitroprusside	100	Pristinamycin	100	Pancuronium	100
						Sodium Thiosulphate	100	Procaine	100	Parecoxib Sodium	100
						Sulfadiazine	100	Procarbazine	100	Pentamidine Isethionate	100
						Tazobactam	100	Propofol	100	Perfluorooctane	100
						Teicoplanin	100	Pyrazinamide	100	Piperacillin	100
						Temocillin	100	Quinupristin	100	Posaconazole	100
						Tenofovir	100	Remifentanil	100	Pralidoxime	100
						Thiopentone	100	Ribavirin	100	Primaquine	100
						Tigecycline	100	Rocuronium	100	Pristinamycin	100
						Tropicamide	100	Ropivacaine	100	Procaine	100
						Vecuronium	100	Saquinavir	100	Procarbazine	100
						Voriconazole	100	Sodium Clodronate	100	Propofol	100
						Zidovudine	100	Sodium Fusidate	100	Pyrazinamide	100
						Ceftazidime	100	Sodium Nitroprusside	100	Raltegravir	100

Contributions =100%											
QEII		CAB		IPS		PC		PA		RBWH	
						Suxamethonium	100	Sodium Tetradecyl sulphate	100	Remifentanil	100
						Cefoxitin	100	Sodium Thiosulphate	100	Rocuronium	100
						Ephedrine	100	Streptomycin Sulfate	100	Ropivacaine	100
								Sufentanyl	100	Saquinavir	100
								Sulfadiazine	100	Sodium Clodronate	100
								Tazobactam	100	Sodium Fusidate	100
								Teicoplanin	100	Sodium Nitroprusside	100
								Tenofovir	100	Sodium Phenylbutyrate	100
								Terlipressin	100	Sodium Tetradecyl sulphate	100
								Thiopentone	100	Sodium Thiosulphate	100
								Tigecycline	100	Succimer	100
								Topotecan	100	Sufentanyl	100
								Trometamol	100	Tazobactam	100
								Tropicamide	100	Teicoplanin	100
								Vecuronium	100	Tenofovir	100
								Voriconazole	100	Terlipressin	100
								Zidovudine	100	Tetracycline	100
								Ceftazidime	100	Thiopentone	100
								Cefoxitin	100	Tigecycline	100
								Suxamethonium	100	Tipranavir	100
										Tropicamide	100
										Vecuronium	100
										Voriconazole	100
										Zidovudine	100
										Suxamethonium	100
										Ephedrine	100
										Cefoxitin	100
										Ceftazidime	100

Table SI 8. Compounds for which the contribution of hospitals ranges from 50 to 100%.

50 ≤ Contributions < 100%											
QEII		**CAB**		**IPS**		**PC**		**PA**		**RBWH**	
Suxamethonium	99	Tenecteplase	99	Tenecteplase	99	Cefazolin	99	Carmustine	99	Thiotepa	99
Ceftazidime	99	Ketorolac	99	Ketorolac	98	Ritonavir	98	Ivabradine	99	Carmustine	99
Ephedrine	98	Ampicillin	99	Indinavir	98	Phentolamine Mesylate	96	Vincristine Sulphate	99	Infliximab	99
Cefoxitin	97	Gentamicin Sulphate	95	Ampicillin	98	Tirofiban	95	Infliximab	98	Vincristine Sulphate	99
Ampicillin	93	Lincomycin	95	Ritonavir	98	Tobramycin	95	Ephedrine	98	Acetylcholine Chloride	98
Tenecteplase	89	Midazolam	94	Midazolam	97	Valganciclovir	93	Ritonavir	97	Docetaxel	98
Framycetin Sulphate	87	Ceftriaxone	94	Phentolamine Mesylate	96	Ganciclovir	93	Bevacizumab	96	Ritonavir	97
Gentamicin Sulphate	80	Cefotaxime	93	Sunitinib	95	Sulfadoxine	93	Ampicillin	93	Phentolamine Mesylate	97
Lincomycin	72	Benzylpenicillin	92	Lincomycin	95	Ampicillin	85	Midazolam	92	Midazolam	96
Latanoprost	70	Tirofiban	88	Cefazolin	95	Midazolam	84	Docetaxel	92	Indinavir	95
Imiquimod	70	Naloxone Hydrochloride	87	Gentamicin Sulphate	95	Latanoprost	83	Valganciclovir	92	Ampicillin	95
Cefazolin	68	Latanoprost	87	Acetylcholine Chloride	94	Ketorolac	83	Cytarabine	91	Ganciclovir	94
Naloxone Hydrochloride	67	Granisetron	85	Ticarcillin	93	Gentamicin Sulphate	77	Lincomycin	89	Lincomycin	94
Ticarcillin	61	Cefazolin	84	Latanoprost	93	Abciximab	76	Phentolamine Mesylate	89	Idarubicin Hydrochloride	91
		Cefalotin	84	Naloxone Hydrochloride	89	Lincomycin	73	Ticarcillin	89	Deferasirox	90
		Ticarcillin	83	Benzylpenicillin	88	Cefepime	71	Deferasirox	88	Cytarabine	89
		Chloral Hydrate	83	Tirofiban	83	Tenecteplase	70	Cefazolin	87	Gentamicin Sulphate	89
		Tobramycin	83	Nicorandil	81	Ticarcillin	69	Latanoprost	86	Eptifibatide	89
		Pethidine Hydrochloride	74	Vancomycin	81	Epoprostenol	69	Gentamicin Sulphate	86	Latanoprost	85
		Moxifloxacin	72	Pethidine Hydrochloride	80	Nicorandil	68	Idarubicin Hydrochloride	84	Ticarcillin	85
		Nicorandil	70	Ceftriaxone	79	Stavudine	68	Ganciclovir	83	Fludarabine Phosphate	84
		Zuclopenthixol	68	Granisetron	77	Vancomycin	68	Vancomycin	81	Vancomycin	84

50 ≤ Contributions < 100%											
QEII		CAB		IPS		PC		PA		RBWH	
		Vancomycin	67	Cefotaxime	75	Deferasirox	67	Imiquimod	78	Benzylpenicillin	83
		Misoprostol	66	Imiquimod	73	Naloxone Hydrochloride	65	Melphalan	76	Cefazolin	81
		Acetylcysteine	65	Moxifloxacin	68	Benzylpenicillin	58	Ifosfamide	75	Melphalan	81
		Methylprednisolone	58	Desferrioxamine	68	Chloral Hydrate	51	Benzylpenicillin	74	Naloxone Hydrochloride	78
		Flupenthixol	58	Pimozide	67	Ceftriaxone	50	Atovaquone	71	Filgrastim	78
		Cefepime	56	Enoxaparin	63			Nicorandil	66	Tobramycin	76
		Enoxaparin	55	Acetylcysteine	63			Fludarabine Phosphate	61	Ketorolac	75
		Disodium Pamidronate	54	Tobramycin	60			Etoposide	61	Cefotaxime	75
		Silver Sulfadiazine	50	Neomycin	60			Methylprednisolone	59	Bevacizumab	75
				Thioridazine Hydrochloride	60			Neomycin	52	Tirofiban	73
				Silver Sulfadiazine	57			Epirubicin Hydrochloride	52	Silver Sulfadiazine	71
				Proguanil	53			Pyrimethamine	51	Cefalotin	69
								Naloxone Hydrochloride	51	Granisetron	69
								Tobramycin	50	Methylprednisolone	69
										Pethidine Hydrochloride	68
										Valganciclovir	67
										Etoposide	64
										Ceftriaxone	63
										Tenecteplase	62
										Acetylcysteine	60
										Cefepime	60
										Abciximab	59
										Riluzole	58
										Nicorandil	52
										Ifosfamide	51

Table SI 9. Compounds for which the contribution of hospitals ranges from 15 to 50%

15 ≤ Contributions < 50%											
QEII		**CAB**		**IPS**		**PC**		**PA**		**RBWH**	
Midazolam	41	Benzathine Penicillin	48	Disodium Pamidronate	49	Acetylcysteine	45	Granisetron	48	Deferiprone	49
Ketorolac	40	Metronidazole	43	Hydromorphone	48	Moxifloxacin	44	Ceftriaxone	44	Bivalirudin	48
Benzylpenicillin	36	Butylscopolamine	41	Misoprostol	47	Imiquimod	41	Cisplatin	43	Moxifloxacin	45
Desferrioxamine	36	Dexamethasone	39	Methylprednisolone	47	Methylprednisolone	41	Moxifloxacin	42	Cyclophosphamide	45
Ceftriaxone	31	Filgrastim	38	Atovaquone	45	Bivalirudin	40	Apraclonidine	41	Disodium Pamidronate	44
Nicorandil	30	Framycetin Sulphate	38	Oxpentifylline	43	Cefalotin	34	Cyclophosphamide	40	Calcium Folinate	43
Vancomycin	29	Fluphenazine Decanoate	37	Apomorphine	43	Cefotaxime	31	Fulvestrant	38	Chloral Hydrate	40
Benzathine Penicillin	28	Haloperidol	35	Azithromycin	42	Atovaquone	30	Tacrolimus	36	Cisplatin	39
Granisetron	25	Flucloxacillin	33	Flucloxacillin	41	Tacrolimus	28	Cinacalcet	36	Clioquinol	39
Methylprednisolone	23	Probenecid	32	Framycetin Sulphate	36	Azithromycin	26	Proguanil	36	Stavudine	39
Enoxaparin	21	Glucagon Hydrochloride	32	Butylscopolamine	35	Everolimus	24	Ofloxacin	35	Ondansetron	38
Cefotaxime	18	Rifampicin	31	Dexamethasone	35	Bosentan	23	Disodium Pamidronate	34	Dexamethasone	37
Cefalotin	18	Azithromycin	28	Dipyridamole	34	Granisetron	23	Homatropine Hydrobromide	32	Epoprostenol	33
Acetylcysteine	18	Dipyridamole	27	Glucagon Hydrochloride	33	Neomycin	23	Alprostadil	32	Apraclonidine	32
Dipyridamole	17	Chlorpromazine Hydrochloride	27	Benzathine Penicillin	33	Papaverine	22	Ondansetron	30	Atovaquone	30
Butylscopolamine	15	Ondansetron	26	Fentanyl	29	Phenoxybenzamine Hydrochloride	22	Calcium Folinate	29	Mitozantrone	28
		Dicloxacillin	21	Etoposide	29	Desferrioxamine	21	Acetylcysteine	29	Pyrimethamine	28
		Roxithromycin	19	Alprostadil	28	Cladribine	21	Praziquantel	28	Isoniazid	28
		Clindamycin	16	Metronidazole	25	Rifampicin	21	Pimozide	27	Didanosine	27
		Fentanyl	16	Ataztrimethoprim anavir	23	Bumetanide	19	Benzathine Penicillin	26	Rifampicin	23
		Olanzapine	16	Clozapine	22	Rifabutin	19	Tirofiban	26	Glucagon Hydrochloride	22
		Neomycin	15	Bumetanide	21	Enoxaparin	18	Oxpentifylline	23	Hydromorphone	22

15 ≤ Contributions < 50%											
QEII		**CAB**		**IPS**		**PC**		**PA**		**RBWH**	
		Clozapine	15	Buprenorphine	21	Disodium Pamidronate	18	Enoxaparin	23	Cetuximab	21
				Zuclopenthixol	21	Isoniazid	18	Ketorolac	23	Alprostadil	21
				Haloperidol	19	Cyclosporin	16	Dexamethasone	22	Thioguanine	21
				Rifampicin	19	Ondansetron	15	Sevelamer	22	Bleomycin	21
				Bevacizumab	19			Cyclosporin	20	Enoxaparin	21
				Flupenthixol	16			Fentanyl	20	Neomycin	20
				Trastuzumab	16			Hydromorphone	20	Misoprostol	20
				Ethacrynic Acid	16			Isoniazid	19	Efavirenz	19
								Filgrastim	19	Amphotericin	18
								Flucloxacillin	17	Flucloxacillin	18
								Cladribine	16	Azithromycin	17
								Carmellose Sodium	16	Methotrexate	17
								Phenindione	15	Ofloxacin	17
								Amphotericin	15	Buprenorphine	16
								Dipyridamole	15	Butylscopolamine	16
										Apomorphine	15
										Cyclosporin	15

Table SI 10. Compounds for which the contribution of hospitals ranges from 5 to 15%.

5 ≤ Contributions < 15%											
QEII		**CAB**		**IPS**		**PC**		**PA**		**RBWH**	
Azithromycin	14	Benztropine Mesylate	14	Clavulanic Acid	14	Hydromorphone	14	Glucagon Hydrochloride	14	Tacrolimus	14
Podophyllotoxin	13	Clavulanic Acid	14	Aripiprazole	14	Butylscopolamine	13	Lamivudine	13	Temozolomide	13
Quetiapine	12	Amisulpride	13	Risperidone	14	Sildenafil	12	Rituximab	13	Fentanyl	13
Dicloxacillin	11	Risperidone	13	Benztropine Mesylate	14	Erlotinib	12	Rifampicin	13	Homatropine Hydrobromide	13
Disodium Pamidronate	11	Trimethoprim	13	Trimethoprim	13	Mexiletine Hydrochloride	12	Vinblastine Sulphate	13	Imiquimod	13
Silver Sulfadiazine	10	Buprenorphine	12	Roxithromycin	13	Clozapine	11	Silver Sulfadiazine	12	Benzathine Penicillin	13
Moxifloxacin	9	Diazepam	12	Clindamycin	12	Flucloxacillin	11	Phenoxybenzamine Hydrochloride	12	Lamivudine	13
Haloperidol	8	Ivermectin	11	Isoniazid	12	Dipyridamole	11	Pethidine Hydrochloride	12	Rituximab	12
Flucloxacillin	8	Ciprofloxacin	11	Olanzapine	12	Tetrabenazine	10	Gabapentin	12	Gabapentin	12
Griseofulvin	8	Phenobarbitone	10	Ciprofloxacin	12	Filgrastim	10	Chloroquine	11	Desferrioxamine	12
Glucagon Hydrochloride	7	Tranexamic Acid	9	Lamivudine	10	Tranexamic Acid	10	Dantrolene Sodium	11	Metronidazole	12
Propantheline	6	Aripiprazole	9	Cyclophosphamide	9	Atazanavir	10	Bleomycin	11	Cinacalcet	12
Metronidazole	6	Bumetanide	9	Dantrolene Sodium	9	Dexamethasone	10	Methotrexate	11	Bacitracin	11
Fentanyl	6	Alprostadil	9	Vigabatrin	8	Alprostadil	9	Clindamycin	11	Dasatinib	11
Probenecid	6	Trifluoperazine Hydrochloride	8	Ziprasidone	8	Fentanyl	9	Trastuzumab	11	Vinblastine Sulphate	11
Flupenthixol	5	Mupirocin	8	Oxycodone	8	Probenecid	8	Ivermectin	11	Haloperidol	11
Mirtazapine	5	Quetiapine	7	Chlorpromazine Hydrochloride	8	Itraconazole	8	Doxorubicin Hydrochloride	11	Framycetin Sulphate	11
Tobramycin	5	Primidone	7	Filgrastim	8	Tiagabine	8	Rifabutin	9	Dipyridamole	10
Trimethoprim	5	Amoxycillin	7	Dicloxacillin	8	Oxpentifylline	8	Zuclopenthixol	9	Doxorubicin Hydrochloride	10
		Betamethasone	6	Hydralazine Hydrochloride	7	Dantrolene Sodium	8	Butylscopolamine	9	Mupirocin	10
		Prochlorperazine	6	Orphenadrine Citrate	7	Didanosine	8	Azithromycin	9	Carmellose Sodium	10
		Tramadol	6	Methadone Hydrochloride	7	Ciprofloxacin	7	Ethacrynic Acid	9	Ciprofloxacin	9
		Orphenadrine Citrate	6	Carmellose Sodium	7	Bromhexine Hydrochloride	7	Epoprostenol	9	Ursodeoxycholic Acid	9

5 ≤ Contributions < 15%											
QEII		CAB		IPS		PC		PA		RBWH	
		Omeprazole	6	Mianserin Hydrochloride	7	Flupenthixol	7	Mitozantrone	9	Clozapine	9
		Albendazole	6	Omeprazole	7	Pethidine Hydrochloride	7	Sirolimus	8	Eplerenone	9
		Morphine	6	Morphine	6	Haloperidol	6	Haloperidol	8	Tranexamic Acid	9
		Oxycodone	6	Gramicidin	6	Efavirenz	6	Clozapine	8	Pegfilgrastim	9
		Hydralazine Hydrochloride	5	Podophyllotoxin	6	Benzathine Penicillin	6	Baclofen	8	Clindamycin	8
		Methadone Hydrochloride	5	Digoxin	6	Glucagon Hydrochloride	6	Bumetanide	7	Chlorpromazine Hydrochloride	8
		Cefalexin	5	Naltrexone	6	Ziprasidone	5	Ciprofloxacin	7	Gramicidin	8
		Salmeterol	5	Benserazide	6	Amphotericin	5	Atazanavir	7	Flupenthixol	7
		Sulphamethoxazole	5	Ondansetron	6	Zoledronic Acid	5	Cefepime	7	Nevirapine	7
		Frusemide	5	Bromhexine Hydrochloride	6	Cefuroxime	5	Albendazole	7	Carboplatin	7
		Labetalol Hydrochloride	5	Quetiapine	6	Zuclopenthixol	5	Metronidazole	7	Zoledronic Acid	7
		Pyridostigmine Bromide	5	Phenytoin	6	Amisulpride	5	Framycetin Sulphate	7	Paclitaxel	7
		Sodium Valproate	5	Sulphamethoxazole	6	Buprenorphine	5	Bacitracin	7	Dicloxacillin	7
		Acamprosate	5	Frusemide	6	Framycetin Sulphate	5	Clavulanic Acid	7	Atazanavir	6
		Nitrofurantoin	5	Clobazam	6			Flupenthixol	7	Sulphamethoxazole	6
				Carboplatin	5			Buprenorphine	6	Epirubicin Hydrochloride	6
				Phenobarbitone	5			Acetazolamide	6	Diazepam	6
				Dihydroergotamine	5			Hydralazine Hydrochloride	6	Clonidine	6
				Sodium Valproate	5			Sulphamethoxazole	6	Lorazepam	6
				Amiodarone Hydrochloride	5			Oxycodone	6	Ivabradine	6
				Gabapentin	5			Abciximab	6	Clavulanic Acid	5
				Amoxycillin	5			Apomorphine	5	Oxpentifylline	5
				Nortriptyline Hydrochloride	5			Methadone Hydrochloride	5	Zuclopenthixol	5
				Bisoprolol	5			Everolimus	5	Oxycodone	5
				Salmeterol	5			Eplerenone	5	Acetazolamide	5

5 ≤ Contributions < 15%											
QEII		CAB		IPS		PC		PA		RBWH	
				Betamethasone	5			Dicloxacillin	5	Papaverine	5
								Zoledronic Acid	5	Trimethoprim	5
								Risperidone	5	Albendazole	5
										Morphine	5
										Vinorelbine	5

Table SI 11. Compounds for which the contribution of hospitals ranges from 0 to 5%

0 ≤ Contributions < 5%											
QEII		CAB		IPS		PC		PA		RBWH	
Zuclopenthixol	4	Phenytoin	4	Dipivefrine	4	Carmellose Sodium	4	Trimethoprim	4	Sevelamer	4
Orphenadrine Citrate	4	Temazepam	4	Procaine Penicillin	4	Baclofen	4	Bortezomib	4	Phenobarbitone	4
Flumethasone Pivalate	4	Bromhexine Hydrochloride	4	Cefalexin	4	Silver Sulfadiazine	4	Chlorpromazine Hydrochloride	4	Risperidone	4
Clioquinol	4	Propantheline	4	Amisulpride	4	Amiodarone Hydrochloride	4	Diazepam	4	Thalidomide	4
Pethidine Hydrochloride	4	Digoxin	4	Cefuroxime	4	Clindamycin	4	Thalidomide	4	Sulthiame	4
Aripiprazole	4	Apomorphine	4	Lorazepam	4	Oseltamivir	4	Carboplatin	4	Omeprazole	4
Tirofiban	4	Domperidone	4	Entacapone	4	Vigabatrin	4	Amiodarone Hydrochloride	4	Phenytoin	4
Vigabatrin	4	Gramicidin	4	Clonazepam	4	Eplerenone	4	Fludrocortisone Acetate	4	Valaciclovir	4
Buprenorphine	3	Amiodarone Hydrochloride	3	Cyproterone Acetate	4	Melphalan	4	Dorzolamide	3	Oseltamivir	4
Rifampicin	3	Erythromycin	3	Temazepam	4	Olanzapine	3	Temazepam	3	Labetalol Hydrochloride	4
Clavulanic Acid	3	Lamivudine	3	Norfloxacin	4	Sirolimus	3	Eptifibatide	3	Amisulpride	4
Benztropine Mesylate	3	Doxycycline	3	Clopidogrel	4	Clavulanic Acid	3	Olanzapine	3	Fluphenazine Decanoate	4
Olanzapine	3	Gabapentin	3	Tranexamic Acid	4	Dicloxacillin	3	Phenytoin	3	Benztropine Mesylate	4
Clindamycin	3	Norfloxacin	3	Diazepam	4	Ifosfamide	3	Nevirapine	3	Dorzolamide	3
Amitriptyline Hydrochloride	3	Clonidine	3	Nitrofurantoin	3	Chlorpromazine Hydrochloride	3	Omeprazole	3	Aripiprazole	3
Roxithromycin	3	Oseltamivir	3	Paliperidone	3	Gabapentin	3	Lorazepam	3	Dantrolene Sodium	3
Benserazide	3	Clonazepam	3	Trifluoperazine Hydrochloride	3	Omeprazole	3	Quetiapine	3	Methadone Hydrochloride	3
Captopril	3	Diphenoxylate Hydrochloride	3	Biperiden Hydrochloride	3	Lamivudine	3	Frusemide	3	Norfloxacin	3
Tetrabenazine	3	Ursodeoxycholic Acid	3	Mefloquine	3	Misoprostol	3	Pyridostigmine Bromide	3	Temazepam	3
Risperidone	3	Cefuroxime	3	Amantadine Hydrochloride	3	Risperidone	3	Pegfilgrastim	3	Fluorouracil	3
Chloroquine	2	Metoprolol	2	Prochlorperazine	3	Sulphamethoxazole	3	Aripiprazole	3	Acamprosate	3
Hydromorphone	2	Clopidogrel	2	Primidone	3	Trimethoprim	3	Perhexiline Maleate	3	Betamethasone	3

0 ≤ Contributions < 5%											
QEII		CAB		IPS		PC		PA		RBWH	
Dexamethasone	2	Lamotrigine	2	Baclofen	3	Ursodeoxycholic Acid	3	Efavirenz	3	Hydralazine Hydrochloride	2
Sulphamethoxazole	2	Hydromorphone	2	Levodopa	3	Vinorelbine	3	Morphine	3	Chloroquine	2
Amisulpride	2	Mirtazapine	2	Chloroquine	3	Hydralazine Hydrochloride	3	Cefotaxime	3	Gemcitabine	2
Tramadol	2	Nortriptyline Hydrochloride	2	Tramadol	3	Benztropine Mesylate	3	Entacapone	3	Entacapone	2
Gabapentin	2	Perhexiline Maleate	2	Mirtazapine	3	Metronidazole	3	Benztropine Mesylate	3	Mercaptopurine	2
Misoprostol	2	Spironolactone	2	Eplerenone	3	Quetiapine	3	Pilocarpine	3	Hydroxyurea	2
Mupirocin	2	Isosorbide Mononitrate	2	Oxaliplatin	3	Methadone Hydrochloride	3	Labetalol Hydrochloride	3	Amiodarone Hydrochloride	2
Omeprazole	2	Baclofen	2	Gemcitabine	3	Digoxin	3	Dapsone	3	Bumetanide	2
Acetazolamide	2	Clarithromycin	2	Albendazole	3	Frusemide	3	Oseltamivir	2	Pilocarpine	2
Doxepin Hydrochloride	2	Oxazepam	2	Oxazepam	3	Amantadine Hydrochloride	3	Paclitaxel	2	Digoxin	2
Ivermectin	2	Phenoxymethylpenicillin	2	Acetazolamide	3	Spironolactone	2	Probenecid	2	Quetiapine	2
Gramicidin	2	Topiramate	2	Erythromycin	3	Chloroquine	2	Adefovir Dipivoxil	2	Domperidone	2
Ciprofloxacin	2	Pioglitazone Hydrochloride	2	Isosorbide Mononitrate	3	Apomorphine	2	Itraconazole	2	Strontium Ranelate	2
Chlorpromazine Hydrochloride	2	Warfarin	2	Oseltamivir	3	Levetiracetam	2	Betamethasone	2	Baclofen	2
Cefuroxime	2	Carbamazepine	2	Metoprolol	3	Roxithromycin	2	Tramadol	2	Olanzapine	2
Nevirapine	2	Venlafaxine	2	Spironolactone	3	Pemetrexed	2	Amisulpride	2	Roxithromycin	2
Cefalexin	2	Citalopram	2	Sevelamer	2	Clonazepam	2	Bisoprolol	2	Etanercept	2
Bethanechol Chloride	2	Mianserin Hydrochloride	2	Acamprosate	2	Lamotrigine	2	Nitrofurantoin	2	Bisoprolol	2
Amoxycillin	2	Propylthiouracil	2	Fludrocortisone Acetate	2	Entacapone	2	Timolol	2	Frusemide	2
Diazepam	2	Glimepiride	2	Carbimazole	2	Domperidone	2	Oxybutynin	2	Imatinib	2
Biperiden Hydrochloride	2	Levetiracetam	2	Pilocarpine	2	Oxycodone	2	Mexiletine Hydrochloride	2	Chlorambucil	2
Piroxicam	2	Carbidopa	2	Topiramate	2	Lorazepam	2	Norfloxacin	2	Tramadol	2
Phenytoin	2	Eplerenone	2	Eformoterol	2	Fotemustine	2	Valaciclovir	2	Ivermectin	2
Isoniazid	2	Carvedilol	2	Domperidone	2	Naltrexone	2	Fluorouracil	2	Buspirone Hydrochloride	2
Bisoprolol	1	Tinidazole	2	Perhexiline Maleate	2	Galantamine	2	Benzhexol Hydrochloride	2	Prochlorperazine	2

0 ≤ Contributions < 5%											
QEII		CAB		IPS		PC		PA		RBWH	
Citalopram	1	Diltiazem Hydrochloride	2	Strontium Ranelate	2	Morphine	2	Cefuroxime	2	Spironolactone	2
Amiodarone Hydrochloride	1	Levodopa	2	Clonidine	2	Phenobarbitone	2	Levetiracetam	2	Timolol	2
Salmeterol	1	Eformoterol	2	Valaciclovir	2	Topiramate	2	Digoxin	2	Clarithromycin	2
Doxycycline	1	Benserazide	1	Oxybutynin	2	Perhexiline Maleate	2	Gemcitabine	2	Benserazide	2
Levodopa	1	Methyldopa	1	Benzhexol Hydrochloride	2	Bisoprolol	2	Bromhexine Hydrochloride	2	Erythromycin	2
Mianserin Hydrochloride	1	Benzathine Phenoxymethylpenicillin	1	Citalopram	2	Aripiprazole	2	Tranexamic Acid	2	Clonazepam	2
Cyproterone Acetate	1	Dothiepin Hydrochloride	1	Imipramine Hydrochloride	2	Diazepam	2	Desmopressin	2	Rifabutin	2
Fluvoxamine	1	Allopurinol	1	Probenecid	2	Phenytoin	1	Benserazide	2	Phenoxybenzamine Hydrochloride	2
Dothiepin Hydrochloride	1	Fluvoxamine	1	Timolol	2	Isosorbide Mononitrate	1	Misoprostol	2	Ethacrynic Acid	1
Digoxin	1	Bisoprolol	1	Oxcarbazepine	2	Norfloxacin	1	Propylthiouracil	2	Primidone	1
Chlorambucil	1	Ramipril	1	Sotalol Hydrochloride	2	Sodium Valproate	1	Azathioprine	2	Bicalutamide	1
Baclofen	1	Esomeprazole	1	Triamterene	2	Salmeterol	1	Hydroxyurea	2	Itraconazole	1
Pericyazine	1	Colchicine	1	Pegfilgrastim	2	Oxybutynin	1	Erlotinib	2	Sodium Valproate	1
Bromocriptine Mesylate	1	Dantrolene Sodium	1	Itraconazole	2	Temazepam	1	Acitretin	2	Proguanil	1
Oxycodone	1	Valaciclovir	1	Gemfibrozil	2	Fludrocortisone Acetate	1	Bivalirudin	1	Mexiletine Hydrochloride	1
Prochlorperazine	1	Nevirapine	1	Glipizide	2	Prochlorperazine	1	Mupirocin	1	Cefuroxime	1
Dorzolamide	1	Amitriptyline Hydrochloride	1	Pergolide	2	Procaine Penicillin	1	Sodium Valproate	1	Dapsone	1
Sodium Valproate	1	Hydroxychloroquine Sulphate	1	Colchicine	2	Atomoxetine	1	Cyproterone Acetate	1	Levetiracetam	1
Procaine Penicillin	1	Nitrazepam	1	Labetalol Hydrochloride	2	Benserazide	1	Isosorbide Mononitrate	1	Tinidazole	1
Hydralazine Hydrochloride	1	Sertraline	1	Amphotericin	2	Metoprolol	1	Metoprolol	1	Acitretin	1
Venlafaxine	1	Amantadine Hydrochloride	1	Levetiracetam	2	Clopidogrel	1	Roxithromycin	1	Metoprolol	1
Amphotericin	1	Amlodipine	1	Phenoxymethylpenicillin	2	Tramadol	1	Glipizide	1	Oxazepam	1
Morphine	1	Terbutaline Sulphate	1	Carbamazepine	2	Dorzolamide	1	Carbimazole	1	Cyproterone Acetate	1
Zoledronic Acid	1	Cyproterone Acetate	1	Atorvastatin	2	Losartan	1	Amoxycillin	1	Amoxycillin	1

0 ≤ Contributions < 5%											
QEII		CAB		IPS		PC		PA		RBWH	
Frusemide	1	Oxybutynin	1	Carvedilol	2	Primidone	1	Salmeterol	1	Dihydroergotamine	1
Betamethasone	1	Prazosin Hydrochloride	1	Venlafaxine	2	Cyproterone Acetate	1	Colchicine	1	Cefalexin	1
Entacapone	1	Sotalol Hydrochloride	1	Nitrazepam	1	Eformoterol	1	Nortriptyline Hydrochloride	1	Carvedilol	1
Ethacrynic Acid	1	Carbimazole	1	Lamotrigine	1	Mirtazapine	1	Oxazepam	1	Tetrabenazine	1
Norfloxacin	1	Paroxetine	1	Doxycycline	1	Acamprosate	1	Clonazepam	1	Oxybutynin	1
Pilocarpine	1	Atorvastatin	1	Cabergoline	1	Biperiden Hydrochloride	1	Galantamine	1	Sildenafil	1
Rosiglitazone	1	Sulphasalazine	1	Dothiepin Hydrochloride	1	Warfarin	1	Domperidone	1	Balsalazide	1
Clonidine	1	Timolol	1	Amitriptyline Hydrochloride	1	Amoxycillin	1	Clonidine	1	Amitriptyline Hydrochloride	1
Oxybutynin	1	Gliclazide	1	Colestyramine	1	Carbamazepine	1	Dipivefrine	1	Levodopa	1
Tamsulosin	1	Fluoxetine Hydrochloride	1	Fluvoxamine	1	Tropisetron	1	Vinorelbine	1	Isosorbide Mononitrate	1
Nitrofurantoin	1	Propranolol Hydrochloride	1	Warfarin	1	Carvedilol	1	Mirtazapine	1	Adefovir Dipivoxil	1
Erythromycin	1	Captopril	1	Disopyramide	1	Mupirocin	1	Gramicidin	1	Flumethasone Pivalate	1
Terbutaline Sulphate	1	Olsalazine Sodium	1	Allopurinol	1	Sotalol Hydrochloride	1	Podophyllotoxin	1	Vigabatrin	1
Glimepiride	1	Rosiglitazone	1	Alendronate Sodium	1	Nevirapine	1	Spironolactone	1	Perhexiline Maleate	1
Phenoxymethylpenicillin	1	Cyclophosphamide	1	Clarithromycin	1	Bromocriptine Mesylate	1	Amantadine Hydrochloride	1	Nitrofurantoin	1
Phenobarbitone	1	Clomipramine Hydrochloride	1	Ramipril	1	Acetazolamide	1	Amitriptyline Hydrochloride	1	Salmeterol	1
Cabergoline	1	Doxepin Hydrochloride	1	Terbutaline Sulphate	1	Clonidine	1	Acamprosate	1	Fludrocortisone Acetate	1
Carbamazepine	1	Nifedipine	1	Pericyazine	1	Fluphenazine Decanoate	1	Levodopa	1	Naltrexone	1
Domperidone	1	Lercanidipine	1	Hydroxyurea	1	Citalopram	1	Sildenafil	1	Bromocriptine Mesylate	1
Moclobemide	1	Colestyramine	1	Benzathine Phenoxymethylpenicillin	1	Glipizide	1	Fluorometholone	1	Captopril	1
Sertraline	1	Itraconazole	1	Mupirocin	1	Oxazepam	1	Oxcarbazepine	1	Exemestane	1
Glipizide	1	Pericyazine	1	Amlodipine	1	Strontium Ranelate	1	Prochlorperazine	1	Clopidogrel	1
Fluoxetine Hydrochloride	1	Tacrolimus	1	Letrozole	1	Levodopa	1	Tiotropium	1	Citalopram	1
Ondansetron	1	Perindopril	1	Glimepiride	1	Donepezil	1	Citalopram	1	Methyldopa	1

0 ≤ Contributions < 5%											
QEII		CAB		IPS		PC		PA		RBWH	
Diphenoxylate Hydrochloride	1	Pravastatin	1	Tinidazole	1	Propranolol Hydrochloride	1	Bethanechol Chloride	1	Diphenoxylate Hydrochloride	1
Sumatriptan	1	Lorazepam	1	Risedronate Sodium	1	Ethacrynic Acid	1	Warfarin	1	Nitrazepam	1
Colchicine	1	Raloxifene	1	Mercaptopurine	1	Colchicine	1	Cefalexin	1	Topiramate	1
Adefovir Dipivoxil	1	Hydroxyurea	1	Mexiletine Hydrochloride	1	Alendronate Sodium	1	Colestyramine	1	Hydroxychloroquine Sulphate	1
Levetiracetam	1	Dorzolamide	1	Brimonidine	1	Cefalexin	1	Carbamazepine	1	Glipizide	1
Imipramine Hydrochloride	1	Lisinopril	1	Rosiglitazone	1	Atorvastatin	1	Phenobarbitone	1	Mirtazapine	1
Sulphasalazine	1	Pindolol	1	Desmopressin	1	Nitrazepam	1	Bimatoprost	1	Letrozole	1
Azathioprine	1	Acetazolamide	1	Sertraline	1	Captopril	1	Hydroxychloroquine Sulphate	1	Carbamazepine	1
Bumetanide	1	Imipramine Hydrochloride	1	Methyldopa	1	Valaciclovir	1	Entecavir	1	Propantheline	1
Methadone Hydrochloride	1	Amphotericin	1	Paroxetine	1	Timolol	1	Eformoterol	1	Atorvastatin	1
Isosorbide Mononitrate	1	Metformin Hydrochloride	1	Bromocriptine Mesylate	1	Trastuzumab	1	Brimonidine	1	Colchicine	1
Apraclonidine	1	Calcium Folinate	1	Cyclosporin	1	Benzhexol Hydrochloride	1	Amlodipine	1	Eformoterol	1
Clopidogrel	1	Simvastatin	1	Prazosin Hydrochloride	1	Terbutaline Sulphate	0.5	Gliclazide	1	Galantamine	1
Benzhexol Hydrochloride	1	Glibenclamide	1	Carbidopa	1	Nitrofurantoin	0.5	Trifluoperazine Hydrochloride	1	Bromhexine Hydrochloride	1
Homatropine Hydrobromide	1	Verapamil Hydrochloride	1	Lisinopril	1	Carbidopa	0.5	Ursodeoxycholic Acid	1	Carbidopa	1
Famciclovir	1	Tamoxifen	1	Perindopril	1	Carbimazole	0.5	Carvedilol	1	Oxcarbazepine	1
Paroxetine	0.5	Alprazolam	1	Propylthiouracil	1	Gramicidin	0.5	Methyldopa	1	Venlafaxine	1
Amantadine Hydrochloride	0.5	Azathioprine	1	Diltiazem Hydrochloride	1	Cabergoline	0.5	Carbidopa	1	Fluvoxamine	1
Perhexiline Maleate	0.5	Atenolol	1	Nifedipine	1	Amiloride Hydrochloride	0.5	Propantheline	1	Carbimazole	1
Atorvastatin	0.5	Fludrocortisone Acetate	1	Esomeprazole	1	Carboplatin	0.5	Cefalotin	1	Warfarin	1
Temazepam	0.5	Candesartan	1	Fluphenazine Decanoate	1	Bicalutamide	0.4	Clarithromycin	1	Propranolol Hydrochloride	1
Tranexamic Acid	0.5	Entacapone	1	Hydroxychloroquine Sulphate	1	Ramipril	0.4	Letrozole	1	Trifluoperazine Hydrochloride	1
Escitalopram	0.5	Bromocriptine Mesylate	0.5	Gliclazide	1	Doxycycline	0.4	Clopidogrel	1	Lamotrigine	1
Timolol	0.4	Risedronate Sodium	0.5	Metformin Hydrochloride	1	Risedronate Sodium	0.4	Atorvastatin	1	Allopurinol	1

0 ≤ Contributions < 5%											
QEII		**CAB**		**IPS**		**PC**		**PA**		**RBWH**	
Mefloquine	0.4	Alendronate Sodium	0.4	Verapamil Hydrochloride	1	Gemfibrozil	0.4	Chlorambucil	1	Risedronate Sodium	1
Warfarin	0.4	Flecainide Acetate	0.4	Fluoxetine Hydrochloride	1	Gliclazide	0.4	Strontium Ranelate	1	Amlodipine	1
Carbimazole	0.4	Fosinopril	0.4	Clomipramine Hydrochloride	1	Amlodipine	0.4	Primidone	1	Azathioprine	1
Eformoterol	0.4	Carmellose Sodium	0.4	Simvastatin	1	Disopyramide	0.4	Topiramate	1	Pyridostigmine Bromide	1
Methysergide	0.4	Felodipine	0.4	Flecainide Acetate	1	Pilocarpine	0.4	Allopurinol	1	Dipivefrine	1
Carvedilol	0.4	Gemfibrozil	0.4	Atenolol	1	Clarithromycin	0.4	Sulphasalazine	1	Entecavir	1
Prazosin Hydrochloride	0.4	Amiloride Hydrochloride	0.4	Raloxifene	1	Venlafaxine	0.4	Pericyazine	1	Adalimumab	1
Propranolol Hydrochloride	0.4	Ezetimibe	0.4	Propranolol Hydrochloride	1	Amitriptyline Hydrochloride	0.4	Lisinopril	1	Colestyramine	1
Metformin Hydrochloride	0.4	Mesalazine	0.4	Glibenclamide	1	Sertraline	0.4	Alendronate Sodium	1	Benzhexol Hydrochloride	1
Gliclazide	0.4	Anastrozole	0.3	Pravastatin	1	Verapamil Hydrochloride	0.4	Diltiazem Hydrochloride	1	Irinotecan	1
Fenofibrate	0.4	Hydrochlorothiazide	0.3	Ezetimibe	1	Sevelamer	0.4	Glimepiride	1	Tropisetron	1
Clonazepam	0.4	Fenofibrate	0.3	Hydrochlorothiazide	1	Lisinopril	0.4	Paliperidone	1	Alendronate Sodium	1
Fludrocortisone Acetate	0.4	Irbesartan	0.3	Leflunomide	1	Perindopril	0.4	Bosentan	1	Esomeprazole	1
Meloxicam	0.4	Famciclovir	0.3	Doxepin Hydrochloride	1	Betamethasone	0.4	Solifenacin Succinate	1	Gliclazide	1
Fosinopril	0.4	Bimatoprost	0.3	Amiloride Hydrochloride	1	Flecainide Acetate	0.4	Mercaptopurine	1	Propylthiouracil	1
Lamotrigine	0.4	Indapamide	0.3	Moclobemide	1	Esomeprazole	0.3	Pioglitazone Hydrochloride	1	Bosentan	1
Metoprolol	0.4	Fluorometholone	0.3	Irbesartan	1	Calcium Folinate	0.3	Bicalutamide	1	Gemfibrozil	1
Lisinopril	0.4	Cefaclor	0.3	Azathioprine	1	Allopurinol	0.3	Venlafaxine	1	Podophyllotoxin	1
Lamivudine	0.3	Betahistine	0.3	Donepezil	1	Leflunomide	0.3	Sertraline	1	Ramipril	1
Gemfibrozil	0.3	Oxcarbazepine	0.3	Lercanidipine	0.5	Diltiazem Hydrochloride	0.3	Ramipril	1	Prazosin Hydrochloride	1
Risedronate Sodium	0.3	Triamterene	0.2	Betaxolol Hydrochloride	0.5	Acitretin	0.3	Atenolol	0.5	Cabergoline	1
Spironolactone	0.3	Brimonidine	0.2	Tamoxifen	0.5	Atenolol	0.3	Ziprasidone	0.5	Dothiepin Hydrochloride	1
Sotalol Hydrochloride	0.3	Montelukast	0.2	Pioglitazone Hydrochloride	0.5	Pravastatin	0.3	Phenelzine Sulphate	0.5	Terbutaline Sulphate	0.5
Allopurinol	0.3	Cabergoline	0.2	Captopril	0.5	Cisplatin	0.3	Fluphenazine Decanoate	0.5	Imipramine Hydrochloride	0.5

0 ≤ Contributions < 5%											
QEII		**CAB**		**IPS**		**PC**		**PA**		**RBWH**	
Amlodipine	0.3	Methotrexate	0.2	Pindolol	0.5	Dapsone	0.3	Moclobemide	0.5	Sertraline	0.5
Perindopril	0.3	Chlorthalidone	0.2	Fosinopril	0.4	Moclobemide	0.3	Erythromycin	0.5	Lisinopril	0.5
Topiramate	0.3	Biperiden Hydrochloride	0.2	Fluvastatin	0.4	Paroxetine	0.3	Prazosin Hydrochloride	0.5	Phenelzine Sulphate	0.5
Olsalazine Sodium	0.3	Benzhexol Hydrochloride	0.2	Cefaclor	0.3	Ofloxacin	0.3	Pregabalin	0.5	Atenolol	0.4
Mebeverine Hydrochloride	0.3	Glipizide	0.1	Felodipine	0.3	Fluvoxamine	0.3	Verapamil Hydrochloride	0.5	Diltiazem Hydrochloride	0.4
Alendronate Sodium	0.3	Strontium Ranelate	0.1	Alprazolam	0.3	Hydroxyurea	0.3	Exemestane	0.5	Clomipramine Hydrochloride	0.4
Clomipramine Hydrochloride	0.3	Letrozole	0.1	Candesartan	0.3	Erythromycin	0.3	Betahistine	0.5	Pioglitazone Hydrochloride	0.4
Glibenclamide	0.3	Piroxicam	0.1	Sulphasalazine	0.3	Fluoxetine Hydrochloride	0.3	Tamoxifen	0.5	Doxycycline	0.4
Neomycin	0.3	Pregabalin	0.1	Famciclovir	0.2	Pioglitazone Hydrochloride	0.3	Vigabatrin	0.5	Probenecid	0.4
Carbidopa	0.2	Dexamphetamine Sulphate	0.1	Diphenoxylate Hydrochloride	0.2	Hydroxychloroquine Sulphate	0.3	Sotalol Hydrochloride	0.5	Doxepin Hydrochloride	0.4
Minocycline	0.2	Moclobemide	0.09	Indapamide	0.2	Danazol	0.3	Terbutaline Sulphate	0.5	Fluoxetine Hydrochloride	0.4
Cimetidine	0.2	Leflunomide	0.09	Piroxicam	0.2	Methyldopa	0.3	Biperiden Hydrochloride	0.4	Sulphasalazine	0.4
Amiloride Hydrochloride	0.2	Tamsulosin	0.07	Bimatoprost	0.2	Trifluoperazine Hydrochloride	0.3	Fluvoxamine	0.4	Danazol	0.4
Galantamine	0.2	Sumatriptan	0.05	Propantheline	0.2	Labetalol Hydrochloride	0.3	Doxepin Hydrochloride	0.4	Perindopril	0.4
Atenolol	0.2	Cyclosporin	0.05	Olsalazine Sodium	0.2	Mianserin Hydrochloride	0.3	Losartan	0.4	Ziprasidone	0.4
Carmellose Sodium	0.2	Methylphenidate	0.02	Anastrozole	0.2	Nortriptyline Hydrochloride	0.3	Metformin Hydrochloride	0.4	Sotalol Hydrochloride	0.4
Mercaptopurine	0.2	Desmopressin	0.01	Methotrexate	0.2	Albendazole	0.2	Nifedipine	0.4	Tamoxifen	0.4
Tamoxifen	0.2	Abciximab	0	Ursodeoxycholic Acid	0.1	Lercanidipine	0.2	Pindolol	0.4	Pericyazine	0.4
Diltiazem Hydrochloride	0.2	Acetylcholine Chloride	0	Zopiclone	0.1	Dothiepin Hydrochloride	0.2	Lamotrigine	0.4	Moclobemide	0.4
Simvastatin	0.2	Acitretin	0	Pregabalin	0.05	Pegfilgrastim	0.2	Captopril	0.4	Glibenclamide	0.4
Betaxolol Hydrochloride	0.2	Adalimumab	0	Dexamphetamine Sulphate	0.04	Glimepiride	0.2	Betaxolol Hydrochloride	0.4	Brimonidine	0.4
Raloxifene	0.2	Adefovir Dipivoxil	0	Mesalazine	0.03	Simvastatin	0.2	Lercanidipine	0.4	Mianserin Hydrochloride	0.4

0 ≤ Contributions < 5%											
QEII		CAB		IPS		PC		PA		RBWH	
Strontium Ranelate	0.2	Apraclonidine	0	Tiotropium	0.01	Tiotropium	0.2	Cimetidine	0.4	Glimepiride	0.4
Chlorthalidone	0.2	Atazanavir	0	Sildenafil	0.01	Sulphasalazine	0.2	Diphenoxylate Hydrochloride	0.4	Nifedipine	0.4
Benzathine Phenoxymethylpenicillin	0.2	Atomoxetine	0	Sumatriptan	0.004	Mefloquine	0.2	Adalimumab	0.4	Donepezil	0.4
Verapamil Hydrochloride	0.2	Atovaquone	0	Methylphenidate	0.001	Olsalazine Sodium	0.2	Nitrazepam	0.4	Flecainide Acetate	0.4
Irbesartan	0.2	Auranofin	0	Abciximab	1	Gemcitabine	0.2	Alprazolam	0.4	Leflunomide	0.4
Pravastatin	0.2	Bacitracin	0	Acitretin	0	Pericyazine	0.2	Pemetrexed	0.4	Nortriptyline Hydrochloride	0.4
Indapamide	0.2	Balsalazide	0	Adalimumab	0	Metformin Hydrochloride	0.2	Amiloride Hydrochloride	0.4	Oxaliplatin	0.4
Oxazepam	0.2	Betaxolol Hydrochloride	0	Adefovir Dipivoxil	0	Hydrochlorothiazide	0.2	Leflunomide	0.4	Lercanidipine	0.3
Ramipril	0.2	Bethanechol Chloride	0	Apraclonidine	0	Clomipramine Hydrochloride	0.2	Dothiepin Hydrochloride	0.4	Betaxolol Hydrochloride	0.3
Tinidazole	0.2	Bevacizumab	0	Atomoxetine	0	Prazosin Hydrochloride	0.2	Raloxifene	0.4	Phenoxymethylpenicillin	0.3
Oxcarbazepine	0.2	Bicalutamide	0	Auranofin	0	Ezetimibe	0.2	Esomeprazole	0.4	Mefloquine	0.3
Candesartan	0.2	Bivalirudin	0	Bacitracin	0	Glibenclamide	0.2	Paroxetine	0.4	Anastrozole	0.3
Dantrolene Sodium	0.2	Bleomycin	0	Balsalazide	0	Betaxolol Hydrochloride	0.2	Propranolol Hydrochloride	0.4	Olsalazine Sodium	0.3
Hydrochlorothiazide	0.2	Bortezomib	0	Betahistine	0	Fenofibrate	0.2	Brinzolamide	0.3	Paroxetine	0.3
Nifedipine	0.2	Bosentan	0	Bethanechol Chloride	0	Escitalopram	0.2	Mianserin Hydrochloride	0.3	Verapamil Hydrochloride	0.3
Labetalol Hydrochloride	0.2	Brinzolamide	0	Bicalutamide	0	Diphenoxylate Hydrochloride	0.2	Perindopril	0.3	Bimatoprost	0.3
Pioglitazone Hydrochloride	0.2	Bromazepam	0	Bivalirudin	0	Propylthiouracil	0.2	Donepezil	0.3	Pravastatin	0.3
Hydroxychloroquine Sulphate	0.1	Bupropion	0	Bleomycin	0	Brimonidine	0.2	Pravastatin	0.3	Finasteride	0.3
Flecainide Acetate	0.1	Buspirone Hydrochloride	0	Bortezomib	0	Raloxifene	0.2	Imatinib	0.3	Reboxetine	0.3
Nitrazepam	0.1	Carboplatin	0	Bosentan	0	Auranofin	0.2	Fluoxetine Hydrochloride	0.3	Fluvastatin	0.3
Lercanidipine	0.1	Carmustine	0	Brinzolamide	0	Candesartan	0.2	Simvastatin	0.3	Metformin Hydrochloride	0.3
Methotrexate	0.1	Celecoxib	0	Bromazepam	0	Nifedipine	0.2	Tamsulosin	0.3	Alprazolam	0.3
Dexamphetamine Sulphate	0.1	Cetuximab	0	Bupropion	0	Colestyramine	0.2	Naltrexone	0.3	Simvastatin	0.3

0 ≤ Contributions < 5%											
QEII		CAB		IPS		PC		PA		RBWH	
Nortriptyline Hydrochloride	0.1	Chlorambucil	0	Buspirone Hydrochloride	0	Fosinopril	0.2	Risedronate Sodium	0.3	Hydrochlorothiazide	0.2
Felodipine	0.1	Chloroquine	0	Calcium Folinate	0	Cimetidine	0.2	Mesalazine	0.3	Pindolol	0.2
Esomeprazole	0.1	Ciclesonide	0	Carmustine	0	Ciclesonide	0.2	Oxaliplatin	0.3	Rosiglitazone	0.2
Valaciclovir	0.1	Cimetidine	0	Cefalotin	0	Irbesartan	0.2	Clobazam	0.3	Orphenadrine Citrate	0.2
Clarithromycin	0.1	Cinacalcet	0	Cefepime	0	Propantheline	0.1	Trandolapril	0.3	Amiloride Hydrochloride	0.2
Lansoprazole	0.1	Cisapride	0	Cefoxitin	0	Bimatoprost	0.1	Doxycycline	0.3	Raloxifene	0.2
Letrozole	0.1	Cisplatin	0	Celecoxib	0	Anastrozole	0.1	Olsalazine Sodium	0.3	Irbesartan	0.2
Ezetimibe	0.1	Cladribine	0	Cetuximab	0	Felodipine	0.1	Finasteride	0.3	Clobazam	0.2
Hydroxyurea	0.1	Clioquinol	0	Chloral Hydrate	0	Tamoxifen	0.1	Irbesartan	0.3	Candesartan	0.2
Leflunomide	0.1	Clobazam	0	Chlorambucil	0	Benzathine Phenoxymethylpenicillin	0.1	Anastrozole	0.3	Fosinopril	0.2
Tiotropium	0.1	Clomiphene Citrate	0	Chlorthalidone	0	Phenoxymethylpenicillin	0.1	Quinapril	0.3	Fenofibrate	0.2
Desmopressin	0.1	Cytarabine	0	Ciclesonide	0	Fluorouracil	0.1	Flumethasone Pivalate	0.2	Selegiline Hydrochloride	0.2
Tacrolimus	0.1	Dalteparin	0	Cimetidine	0	Doxepin Hydrochloride	0.1	Clioquinol	0.2	Escitalopram	0.2
Trifluoperazine Hydrochloride	0.1	Danazol	0	Cinacalcet	0	Rosiglitazone	0.1	Escitalopram	0.2	Amantadine Hydrochloride	0.2
Brimonidine	0.1	Dapsone	0	Cisapride	0	Sumatriptan	0.1	Selegiline Hydrochloride	0.2	Dextropropoxyphene	0.2
Donepezil	0.1	Dasatinib	0	Cisplatin	0	Methotrexate	0.1	Ezetimibe	0.2	Griseofulvin	0.2
Celecoxib	0.1	Deferasirox	0	Cladribine	0	Rivastigmine	0.1	Famciclovir	0.2	Ezetimibe	0.2
Mesalazine	0.1	Deferiprone	0	Clioquinol	0	Mesalazine	0.1	Fosinopril	0.2	Desmopressin	0.1
Clozapine	0.1	Desferrioxamine	0	Clomiphene Citrate	0	Chlorthalidone	0.1	Tinidazole	0.2	Sumatriptan	0.1
Methyldopa	0.1	Dextropropoxyphene	0	Cytarabine	0	Indapamide	0.1	Cabergoline	0.2	Felodipine	0.1
Ursodeoxycholic Acid	0.05	Didanosine	0	Dalteparin	0	Azathioprine	0.1	Fenofibrate	0.2	Mesalazine	0.1
Mexiletine Hydrochloride	0.05	Dihydroergotamine	0	Danazol	0	Imipramine Hydrochloride	0.1	Fluvastatin	0.2	Fluorometholone	0.1
Primidone	0.04	Dipivefrine	0	Dapsone	0	Oxaliplatin	0.1	Flecainide Acetate	0.2	Lapatinib	0.1
Cyclosporin	0.04	Disopyramide	0	Dasatinib	0	Alprazolam	0.1	Bromocriptine Mesylate	0.2	Biperiden Hydrochloride	0.1

0 ≤ Contributions < 5%											
QEII		CAB		IPS		PC		PA		RBWH	
Brinzolamide	0.04	Docetaxel	0	Deferasirox	0	Piroxicam	0.1	Imipramine Hydrochloride	0.2	Procaine Penicillin	0.1
Lorazepam	0.04	Donepezil	0	Deferiprone	0	Rituximab	0.1	Felodipine	0.2	Minocycline	0.1
Betahistine	0.03	Doxorubicin Hydrochloride	0	Dextropropoxyphene	0	Irinotecan	0.1	Ciclesonide	0.2	Famciclovir	0.1
Anastrozole	0.03	Duloxetine	0	Didanosine	0	Letrozole	0.1	Hydrochlorothiazide	0.2	Indapamide	0.1
Colestyramine	0.03	Efavirenz	0	Docetaxel	0	Famciclovir	0.1	Irinotecan	0.2	Cimetidine	0.1
Alprazolam	0.03	Enalapril Maleate	0	Dorzolamide	0	Desmopressin	0.1	Gemfibrozil	0.2	Betahistine	0.1
Rosuvastatin	0.03	Entecavir	0	Doxorubicin Hydrochloride	0	Fluorometholone	0.04	Reboxetine	0.2	Montelukast	0.1
Bimatoprost	0.02	Epirubicin Hydrochloride	0	Duloxetine	0	Tinidazole	0.04	Enalapril Maleate	0.2	Piroxicam	0.1
Fluorometholone	0.02	Epoprostenol	0	Efavirenz	0	Cyclophosphamide	0.04	Candesartan	0.2	Benzathine Phenoxymethylpenicillin	0.1
Pyridostigmine Bromide	0.02	Eprosartan Mesylate	0	Enalapril Maleate	0	Pregabalin	0.04	Rosiglitazone	0.2	Telmisartan	0.1
Telmisartan	0.02	Eptifibatide	0	Entecavir	0	Minocycline	0.03	Disopyramide	0.2	Disopyramide	0.05
Methylphenidate	0.02	Erlotinib	0	Epirubicin Hydrochloride	0	Mercaptopurine	0.03	Glibenclamide	0.2	Triamterene	0.04
Enalapril Maleate	0.01	Escitalopram	0	Epoprostenol	0	Betahistine	0.03	Pramipexole	0.2	Rivastigmine	0.03
Dextropropoxyphene	0.01	Etanercept	0	Eprosartan Mesylate	0	Rosuvastatin	0.02	Telmisartan	0.2	Pregabalin	0.03
Eprosartan Mesylate	0.01	Ethacrynic Acid	0	Eptifibatide	0	Triamterene	0.02	Bupropion	0.2	Chlorthalidone	0.02
Trandolapril	0.01	Ethosuximide	0	Erlotinib	0	Brinzolamide	0.02	Tranylcypromine	0.2	Mebeverine Hydrochloride	0.02
Fluvastatin	0.01	Etoposide	0	Escitalopram	0	Pindolol	0.02	Indapamide	0.2	Solifenacin Succinate	0.02
Abciximab	0	Everolimus	0	Etanercept	0	Tamsulosin	0.02	Dexamphetamine Sulphate	0.1	Methylphenidate	0.01
Acamprosate	0	Exemestane	0	Ethosuximide	0	Zolpidem	0.01	Levobunolol	0.1	Dexamphetamine Sulphate	0.01
Acetylcholine Chloride	0	Finasteride	0	Everolimus	0	Fluvastatin	0.01	Phenoxymethylpenicillin	0.1	Lansoprazole	0.01
Acitretin	0	Fludarabine Phosphate	0	Exemestane	0	Olmesartan Medoxomil	0.01	Eprosartan Mesylate	0.1	Atomoxetine	0.004
Adalimumab	0	Flumethasone Pivalate	0	Fenofibrate	0	Cefaclor	0.01	Clomipramine Hydrochloride	0.1	Cefaclor	0.004
Albendazole	0	Flunitrazepam	0	Finasteride	0	Orphenadrine Citrate	0.01	Sumatriptan	0.1	Auranofin	0

0 ≤ Contributions < 5%											
QEII		CAB		IPS		PC		PA		RBWH	
Alprostadil	0	Fluorouracil	0	Fludarabine Phosphate	0	Telmisartan	0.002	Rosuvastatin	0.1	Bethanechol Chloride	0
Apomorphine	0	Flutamide	0	Flumethasone Pivalate	0	Montelukast	0.002	Bromazepam	0.1	Bortezomib	0
Atazanavir	0	Fluvastatin	0	Flunitrazepam	0	Dexamphetamine Sulphate	0	Duloxetine	0.1	Brinzolamide	0
Atomoxetine	0	Fondaparinux Sodium	0	Fluorometholone	0	Acetylcholine Chloride	0	Mefloquine	0.1	Bromazepam	0
Atovaquone	0	Fotemustine	0	Fluorouracil	0	Adalimumab	0	Tetrabenazine	0.1	Bupropion	0
Auranofin	0	Fulvestrant	0	Flutamide	0	Adefovir Dipivoxil	0	Flunitrazepam	0.1	Celecoxib	0
Bacitracin	0	Galantamine	0	Fondaparinux Sodium	0	Apraclonidine	0	Minocycline	0.1	Ciclesonide	0
Balsalazide	0	Ganciclovir	0	Fotemustine	0	Bacitracin	0	Triamterene	0.06	Cisapride	0
Bevacizumab	0	Gemcitabine	0	Fulvestrant	0	Balsalazide	0	Montelukast	0.05	Cladribine	0
Bicalutamide	0	Griseofulvin	0	Galantamine	0	Bethanechol Chloride	0	Chlorthalidone	0.04	Clomiphene Citrate	0
Bivalirudin	0	Homatropine Hydrobromide	0	Ganciclovir	0	Bevacizumab	0	Etanercept	0.03	Dalteparin	0
Bleomycin	0	Ibandronic Acid	0	Griseofulvin	0	Bleomycin	0	Mebeverine Hydrochloride	0.03	Duloxetine	0
Bortezomib	0	Idarubicin Hydrochloride	0	Homatropine Hydrobromide	0	Bortezomib	0	Piroxicam	0.03	Enalapril Maleate	0
Bosentan	0	Ifosfamide	0	Ibandronic Acid	0	Bromazepam	0	Sitagliptin	0.02	Eprosartan Mesylate	0
Bromazepam	0	Imatinib	0	Idarubicin Hydrochloride	0	Bupropion	0	Zolpidem	0.02	Erlotinib	0
Bromhexine Hydrochloride	0	Imiquimod	0	Ifosfamide	0	Buspirone Hydrochloride	0	Methylphenidate	0.02	Ethosuximide	0
Bupropion	0	Indinavir	0	Imatinib	0	Carmustine	0	Zopiclone	0.02	Everolimus	0
Buspirone Hydrochloride	0	Infliximab	0	Infliximab	0	Celecoxib	0	Tropisetron	0.01	Flunitrazepam	0
Calcium Folinate	0	Irinotecan	0	Irinotecan	0	Cetuximab	0	Cefaclor	0.01	Flutamide	0
Carboplatin	0	Isoniazid	0	Ivabradine	0	Chlorambucil	0	Dextropropoxyphene	0.005	Fondaparinux Sodium	0
Carmustine	0	Ivabradine	0	Ivermectin	0	Cinacalcet	0	Griseofulvin	0.004	Fotemustine	0
Cefaclor	0	Lansoprazole	0	Lansoprazole	0	Cisapride	0	Meloxicam	0.001	Fulvestrant	0
Cefepime	0	Lapatinib	0	Lapatinib	0	Clioquinol	0	Acetylcholine Chloride	0	Ibandronic Acid	0
Cetuximab	0	Lenograstim	0	Levobunolol	0	Clobazam	0	Atomoxetine	0	Levobunolol	0

0 ≤ Contributions < 5%											
QEII		CAB		IPS		PC		PA		RBWH	
Chloral Hydrate	0	Levobunolol	0	Losartan	0	Clomiphene Citrate	0	Auranofin	0	Losartan	0
Ciclesonide	0	Losartan	0	Mebeverine Hydrochloride	0	Cytarabine	0	Balsalazide	0	Meloxicam	0
Cinacalcet	0	Mebeverine Hydrochloride	0	Meloxicam	0	Dalteparin	0	Benzathine Phenoxymethylpenicillin	0	Memantine Hydrochloride	0
Cisapride	0	Mefloquine	0	Melphalan	0	Dasatinib	0	Buspirone Hydrochloride	0	Methysergide	0
Cisplatin	0	Meloxicam	0	Memantine Hydrochloride	0	Deferiprone	0	Celecoxib	0	Modafinil	0
Cladribine	0	Melphalan	0	Methysergide	0	Dextropropoxyphene	0	Cetuximab	0	Moxonidine	0
Clobazam	0	Memantine Hydrochloride	0	Minocycline	0	Dihydroergotamine	0	Chloral Hydrate	0	Nandrolone Decanoate	0
Clomiphene Citrate	0	Mercaptopurine	0	Mitozantrone	0	Dipivefrine	0	Cisapride	0	Nilotinib	0
Cyclophosphamide	0	Methysergide	0	Modafinil	0	Docetaxel	0	Clomiphene Citrate	0	Olmesartan Medoxomil	0
Cytarabine	0	Mexiletine Hydrochloride	0	Montelukast	0	Doxorubicin Hydrochloride	0	Dalteparin	0	Paliperidone	0
Dalteparin	0	Minocycline	0	Moxonidine	0	Duloxetine	0	Danazol	0	Pemetrexed	0
Danazol	0	Mitozantrone	0	Nandrolone Decanoate	0	Enalapril Maleate	0	Dasatinib	0	Pergolide	0
Dapsone	0	Modafinil	0	Nevirapine	0	Entecavir	0	Deferiprone	0	Phenindione	0
Dasatinib	0	Moxonidine	0	Nilotinib	0	Epirubicin Hydrochloride	0	Desferrioxamine	0	Pimozide	0
Deferasirox	0	Naltrexone	0	Ofloxacin	0	Eprosartan Mesylate	0	Didanosine	0	Pramipexole	0
Deferiprone	0	Nandrolone Decanoate	0	Olmesartan Medoxomil	0	Eptifibatide	0	Dihydroergotamine	0	Praziquantel	0
Didanosine	0	Nilotinib	0	Paclitaxel	0	Etanercept	0	Ethosuximide	0	Quinapril	0
Dihydroergotamine	0	Ofloxacin	0	Papaverine	0	Ethosuximide	0	Flutamide	0	Rosuvastatin	0
Dipivefrine	0	Olmesartan Medoxomil	0	Pemetrexed	0	Etoposide	0	Fondaparinux Sodium	0	Sirolimus	0
Disopyramide	0	Oxaliplatin	0	Phenelzine Sulphate	0	Exemestane	0	Fotemustine	0	Sitagliptin	0
Docetaxel	0	Oxpentifylline	0	Phenindione	0	Finasteride	0	Ibandronic Acid	0	Sulfadoxine	0
Doxorubicin Hydrochloride	0	Paclitaxel	0	Phenoxybenzamine Hydrochloride	0	Fludarabine Phosphate	0	Indinavir	0	Sulindac	0

0 ≤ Contributions < 5%											
QEII		CAB		IPS		PC		PA		RBWH	
Duloxetine	0	Paliperidone	0	Pramipexole	0	Flumethasone Pivalate	0	Lansoprazole	0	Sunitinib	0
Efavirenz	0	Papaverine	0	Praziquantel	0	Flunitrazepam	0	Lapatinib	0	Tamsulosin	0
Entecavir	0	Pegfilgrastim	0	Pyridostigmine Bromide	0	Flutamide	0	Memantine Hydrochloride	0	Tazarotene	0
Epirubicin Hydrochloride	0	Pemetrexed	0	Pyrimethamine	0	Fondaparinux Sodium	0	Methysergide	0	Thioridazine Hydrochloride	0
Eplerenone	0	Pergolide	0	Quinapril	0	Fulvestrant	0	Modafinil	0	Tiagabine	0
Epoprostenol	0	Phenelzine Sulphate	0	Reboxetine	0	Griseofulvin	0	Moxonidine	0	Tiotropium	0
Eptifibatide	0	Phenindione	0	Rifabutin	0	Homatropine Hydrobromide	0	Nandrolone Decanoate	0	Trandolapril	0
Erlotinib	0	Phenoxybenzamine Hydrochloride	0	Riluzole	0	Ibandronic Acid	0	Nilotinib	0	Tranylcypromine	0
Etanercept	0	Phentolamine Mesylate	0	Rituximab	0	Idarubicin Hydrochloride	0	Olmesartan Medoxomil	0	Trastuzumab	0
Ethosuximide	0	Pilocarpine	0	Rivastigmine	0	Imatinib	0	Orphenadrine Citrate	0	Varenicline	0
Etoposide	0	Pimozide	0	Rosuvastatin	0	Indinavir	0	Papaverine	0	Zanamivir	0
Everolimus	0	Podophyllotoxin	0	Selegiline Hydrochloride	0	Infliximab	0	Pergolide	0	Zolpidem	0
Exemestane	0	Pramipexole	0	Sirolimus	0	Ivabradine	0	Procaine Penicillin	0	Zopiclone	0
Filgrastim	0	Praziquantel	0	Sitagliptin	0	Ivermectin	0	Riluzole	0		
Finasteride	0	Procaine Penicillin	0	Solifenacin Succinate	0	Lansoprazole	0	Rivastigmine	0		
Fludarabine Phosphate	0	Proguanil	0	Stavudine	0	Lapatinib	0	Stavudine	0		
Flunitrazepam	0	Pyrimethamine	0	Sulfadoxine	0	Lenograstim	0	Sulfadoxine	0		
Fluorouracil	0	Quinapril	0	Sulindac	0	Levobunolol	0	Sulindac	0		
Fluphenazine Decanoate	0	Reboxetine	0	Sulthiame	0	Mebeverine Hydrochloride	0	Sulthiame	0		
Flutamide	0	Rifabutin	0	Tacrolimus	0	Meloxicam	0	Sunitinib	0		
Fondaparinux Sodium	0	Riluzole	0	Tamsulosin	0	Memantine Hydrochloride	0	Tazarotene	0		
Fotemustine	0	Ritonavir	0	Tazarotene	0	Methylphenidate	0	Temozolomide	0		
Fulvestrant	0	Rituximab	0	Telmisartan	0	Methysergide	0	Tenecteplase	0		
Ganciclovir	0	Rivastigmine	0	Temozolomide	0	Mitozantrone	0	Thioguanine	0		

0 ≤ Contributions < 5%											
QEII		CAB		IPS		PC		PA		RBWH	
Gemcitabine	0	Rosuvastatin	0	Tetrabenazine	0	Modafinil	0	Thioridazine Hydrochloride	0		
Ibandronic Acid	0	Selegiline Hydrochloride	0	Thalidomide	0	Moxonidine	0	Thiotepa	0		
Idarubicin Hydrochloride	0	Sevelamer	0	Thioguanine	0	Nandrolone Decanoate	0	Tiagabine	0		
Ifosfamide	0	Sildenafil	0	Thiotepa	0	Nilotinib	0	Varenicline	0		
Imatinib	0	Sirolimus	0	Tiagabine	0	Oxcarbazepine	0	Zanamivir	0		
Indinavir	0	Sitagliptin	0	Trandolapril	0	Paclitaxel	0				
Infliximab	0	Solifenacin Succinate	0	Tranylcypromine	0	Paliperidone	0				
Irinotecan	0	Stavudine	0	Tropisetron	0	Pergolide	0				
Itraconazole	0	Sulfadoxine	0	Valganciclovir	0	Phenelzine Sulphate	0				
Ivabradine	0	Sulindac	0	Varenicline	0	Phenindione	0				
Lapatinib	0	Sulthiame	0	Vinblastine Sulphate	0	Pimozide	0				
Levobunolol	0	Sunitinib	0	Vincristine Sulphate	0	Podophyllotoxin	0				
Losartan	0	Tazarotene	0	Vinorelbine	0	Pramipexole	0				
Melphalan	0	Telmisartan	0	Zanamivir	0	Praziquantel	0				
Memantine Hydrochloride	0	Temozolomide	0	Zoledronic Acid	0	Proguanil	0				
Mitozantrone	0	Tetrabenazine	0	Zolpidem	0	Pyridostigmine Bromide	0				
Modafinil	0	Thalidomide	0			Pyrimethamine	0				
Montelukast	0	Thioguanine	0			Quinapril	0				
Moxonidine	0	Thioridazine Hydrochloride	0			Reboxetine	0				
Naltrexone	0	Thiotepa	0			Riluzole	0				
Nandrolone Decanoate	0	Tiagabine	0			Selegiline Hydrochloride	0				
Nilotinib	0	Tiotropium	0			Sitagliptin	0				
Ofloxacin	0	Trandolapril	0			Solifenacin Succinate	0				
Olmesartan Medoxomil	0	Tranylcypromine	0			Sulindac	0				
Oseltamivir	0	Trastuzumab	0			Sulthiame	0				
Oxaliplatin	0	Tropisetron	0			Sunitinib	0				
Oxpentifylline	0	Valganciclovir	0			Tazarotene	0				
Paclitaxel	0	Varenicline	0			Temozolomide	0				

0 ≤ Contributions < 5%											
QEII		CAB		IPS		PC		PA		RBWH	
Paliperidone	0	Vigabatrin	0			Thalidomide	0				
Papaverine	0	Vinblastine Sulphate	0			Thioguanine	0				
Pegfilgrastim	0	Vincristine Sulphate	0			Thioridazine Hydrochloride	0				
Pemetrexed	0	Vinorelbine	0			Thiotepa	0				
Pergolide	0	Zanamivir	0			Trandolapril	0				
Phenelzine Sulphate	0	Ziprasidone	0			Tranylcypromine	0				
Phenindione	0	Zoledronic Acid	0			Varenicline	0				
Phenoxybenzamine Hydrochloride	0	Zolpidem	0			Vinblastine Sulphate	0				
Phentolamine Mesylate	0	Zopiclone	0			Vincristine Sulphate	0				
Pimozide	0					Zanamivir	0				
Pindolol	0					Zopiclone	0				
Pramipexole	0										
Praziquantel	0										
Pregabalin	0										
Proguanil	0										
Propylthiouracil	0										
Pyrimethamine	0										
Quinapril	0										
Reboxetine	0										
Rifabutin	0										
Riluzole	0										
Ritonavir	0										
Rituximab	0										
Rivastigmine	0										
Selegiline Hydrochloride	0										
Sevelamer	0										
Sildenafil	0										
Sirolimus	0										
Sitagliptin	0										
Solifenacin Succinate	0										
Stavudine	0										

0 ≤ Contributions < 5%											
QEII		CAB		IPS		PC		PA		RBWH	
Sulfadoxine	0										
Sulindac	0										
Sulthiame	0										
Sunitinib	0										
Tazarotene	0										
Temozolomide	0										
Thalidomide	0										
Thioguanine	0										
Thioridazine Hydrochloride	0										
Thiotepa	0										
Tiagabine	0										
Tranylcypromine	0										
Trastuzumab	0										
Triamterene	0										
Tropisetron	0										
Valganciclovir	0										
Varenicline	0										
Vinblastine Sulphate	0										
Vincristine Sulphate	0										
Vinorelbine	0										
Zanamivir	0										
Ziprasidone	0										
Zolpidem	0										
Zopiclone	0										

Supporting Information B3: Therapeutic classes covered at Queen Elizabeth II Jubilee Hospital (QEII) - (*Supporting information for Figure 5.a)*

Table SI 12. Compounds for which QEII's contribution is 100%.
Total number of compounds: 54.

Generic	Class Acronym	Therapeutic Class
Bupivacaine	AA	Anaesthetic Agent *(Local)*
Ketamine	AA	Anaesthetic Agent (General)
Levobupivacaine	AA	Anaesthetic Agent (Local)
Oxybuprocaine	AA	Anaesthetic agent (Local)
Propofol	AA	Anaesthetic Agent (General)
Ropivacaine	AA	Anaesthetic agent *(Local)*
Thiopentone	AA	Anaesthetic Agent *(General)*
Ertapenem	AB	Antibiotic
Ethambutol	AB	Antibiotic
Meropenem	AB	Antibiotic
Piperacillin	AB	Antibiotic
Sodium Fusidate	AB	Antibiotic
Tazobactam	AB	Antibiotic
Ceftazidime	AB	Antibiotic
Cefoxitin	AB	Antibiotic
Trimipramine	AD	Antidepressant
Alfentanil	AG	Analgesic
Sodium Nitroprusside	Ahyp	Antihypertensive
Artemether	AM	Antimalarial
Lumefantrine	AM	Antimalarial
Ergotamine	Amig	Antimigraine agent
Mitomycin	AN	Antineoplastic
Droperidol	Apsy	Antipsychotic agent
Levomepromazine	Apsy	Antipsychotic
Glycopyrrolate	ASp	Antispasmodic
Abacavir	AV	Antiviral
Emtricitabine	AV	Antiviral
Tenofovir	AV	Antiviral
Aminophylline	BD	Bronchodilator
Isoprenaline	BD	Bronchodilator
Cyclopentolate	CM	cycloplegic and mydriatic agent
Dicobalt edetate	DxA	Detoxifying agent
Flumazenil	DxA	Detoxifying agent
Pralidoxime	DxA	Detoxifying agent
Lenograstim	HP	Hematopoietic stimulator
Mycophenolate Mofetil	IM	Immunosuppressant
Neostigmine	MS	Muscular stimulant
Tropicamide	MY	Mydriatic
Atracurium	NB	Neuromuscular blocking agent
Cisatracurium	NB	Neuromuscular blocking agent
Pancuronium	NB	Neuromuscular blocking agent
Vecuronium	NB	Neuromuscular blocking agent
Suxamethonium	NB	Neuromuscular blocking agent
Ergometrine	OA	Oxytocic agent
Dexmedetomidine Hydrochloride	S	Sedative
Pyrazinamide	TB	Antitubercular agent
Diazoxide	VA	Antihypertensive
Nimodipine	VA	Vasodilator
Dobutamine	VP	Vasopressor
Dopamine	VP	Vasopressor
Metaraminol tartrate	VP	Vasopressor
Ephedrine	VP	Vasopressor
Adapalene	-	Acne treatment

Table SI 13. Compounds for which QEII's contribution is in the range 15-100%.
Total number of compounds: 26.

Generic	Class Acronym	Therapeutic Class
Ampicillin	AB	Antibiotic
Framycetin Sulphate	AB	Antibiotic
Gentamicin Sulphate	AB	Antibiotic
Lincomycin	AB	Anibiotic
Cefazolin	AB	Antibiotic
Ticarcillin	AB	Antibiotic
Benzylpenicillin	AB	Antibiotic
Ceftriaxone	AB	Antibiotic
Vancomycin	AB	Antibiotic
Benzathine Penicillin	AB	Antibiotic
Cefotaxime	AB	Antibiotic
Cefalotin	AB	Antibiotic
Butylscopolamine	Ach	Abdominal anti-spasmodic
Enoxaparin	Acog	Anticoagulant
Dipyridamole	Acog	Anticoagulant
Granisetron	AE	Antiemetic
Latanoprost	AGl	Antiglaucoma agent
Ketorolac	AI	Anti-inflammatory
Methylprednisolone	AI	Anti-inflammatory
Imiquimod	AV	Antiviral
Midazolam	AX	Anxyolitic
Naloxone Hydrochloride	DxA	Detoxifying agent
Desferrioxamine	DxA	Detoxifying agent
Acetylcysteine	DxA	Detoxifying agent
Tenecteplase	FB	Fibronolytic agent
Nicorandil	VA	Vasodilator

Table SI 14. Compounds for which QEII's contribution is in the range 0-15%.
Total number of compounds: 407.

Generic	Class Acronym	Therapeutic Class
Azithromycin	AB	Antibiotic *(Pneumonia treatment)*
Dicloxacillin	AB	Antibiotic
Silver Sulfadiazine	AB	Antibiotic
Moxifloxacin	AB	Antibiotic
Flucloxacillin	AB	Antibiotic
Metronidazole	AB	Antibiotic
Tobramycin	AB	Antibiotic
Trimethoprim	AB	Antibiotic
Clindamycin	AB	Anitbiotic
Roxithromycin	AB	Antibiotic
Sulphamethoxazole	AB	Antibiotic
Mupirocin	AB	Antibiotic
Gramicidin	AB	Antibiotic
Ciprofloxacin	AB	Antibiotic
Cefuroxime	AB	Antibiotic
Cefalexin	AB	Antibiotic
Amoxycillin	AB	Antibiotic
Doxycycline	AB	Antibiotic
Procaine Penicillin	AB	Antibiotic
Norfloxacin	AB	Antibiotic
Nitrofurantoin	AB	Antibiotic
Erythromycin	AB	Antibiotic
Phenoxymethylpenicillin	AB	Antibiotic
Sulphasalazine	AB	Antibiotic
Neomycin	AB	Antibiotic
Minocycline	AB	Antibiotic
Benzathine Phenoxymethylpenicillin	AB	Antibiotic
Tinidazole	AB	Antibiotic
Bacitracin	AB	Antibiotic
Cefaclor	AB	Antibiotic
Cefepime	AB	Antibiotic
Ofloxacin	AB	Antibiotic
Rifabutin	AB	Antibiotic
Vigabatrin	AC	Anticonvulsant
Gabapentin	AC	Anticonvulsant
Phenytoin	AC	Anticonvulsant
Sodium Valproate	AC	Anticonvulsant
Phenobarbitone	AC	Anticonvulsant
Carbamazepine	AC	Anticonvulsant
Levetiracetam	AC	Anticonvulsant / antiepileptic
Lamotrigine	AC	Anticonvulsant
Topiramate	AC	Anticonvulsant
Oxcarbazepine	AC	Anticonvulsant
Primidone	AC	Anticonvulsant
Ethosuximide	AC	Anticonvulsant
Pregabalin	AC	Anticonvulsant
Sulthiame	AC	Anticonvulsant
Tiagabine	AC	Anticonvulsant
Tirofiban	Acog	Anticoagulant
Clopidogrel	Acog	Anticoagulant / Antithrombotic

Generic	Class Acronym	Therapeutic Class
Warfarin	Acog	Anticoagulant
Abciximab	Acog	Anticoagulant / Antithrombotic
Bivalirudin	Acog	Anticoagulant
Dalteparin	Acog	anticoagulant
Eptifibatide	Acog	Anticoagulant
Fondaparinux Sodium	Acog	Anticoagulant
Phenindione	Acog	Anticoagulant
Mirtazapine	AD	Antidepressant
Amitriptyline Hydrochloride	AD	Antidepressant
Doxepin Hydrochloride	AD	Antidepressant
Citalopram	AD	Antidepressant
Mianserin Hydrochloride	AD	Antidepressant
Fluvoxamine	AD	Antidepressant
Dothiepin Hydrochloride	AD	Antidepressant
Venlafaxine	AD	Antidepressant
Moclobemide	AD	Antidepressant
Sertraline	AD	Antidepressant
Fluoxetine Hydrochloride	AD	Antidepressant
Imipramine Hydrochloride	AD	Antidepressant
Paroxetine	AD	Antidepressant
Escitalopram	AD	Antidepressant
Clomipramine Hydrochloride	AD	Antidepressant
Nortriptyline Hydrochloride	AD	Antidepressant
Bupropion	AD	Antidepressant
Duloxetine	AD	Antidepressant
Phenelzine Sulphate	AD	Antidepressant
Reboxetine	AD	Antidepressant
Tranylcypromine	AD	Antidepressant
Metformin Hydrochloride	AdB	Antidiabetic
Desmopressin	aDI	Antidiuretic
Diphenoxylate Hydrochloride	ADy	Antidiarrheal
Prochlorperazine	AE	Antiemetic
Ondansetron	AE	Antiemetic
Tropisetron	AE	Antiemetic
Griseofulvin	AF	Antifungal
Clioquinol	AF	Anti-fungal agent
Amphotericin	AF	Antifungal
Itraconazole	AF	Antifungal Agent
Fentanyl	AG	Analgesic
Pethidine Hydrochloride	AG	Analgesic
Buprenorphine	AG	Analgesic
Hydromorphone	AG	Analgesic
Tramadol	AG	Analgesic
Oxycodone	AG	Analgesic
Morphine	AG	Analgesic
Methadone Hydrochloride	AG	Analgesic
Dextropropoxyphene	AG	Analgesic
Acetazolamide	AGl	Antiglaucoma agent
Dorzolamide	AGl	Antiglaucoma agent
Pilocarpine	AGl	Antiglaucoma agent
Apraclonidine	AGl	Antiglaucoma agent
Timolol	AGl	Antiglaucoma agent
Brimonidine	AGl	Antiglaucoma agent

Generic	Class Acronym	Therapeutic Class
Brinzolamide	AGl	Antiglaucoma agent
Bimatoprost	AGl	Antiglaucoma agent
Dipivefrine	AGl	Antiglaucoma agent
Albendazole	AH	Anthelminthic
Praziquantel	AH	Antihelminthic
Captopril	Ahyp	Antihypertensive
Hydralazine Hydrochloride	Ahyp	Antihypertensive
Clonidine	Ahyp	Antihypertensive
Prazosin Hydrochloride	Ahyp	Antihypertensive
Fosinopril	Ahyp	Antihypertensive
Lisinopril	Ahyp	Antihypertensive
Amlodipine	Ahyp	Antihypertensive / vasodilator
Perindopril	Ahyp	Antihypertensive
Verapamil Hydrochloride	Ahyp	Antihypertensive
Irbesartan	Ahyp	Antihypertensive
Ramipril	Ahyp	Antihypertensive
Candesartan	Ahyp	Antihypertensive
Nifedipine	Ahyp	Antihypertensive antianginal
Lercanidipine	Ahyp	Antihypertensive agent
Felodipine	Ahyp	Antihypertensive
Methyldopa	Ahyp	Antihypertensive
Telmisartan	Ahyp	Antihypertensive
Enalapril Maleate	Ahyp	Antihypertensive
Eprosartan Mesylate	Ahyp	Antihypertensive
Trandolapril	Ahyp	Antihypertensive
Eplerenone	Ahyp	Antihypertensive
Losartan	Ahyp	Antihypertensive
Moxonidine	Ahyp	Antihypertensive
Olmesartan Medoxomil	Ahyp	Antihypertensive
Phentolamine Mesylate	Ahyp	Antihypertensive
Quinapril	Ahyp	Antihypertensive
Flumethasone Pivalate	AI	Anti-inflammatory
Dexamethasone	AI	Anti-inflammatory
Misoprostol	AI	Anti-inflammatory
Piroxicam	AI	Anti-inflammatory
Betamethasone	AI	Antiasthmatic, Anti-inflammatory
Meloxicam	AI	Anti-inflammatory
Olsalazine Sodium	AI	Anti-inflammatory
Celecoxib	AI	Anti-inflammatory
Mesalazine	AI	Anti-inflammatory
Fluorometholone	AI	Anti-inflammatory
Balsalazide	AI	Anti-inflammatory
Ciclesonide	AI	Anti-inflammatory
Sulindac	AI	Anti-inflammatory
Diazepam	AL	Anxiolytic
Clonazepam	AL	Anxiolytic
Alprazolam	AL	Anxiolytic
Clobazam	AL	Anxiolytic / Anticonvulsant
Dapsone	ALP	Antileprotic, antimalarial
Chloroquine	AM	Antimalarial
Mefloquine	AM	Antimalarial
Hydroxychloroquine Sulphate	AM	Anti-malarial
Atovaquone	AM	Antimalarial

Generic	Class Acronym	Therapeutic Class
Proguanil	AM	Antimalarial
Pyrimethamine	AM	Antimalarial
Sulfadoxine	AM	Antimalarial
Sumatriptan	Amig	Antimigraine agent
Methysergide	Amig	Antimigraine agent
Chlorambucil	AN	Antineoplastic
Mercaptopurine	AN	Antineoplastic
Tamoxifen	AN	Hormonal antineoplastic agent
Methotrexate	AN	Antineoplastic
Letrozole	AN	Antineoplastic
Hydroxyurea	AN	Antineoplastic
Anastrozole	AN	(Hormonal) antineoplastic agent
Bevacizumab	AN	Antineoplastic
Bicalutamide	AN	(Hormonal) antineoplastic agent
Bleomycin	AN	Antineoplastic antibiotic
Bortezomib	AN	Antineoplastic agent
Carboplatin	AN	Antineoplastic
Carmustine	AN	Antineoplastic
Cetuximab	AN	Antineoplastic agent
Cisplatin	AN	Antineoplastic
Cladribine	AN	Antineoplastic
Cyclophosphamide	AN	Antineoplastic
Cytarabine	AN	Antineoplastic
Dasatinib	AN	Antineoplastic agent
Docetaxel	AN	Antineoplastic
Doxorubicin Hydrochloride	AN	Antineoplastic
Epirubicin Hydrochloride	AN	Antineoplastic
Erlotinib	AN	Antineoplastic agent
Etoposide	AN	Antineoplastic agent
Everolimus	AN	Antineoplastic agent
Exemestane	AN	Antineoplastic agent
Fludarabine Phosphate	AN	Antineoplastic
Fluorouracil	AN	Antineoplastic
Flutamide	AN	Antineoplastic agent
Fotemustine	AN	Antineoplastic
Fulvestrant	AN	Antineoplastic
Gemcitabine	AN	Antineoplastic
Idarubicin Hydrochloride	AN	Antineoplastic
Ifosfamide	AN	Antineoplastic
Imatinib	AN	Antineoplastic agent
Irinotecan	AN	Antineoplastic agent
Lapatinib	AN	Antineoplastic agent
Melphalan	AN	Antineoplastic
Mitozantrone	AN	Antineoplastic
Nilotinib	AN	Antineoplastic agent
Oxaliplatin	AN	Antineoplastic
Paclitaxel	AN	Antineoplastic
Pemetrexed	AN	Antineoplastic
Rituximab	AN	Antineoplastic agent
Sunitinib	AN	Antineoplastic
Temozolomide	AN	Antineoplastic
Thalidomide	AN	miscellaneous anti neoplastic
Thioguanine	AN	Antineoplastic

Generic	Class Acronym	Therapeutic Class
Thiotepa	AN	Anineoplastic
Trastuzumab	AN	Antineoplastic agent
Vinblastine Sulphate	AN	Antineoplastic
Vincristine Sulphate	AN	Antineoplastic
Vinorelbine	AN	Antineoplastic
Cyproterone Acetate	Aan	Antiandrogen
Benztropine Mesylate	AP	Antiparkinsonian agent
Benserazide	AP	Antiparkinsonian agent
Biperiden Hydrochloride	AP	Antiparkinsonian agent
Levodopa	AP	Antiparkinsonian agent
Bromocriptine Mesylate	AP	Antiparkinsonian agent
Entacapone	AP	Antiparkinsonian
Cabergoline	AP	Antiparkinsonian agent
Domperidone	AP	Antiparkinsonian agent
Benzhexol Hydrochloride	AP	Antiparkinsonian agent
Amantadine Hydrochloride	AP	Antiparkinsonian, Antiviral
Carbidopa	AP	Antiparkinsonian agent
Apomorphine	AP	Antiparkinsonian agent
Pergolide	AP	Antiparkinsonian agent
Pramipexole	AP	Antiparkinsonian agent
Selegiline Hydrochloride	AP	Antiparkinsonian agent
Acitretin	Apso	Antipsoriatic
Tazarotene	APSo	Antipsioratic
Quetiapine	Apsy	Antipsychotic
Haloperidol	APsy	Antipsychotic
Flupenthixol	APsy	Antipsychotic agent
Zuclopenthixol	APsy	Antipsychotic
Aripiprazole	Apsy	Antipsychotic / antidepressant
Olanzapine	Apsy	Antipsychotic
Risperidone	APsy	Antipsychotic
Amisulpride	APsy	Antipsychotic
Chlorpromazine Hydrochloride	APsy	Antipsychotic, Anxyolitic
Pericyazine	APsy	Antipsychotic
Trifluoperazine Hydrochloride	APsy	Antipsychotic
Clozapine	APsy	Antipsychotic
Fluphenazine Decanoate	APsy	Antipsychotic agent
Paliperidone	APsy	Antipsychotic agent
Pimozide	Apsy	Antipsychotic
Thioridazine Hydrochloride	APsy	Antipsychotic
Ziprasidone	APsy	Antipsychotic
Amiodarone Hydrochloride	AR	Antiarrhythmic agents
Digoxin	AR	Antiarrhythmic
Diltiazem Hydrochloride	AR	Antiarrhythmic
Flecainide Acetate	AR	Antiarrhythmic agent\
Mexiletine Hydrochloride	AR	Antiarrhythmic agents
Disopyramide	AR	Antiarrhythmic agents
Leflunomide	ARh	Antirheumatic agent
Adalimumab	ARh	Antirheumatic, immunomodulator
Auranofin	ARh	Antirheumatic agent
Infliximab	ARh	Antirheumatic agent
Nandrolone Decanoate	AS	Anabolic steroid
Propantheline	ASp	Antispasmodic
Oxybutynin	ASp	Antispasmodic

Generic	Class Acronym	Therapeutic Class
Mebeverine Hydrochloride	ASp	Antispasmodic
Solifenacin Succinate	ASp	(urinary) antispasmodic
Carbimazole	AT	Antithyroid agent
Propylthiouracil	AT	Antithyroid agent
Ivermectin	ATh	Anthelmintic
Omeprazole	AU	Antiulcer agent
Cimetidine	AU	Antiulcer drug
Esomeprazole	AU	Antiulcer agent
Clarithromycin	AU	Antiulcer drug
Lansoprazole	AU	Antiulcer
Podophyllotoxin	AV	Antiviral
Nevirapine	AV	Antiviral
Adefovir Dipivoxil	AV	Antiviral
Famciclovir	AV	Antiviral
Lamivudine	AV	Antiviral
Valaciclovir	AV	Antiviral
Atazanavir	AV	Antiviral
Didanosine	AV	Antiviral
Efavirenz	AV	Antiviral
Entecavir	AV	Antiviral
Ganciclovir	AV	Antiviral
Indinavir	AV	Antiviral
Oseltamivir	AV	Antiviral
Ritonavir	AV	Antiviral
Stavudine	AV	Antiviral
Valganciclovir	AV	Antiviral
Zanamivir	AV	Antiviral
Oxazepam	AX	Anxyolitic
Nitrazepam	AX	Anxyolitic
Lorazepam	AX	Anxyolitic
Bromazepam	AX	Anxyolitic
Buspirone Hydrochloride	AX	Anxyolitic
Galantamine	AZ	Anti Alzheimer's agent
Donepezil	AZ	Anti-Alzheimer's agent
Memantine Hydrochloride	AZ	Anti Alzheimer's agent
Rivastigmine	AZ	Anti Alzheimer's agent
Bisoprolol	βB	Beta-Blocker
Carvedilol	βB	Beta-Blocker
Propranolol Hydrochloride	βB	Beta-Blocker
Metoprolol	βB	Beta-Blocker
Sotalol Hydrochloride	βB	Beta-Blocker
Atenolol	βB	Beta-Blocker
Betaxolol Hydrochloride	βB	Beta-Blocker
Labetalol Hydrochloride	βB	Beta-Blocker
Levobunolol	βB	Beta-Blocker
Pindolol	βB	Beta-Blocker
Salmeterol	BD	Bronchodilator
Terbutaline Sulphate	BD	Bronchodilator
Eformoterol	BD	Bronchodilator
Tiotropium	BD	Bronchodilator
Clavulanic Acid	BLI	Beta-Lactamase Inhibitors *(used in combination with amoxycillin)*
Disodium Pamidronate	BP	Bone resorption inhibitor

Generic	Class Acronym	Therapeutic Class
Zoledronic Acid	BP	Bone resorption inhibitor
Risedronate Sodium	BP	Bone resorption inhibitor
Alendronate Sodium	BP	Bone Resorption inhibitor
Raloxifene	BP	Bone resorption inhibitor
Ibandronic Acid	BP	Calcium regulator
Ursodeoxycholic Acid	BT	Bile therapy
Bethanechol Chloride	ChS	Urinary and GI tract stimulant
Tetrabenazine	CNS	Central nervous system agent
Dexamphetamine Sulphate	CNS	CNS stimulant
Methylphenidate	CNS	CNS Stimulant - Psychostimulant
Atomoxetine	CNS	Central nervous system agent
Riluzole	CNS	Central nervous system agent
Frusemide	DI	Diuretic
Ethacrynic Acid	DI	Diuretic
Bumetanide	DI	Diuretic / antihypertensive
Spironolactone	DI	Diuretic
Amiloride Hydrochloride	DI	Diuretic
Chlorthalidone	DI	Diuretic
Indapamide	DI	Diuretic
Hydrochlorothiazide	DI	Diuretic
Triamterene	DI	Diuretic
Acamprosate	DxA	Detoxifying agent
Calcium Folinate	DxA	Detoxifying agent
Deferasirox	DxA	Detoxifying agent
Deferiprone	DxA	Detoxifying agent
Naltrexone	DxA	Detoxifying agent
Sevelamer	DxA	Detoxifying agent
Cinacalcet	EA	Endocrine and metabolic agent
Allopurinol	EI	Antigout drug
Cisapride	GP	Gastrointestinal stimulant
Rosiglitazone	HA	Hypoglycaemic agents
Glimepiride	HA	Hypoglycaemic agents
Glipizide	HA	Hypoglycaemic agents
Gliclazide	HA	Hypoglycaemic agent
Glibenclamide	HA	Hypoglycaemic agent
Pioglitazone Hydrochloride	HA	Hypoglycaemic agents
Sitagliptin	HA	Hypoglycaemic agents
Atorvastatin	HL	Hypolipidemic agent
Fenofibrate	HL	Hypolipideamic agent
Gemfibrozil	HL	Hypolipidemic agent
Simvastatin	HL	Hypolipidemic agent
Pravastatin	HL	Hypolipidemic agent
Ezetimibe	HL	Hypolipidaemic agent
Colestyramine	HL	Hypolipidemic agent
Rosuvastatin	HL	Hypolipidaemic agent
Fluvastatin	HL	Hypolipidaemic agent
Danazol	HM	Gonadal hormones
Oxpentifylline	HmT	Hematologic agent
Filgrastim	HP	Hematopoietic stimulator
Pegfilgrastim	HP	Hematopoietic stimulator
Tranexamic Acid	HS	Haemostatic Agent
Sildenafil	IA	Impotence agent
Azathioprine	IM	Immunosuppressant

Generic	Class Acronym	Therapeutic Class
Tacrolimus	IM	Immunosupressant
Cyclosporin	IM	Immunosuppressant
Sirolimus	IM	Immunosupressant
Fludrocortisone Acetate	MC	Synthetic mineralocorticoid
Carmellose Sodium	MP/LU	Mucoprotectant / Lubricant
Orphenadrine Citrate	MR	Muscle relaxant
Pyridostigmine Bromide	MS	Muscular stimulant
Bromhexine Hydrochloride	Mu	Expectorant / mucolytic agent
Homatropine Hydrobromide	MY	Mydriatic
Acetylcholine Chloride	MY	Mydriatic
Varenicline	NA	Nicotinic agonist
Baclofen	NB	Neuromuscular blocker / Muscle relaxant / antispasmodic
Clomiphene Citrate	OS	Ovulation stimulant
Flunitrazepam	S	Sedative
Zolpidem	S	Sedative
Zopiclone	S	Sedative
Finasteride	SA	Synthetic antiandrogen
Temazepam	SE	Sedative
Chloral Hydrate	SE	Sedative-hypnotic
Dantrolene Sodium	sMR	Skeletal muscle relaxant
Rifampicin	TB	Antitubercular agent
Isoniazid	TB	Antitubercular agent
Etanercept	TNF	Antiarthritic
Isosorbide Mononitrate	VA	Vasodilator
Perhexiline Maleate	VA	Vasodilators
Betahistine	VA	Vasodilator
Alprostadil	VA	Vasodilator
Bosentan	VA	Antihypertensive, vasodilator
Epoprostenol	VA	Vasodilator
Ivabradine	VA	Vasodilator
Papaverine	VA	Vasodilator
Phenoxybenzamine Hydrochloride	VA	Vasodilator
Dihydroergotamine	VP	Vasopressor
Glucagon Hydrochloride	-	Insulin antagonist
Probenecid	-	Antigout drug
Tamsulosin	-	Anti adrenergic
Colchicine	-	Antigout drug
Strontium Ranelate	-	Antiosteoporotic agent
Modafinil	-	Analeptic / Stimulant
Montelukast	-	Anti asthmatic

Supporting information B4: Therapeutic classes covered at The Royal Brisbane and Women's Hospital (RBWH). *(Supporting information for Figure 5b))*

Table SI 15. Compounds for which RBWH's contribution is 100%.
Total number of compounds: 123.

Generic	Class Acronym	Therapeutic Class
Amethocaine	AA	Anaesthetic agent *(Local)*
Bupivacaine	AA	Anaesthetic Agent *(Local)*
Cocaine	AA	Anaesthetic Agent *(Local)*
Ketamine	AA	Anaesthetic Agent *(General)*
Levobupivacaine	AA	Anaesthetic Agent *(Local)*
Oxybuprocaine	AA	Anaesthetic agent *(Local)*
Perfluorooctane	AA	Anaesthetic agent *(General)*
Procaine	AA	Anaesthetic agent *(Local)*
Propofol	AA	Anaesthetic Agent *(General)*
Remifentanil	AA	Anaesthetic agent *(General)*
Ropivacaine	AA	Anaesthetic agent *(Local)*
Thiopentone	AA	Anaesthetic Agent *(General)*
Amikacin	AB	Antibiotic
Aztreonam	AB	Antibiotic
Colistimethate Sodium	AB	Antibiotic
Demeclocycline	AB	Antibiotic
Ertapenem	AB	Antibiotic
Ethambutol	AB	Antibiotic
Linezolid	AB	Antibiotic
Meropenem	AB	Antibiotic
Nitazoxanide	AB	Antibiotic
Pentamidine Isethionate	AB	Antibiotic
Piperacillin	AB	Antibiotic
Pristinamycin	AB	Antibiotic
Sodium Fusidate	AB	Antibiotic
Tazobactam	AB	Antibiotic
Teicoplanin	AB	Antibiotic
Tetracycline	AB	Antibiotic
Tigecycline	AB	Antibiotic
Cefoxitin	AB	Antibiotic
Ceftazidime	AB	Antibiotic
Danaparoid	Acog	anticoagulant
Defibrotide	Acog	Anticoagulant
Lepirudin	Acog	Anticoagulant
Caspofungin Acetate	AF	Antifungal Agent
Flucytosine	AF	Antifungal agent
Natamycin	AF	Antifungal Agent
Posaconazole	AF	Antifungal
Voriconazole	AF	Antifungal Agent
Alfentanil	AG	Analgesic
Sufentanyl	AG	Analgesic
Guanethidine	Ahyp	Antihypertensive
Sodium Nitroprusside	Ahyp	Antihypertensive
Phentolamine Mesylate	Ahyp	Antihypertensive
Parecoxib Sodium	AI	Anti-inflammatory
Artemether	AM	Antimalarial
Artesunate	AM	Antimalarial

Generic	Class Acronym	Therapeutic Class
Lumefantrine	AM	Antimalarial
Primaquine	AM	Antimalarial
Ergotamine	Amig	Antimigraine agent
Anagrelide	AN	Antineoplastic
Busulfan	AN	Antineoplastic
Capecitabine	AN	Antineoplastic
Dacarbazine	AN	Antineoplastic
Dactinomycin	AN	Antineoplastic
Daunorubicin	AN	Antineoplastic antibiotic
Lomustine	AN	Antineoplastic
Mitomycin	AN	Antineoplastic
Procarbazine	AN	Antineoplastic
Thiotepa	AN	Antineoplastic
Carmustine	AN	Antineoplastic
Vincristine Sulphate	AN	Antineoplastic
Docetaxel	AN	Antineoplastic
Droperidol	Apsy	Antipsychotic agent
Levomepromazine	Apsy	Antipsychotic
Dofetilide	AR	Antiarrhythmic
Infliximab	ARh	Antirheumatic agent
Glycopyrrolate	ASp	Antispasmodic
Abacavir	AV	Antiviral
Cidofovir	AV	Antiviral
Darunavir	AV	Antiviral
Emtricitabine	AV	Antiviral
Enfuvirtide	AV	Antiviral agent
Etravirine	AV	Antiviral
Foscarnet	AV	Antiviral
Lopinavir	AV	Antiviral
Raltegravir	AV	Antiviral
Saquinavir	AV	Antiviral
Tenofovir	AV	Antiviral
Tipranavir	AV	Antiviral
Zidovudine	AV	Antiviral
Ritonavir	AV	Antiviral
Aminophylline	BD	Bronchodilator
Isoprenaline	BD	Bronchodilator
Sodium Clodronate	BP	Bone resorption inhibitor
Levosimendan	CaS	Calcium sensitizers
Succimer	ChA	Chelating agent
Cyclopentolate	CM	Cycloplegic and mydriatic agent
Metolazone	DI	Diuretic
Dicobalt edetate	DxA	Detoxifying agent
Edrophonium	DxA	Detoxifying agent, diagnosis
Flumazenil	DxA	Detoxifying agent
Pralidoxime	DxA	Detoxifying agent
Sodium Thiosulphate	DxA	Detoxifying agent
Lenograstim	HP	Hematopoietic stimulator
Lenalidomide	IM	Immunosuppressant
Mycophenolate Mofetil	IM	Immunosuppressant
Mycophenolate Sodium	IM	Immunosuppressant
Neostigmine	MS	Muscular stimulant
Tropicamide	MY	Mydriatic

Generic	Class Acronym	Therapeutic Class
Acetylcholine Chloride	MY	Mydriatic
Atracurium	NB	Neuromuscular blocking agent
Cisatracurium	NB	Neuromuscular blocking agent
Mivacurium	NB	Neuromuscular blocking agent
Pancuronium	NB	Neuromuscular blocking agent
Rocuronium	NB	Neuromuscular blocking agent
Vecuronium	NB	Neuromuscular blocking agent
Suxamethonium	NB	Neuromuscular blocking agent
Ergometrine	OA	Oxytocic agent
Dexmedetomidine Hydrochloride	S	Sedative
Sodium Tetradecylsulphate	SC	Sclerosing agents
Pyrazinamide	TB	Antitubercular agent
Diazoxide	VA	Antihypertensive
Nimodipine	VA	Vasodilator
Dobutamine	VP	Vasopressor
Dopamine	VP	Vasopressor
Metaraminol tartrate	VP	Vasopressor
Midodrine	VP	Vasopressor
Terlipressin	VP	Vasopressor
Ephedrine	VP	Vasopressor
Esmolol	βB	Cardio selective beta 1 -blocker
Disulfiram	-	Antioxidant
Sodium Phenylbutyrate	-	Orphan drug

Table SI 16. Compounds for which RBWH's contribution is in the range 15-100%.
Total number of compounds: 78.

Generic	Class Acronym	Therapeutic Class
Ampicillin	AB	Antibiotic
Lincomycin	AB	Antibiotic
Gentamicin Sulphate	AB	Antibiotic
Ticarcillin	AB	Antibiotic
Vancomycin	AB	Antibiotic
Benzylpenicillin	AB	Antibiotic
Cefazolin	AB	Antibiotic
Tobramycin	AB	Antibiotic
Cefotaxime	AB	Antibiotic
Silver Sulfadiazine	AB	Antibiotic
Cefalotin	AB	Antibiotic
Ceftriaxone	AB	Antibiotic
Cefepime	AB	Antibiotic
Moxifloxacin	AB	Antibiotic
Neomycin	AB	Antibiotic
Flucloxacillin	AB	Antibiotic
Azithromycin	AB	Antibiotic
Ofloxacin	AB	Antibiotic
Eptifibatide	Acog	Anticoagulant
Tirofiban	Acog	Anticoagulant
Abciximab	Acog	Anticoagulant / Antithrombotic
Bivalirudin	Acog	Anticoagulant
Enoxaparin	Acog	Anticoagulant
Granisetron	AE	Antiemetic
Ondansetron	AE	Antiemetic
Clioquinol	AF	Antifungal agent
Amphotericin	AF	Antifungal
Pethidine Hydrochloride	AG	Analgesic
Hydromorphone	AG	Analgesic
Buprenorphine	AG	Analgesic
Latanoprost	AGl	Antiglaucoma agent
Apraclonidine	AGl	Antiglaucoma agent
Ketorolac	AI	Anti-inflammatory
Methylprednisolone	AI	Anti-inflammatory
Dexamethasone	AI	Anti-inflammatory
Misoprostol	AI	Anti-inflammatory
Atovaquone	AM	Antimalarial
Pyrimethamine	AM	Antimalarial
Idarubicin Hydrochloride	AN	Antineoplastic
Cytarabine	AN	Antineoplastic
Fludarabine Phosphate	AN	Antineoplastic
Melphalan	AN	Antineoplastic
Bevacizumab	AN	Antineoplastic
Etoposide	AN	Antineoplastic agent
Ifosfamide	AN	Antineoplastic
Cyclophosphamide	AN	Antineoplastic
Cisplatin	AN	Antineoplastic
Mitozantrone	AN	Antineoplastic
Cetuximab	AN	Antineoplastic agent

Generic	Class Acronym	Therapeutic Class
Thioguanine	AN	Antineoplastic
Bleomycin	AN	Antineoplastic antibiotic
Methotrexate	AN	Antineoplastic
Apomorphine	AP	Antiparkinsonian agent
Butylscopolamine	ASp	Abdominal anti-spasmodic
Indinavir	AV	Antiviral
Ganciclovir	AV	Antiviral
Valganciclovir	AV	Antiviral
Stavudine	AV	Antiviral
Didanosine	AV	Antiviral
Efavirenz	AV	Antiviral
Midazolam	AX	Anxyolitic
Disodium Pamidronate	BP	Bone resorption inhibitor
Riluzole	CNS	Central nervous system agent
Deferasirox	DxA	Detoxifying agent
Naloxone Hydrochloride	DxA	Detoxifying agent
Acetylcysteine	DxA	Detoxifying agent
Deferiprone	DxA	Detoxifying agent
Calcium Folinate	DxA	Detoxifying agent
Tenecteplase	FB	Fibronolytic agent
Filgrastim	HP	Hematopoietic stimulator
Cyclosporin	IM	Immunosuppressant
Chloral Hydrate	SE	Sedative-hypnotic
Isoniazid	TB	Antitubercular agent
Rifampicin	TB	Antitubercular agent
Nicorandil	VA	Vasodilator
Epoprostenol	VA	Vasodilator
Alprostadil	VA	Vasodilator
Glucagon Hydrochloride	-	Insulin antagonist

Table SI 17. Compounds for which RBWH's contribution is in the range 0-15%.
Total number of compounds: 347.

Generic	Class Acronym	Therapeutic Class
Benzathine Penicillin	AB	Antibiotic
Metronidazole	AB	Antibiotic
Bacitracin	AB	Antibiotic
Framycetin Sulphate	AB	Antibiotic
Mupirocin	AB	Antibiotic
Ciprofloxacin	AB	Antibiotic
Clindamycin	AB	Anitbiotic
Gramicidin	AB	Antibiotic
Dicloxacillin	AB	Antibiotic
Sulphamethoxazole	AB	Antibiotic
Trimethoprim	AB	Antibiotic
Norfloxacin	AB	Antibiotic
Roxithromycin	AB	Antibiotic
Erythromycin	AB	Antibiotic
Rifabutin	AB	Antibiotic
Cefuroxime	AB	Antibiotic
Tinidazole	AB	Antibiotic
Amoxycillin	AB	Antibiotic
Cefalexin	AB	Antibiotic
Nitrofurantoin	AB	Antibiotic
Doxycycline	AB	Antibiotic
Sulphasalazine	AB	Antibiotic
Phenoxymethylpenicillin	AB	Antibiotic
Procaine Penicillin	AB	Antibiotic
Minocycline	AB	Antibiotic
Benzathine Phenoxymethylpenicillin	AB	Antibiotic
Cefaclor	AB	Anitbiotic
Gabapentin	AC	Anticonvulsant
Phenobarbitone	AC	Anticonvulsant
Sulthiame	AC	Anticonvulsant
Phenytoin	AC	Anticonvulsant
Primidone	AC	Anticonvulsant
Sodium Valproate	AC	Anticonvulsant
Levetiracetam	AC	Anticonvulsant / antiepileptic
Vigabatrin	AC	Anticonvulsant
Topiramate	AC	Anticonvulsant
Carbamazepine	AC	Anticonvulsant
Oxcarbazepine	AC	Anticonvulsant
Lamotrigine	AC	Anticonvulsant
Pregabalin	AC	Anticonvulsant
Ethosuximide	AC	Anticonvulsant
Tiagabine	AC	Anticonvulsant
Dipyridamole	Acog	Anticoagulant
Clopidogrel	Acog	Anticoagulant / Antithrombotic
Warfarin	Acog	Anticoagulant
Dalteparin	Acog	anticoagulant
Fondaparinux Sodium	Acog	Anticoagulant
Phenindione	Acog	Anticoagulant

Generic	Class Acronym	Therapeutic Class
Amitriptyline Hydrochloride	AD	Antidepressant
Citalopram	AD	Antidepressant
Mirtazapine	AD	Antidepressant
Venlafaxine	AD	Antidepressant
Fluvoxamine	AD	Antidepressant
Dothiepin Hydrochloride	AD	Antidepressant
Imipramine Hydrochloride	AD	Antidepressant
Sertraline	AD	Antidepressant
Phenelzine Sulphate	AD	Antidepressant
Clomipramine Hydrochloride	AD	Antidepressant
Doxepin Hydrochloride	AD	Antidepressant
Fluoxetine Hydrochloride	AD	Antidepressant
Moclobemide	AD	Antidepressant
Mianserin Hydrochloride	AD	Antidepressant
Nortriptyline Hydrochloride	AD	Antidepressant
Paroxetine	AD	Antidepressant
Reboxetine	AD	Antidepressant
Escitalopram	AD	Antidepressant
Bupropion	AD	Antidepressant
Duloxetine	AD	Antidepressant
Tranylcypromine	AD	Antidepressant
Metformin Hydrochloride	AdB	Antidiabetic
Desmopressin	aDI	Antidiuretic
Diphenoxylate Hydrochloride	ADy	Antidiarrheal
Prochlorperazine	AE	Antiemetic
Tropisetron	AE	Antiemetic
Itraconazole	AF	Antifungal Agent
Griseofulvin	AF	Antifungal
Fentanyl	AG	Analgesic
Oxycodone	AG	Analgesic
Morphine	AG	Analgesic
Methadone Hydrochloride	AG	Analgesic
Tramadol	AG	Analgesic
Dextropropoxyphene	AG	Analgesic
Acetazolamide	AGl	Antiglaucoma agent
Dorzolamide	AGl	Antiglaucoma agent
Pilocarpine	AGl	Antiglaucoma agent
Timolol	AGl	Antiglaucoma agent
Dipivefrine	AGl	Antiglaucoma agent
Brimonidine	AGl	Antiglaucoma agent
Bimatoprost	AGl	Antiglaucoma agent
Brinzolamide	AGl	Antiglaucoma agent
Albendazole	AH	Anthelminthic
Praziquantel	AH	Antihelminthic
Eplerenone	Ahyp	Antihypertensive
Clonidine	Ahyp	Antihypertensive
Hydralazine Hydrochloride	Ahyp	Antihypertensive
Captopril	Ahyp	Antihypertensive
Methyldopa	Ahyp	Antihypertensive
Amlodipine	Ahyp	Antihypertensive / vasodilator
Ramipril	Ahyp	Antihypertensive
Prazosin Hydrochloride	Ahyp	Antihypertensive
Lisinopril	Ahyp	Antihypertensive

Generic	Class Acronym	Therapeutic Class
Perindopril	Ahyp	Antihypertensive
Nifedipine	Ahyp	Antihypertensive antianginal
Lercanidipine	Ahyp	Antihypertensive agent
Verapamil Hydrochloride	Ahyp	Anihypertensive
Irbesartan	Ahyp	Antihypertensive
Candesartan	Ahyp	Antihypertensive
Fosinopril	Ahyp	Antihypertensive
Felodipine	Ahyp	Antihypertensive
Telmisartan	Ahyp	Antihypertensive
Enalapril Maleate	Ahyp	Antihypertensive
Eprosartan Mesylate	Ahyp	Anihypertensive
Losartan	Ahyp	Antihypertensive
Moxonidine	Ahyp	Antihypertensive
Olmesartan Medoxomil	Ahyp	Antihypertensive
Quinapril	Ahyp	Antihypertensive
Trandolapril	Ahyp	Antihypertensive
Betamethasone	AI	Antiasthmatic, anti-inflammatory
Balsalazide	AI	Anti-inflammatory
Flumethasone Pivalate	AI	Anti-inflammatory
Olsalazine Sodium	AI	Anti-inflammatory
Mesalazine	AI	Anti-inflammatory
Fluorometholone	AI	Anti-inflammatory
Piroxicam	AI	Anti-inflammatory
Celecoxib	AI	Anti-inflammatory
Ciclesonide	AI	Anti-inflammatory
Meloxicam	AI	Anti-inflammatory
Sulindac	AI	Anti-inflammatory
Dapsone	ALP	Antileprotic, antimalarial
Chloroquine	AM	Antimalarial
Proguanil	AM	Antimalarial
Hydroxychloroquine Sulphate	AM	Antimalarial
Mefloquine	AM	Antimalarial
Sulfadoxine	AM	Antimalarial
Sumatriptan	Amig	Antimigraine
Methysergide	Amig	Anti migraine agent
Temozolomide	AN	Antineoplastic
Rituximab	AN	Antineoplastic agent
Dasatinib	AN	Anineoplastic agent
Vinblastine Sulphate	AN	Antineoplastic
Doxorubicin Hydrochloride	AN	Antineoplastic
Carboplatin	AN	Antineoplastic
Paclitaxel	AN	Antineoplastic
Epirubicin Hydrochloride	AN	Antineoplastic
Vinorelbine	AN	Antineoplastic
Thalidomide	AN	miscellaneous anti neoplastic
Fluorouracil	AN	Antineoplastic
Gemcitabine	AN	Antineoplastic
Mercaptopurine	AN	Antineoplastic
Hydroxyurea	AN	Antineoplastic
Imatinib	AN	Antineoplastic agent
Chlorambucil	AN	Antineoplastic
Bicalutamide	AN	(Hormonal) antineoplastic agent
Exemestane	AN	Antineoplastic agent

Generic	Class Acronym	Therapeutic Class
Letrozole	AN	Antineoplastic
Irinotecan	AN	Antineoplastic agent
Tamoxifen	AN	Hormonal antineoplastic agent
Oxaliplatin	AN	Antineoplastic
Anastrozole	AN	(Hormonal) antineoplastic agent
Lapatinib	AN	Antineoplastic agent
Bortezomib	AN	Antineoplastic agent
Cladribine	AN	Anineoplastic
Erlotinib	AN	Antineoplastic agent
Everolimus	AN	Antineoplastic agent
Flutamide	AN	Antineoplastic agent
Fotemustine	AN	Antineoplastic
Fulvestrant	AN	Antineoplastic
Nilotinib	AN	Antineoplastic agent
Pemetrexed	AN	Antineoplastic
Sunitinib	AN	Antineoplastic
Trastuzumab	AN	Antineoplastic agent
Cyproterone Acetate	Aan	Antiandrogen
Benztropine Mesylate	AP	Antiparkinsonian agent
Entacapone	AP	Antiparkinsonian
Domperidone	AP	Antiparkinsonian agent
Benserazide	AP	Antiparkinsonian agent
Levodopa	AP	Antiparkinsonian agent
Bromocriptine Mesylate	AP	Antiparkinsonian agent
Carbidopa	AP	Antiparkinsonian agent
Benzhexol Hydrochloride	AP	Antiparkinsonian agent
Cabergoline	AP	Antiparkinsonian agent
Selegiline Hydrochloride	AP	Antiparkinsonian agent
Amantadine Hydrochloride	AP	Antiparkinsonian, Antiviral
Biperiden Hydrochloride	AP	Antiparkinsonian agent
Pergolide	AP	Antiparkinsonian agent
Pramipexole	AP	Antiparkinsonian agent
Acitretin	Apso	Antipsoriatic
Tazarotene	APSo	Antipsioratic
Haloperidol	APsy	Antipsychotic
Clozapine	APsy	Antipsychotic
Chlorpromazine Hydrochloride	APsy	Antipsychotic, Anxyolitic
Flupenthixol	APsy	Antipsychotic agent
Zuclopenthixol	APsy	Antipsychotic
Risperidone	APsy	Antipsychotic
Amisulpride	APsy	Antipsychotic
Fluphenazine Decanoate	APsy	Antipsychotic agent
Aripiprazole	Apsy	Antipsychotic / antidepressant
Quetiapine	Apsy	Antipsychotic
Olanzapine	Apsy	Antipsychotic
Trifluoperazine Hydrochloride	APsy	Antipsychotic
Ziprasidone	APsy	Antipsychotic
Pericyazine	APsy	Antipsychotic
Paliperidone	APsy	Antipsychotic agent
Pimozide	Apsy	Antipsychotic
Thioridazine Hydrochloride	APsy	Antipsychotic
Amiodarone Hydrochloride	AR	Antiarrhythmic agents
Digoxin	AR	Antiarrhythmic

Generic	Class Acronym	Therapeutic Class
Mexiletine Hydrochloride	AR	Antiarrhythmic agents
Diltiazem Hydrochloride	AR	Antiarrhythmic
Flecainide Acetate	AR	Antiarrhythmic agent
Disopyramide	AR	Antiarrhythmic agents
Adalimumab	ARh	Antirheumatic, immunomodulator
Leflunomide	ARh	Antirheumatic agent
Auranofin	ARh	Antirheumatic agent
Nandrolone Decanoate	AS	Anabolic steroid
Oxybutynin	ASp	Antispasmodic
Propantheline	ASp	Antispasmodic
Mebeverine Hydrochloride	ASp	Antispasmodic
Solifenacin Succinate	ASp	(urinary) antispasmodic
Carbimazole	AT	Antithyroid agent
Propylthiouracil	AT	Antithyroid agent
Ivermectin	ATh	Antihelmintic
Omeprazole	AU	Antiulcer drug
Clarithromycin	AU	Antiulcer drug
Esomeprazole	AU	Antiulcer drug
Cimetidine	AU	Antiulcer drug
Lansoprazole	AU	Antiulcer drug
Imiquimod	AV	Antiviral
Lamivudine	AV	Antiviral
Nevirapine	AV	Antiviral
Atazanavir	AV	Antiviral
Valaciclovir	AV	Antiviral
Oseltamivir	AV	Antiviral
Adefovir Dipivoxil	AV	Antiviral
Entecavir	AV	Antiviral
Podophyllotoxin	AV	Antiviral
Famciclovir	AV	Antiviral
Zanamivir	AV	Antiviral
Diazepam	AL	Anxiolytic
Clonazepam	AL	Anxiolytic
Alprazolam	AL	Anxiolytic
Clobazam	AL	Anxiolytic / Anticonvulsant
Lorazepam	AX	Anxyolitic
Buspirone Hydrochloride	AX	Anxyolitic
Oxazepam	AX	Anxyolitic
Nitrazepam	AX	Anxyolitic
Bromazepam	AX	Anxyolitic
Galantamine	AZ	Anti Alzheimer's agent
Donepezil	AZ	Anti-Alzheimer's agent
Rivastigmine	AZ	Anti Alzheimer's agent
Memantine Hydrochloride	AZ	Anti Alzheimer's agent
Salmeterol	BD	Bronchodilator
Eformoterol	BD	Bronchodilator
Terbutaline Sulphate	BD	Bronchodilator
Tiotropium	BD	Bronchodilator
Clavulanic Acid	BLI	Beta-Lactamase Inhibitors (used in combination with amoxycillin)
Zoledronic Acid	BP	Bone resorption inhibitor
Risedronate Sodium	BP	Bone resorption inhibitor
Alendronate Sodium	BP	Bone Resorption inhibitor

Generic	Class Acronym	Therapeutic Class
Raloxifene	BP	Bone resorption inhibitor
Ibandronic Acid	BP	Calcium regulator
Ursodeoxycholic Acid	BT	Bile therapy
Bethanechol Chloride	ChS	Urinary and GI tract stimulant
Tetrabenazine	CNS	Central nervous system agent
Methylphenidate	CNS	CNS Stimulant - Psychostimulant
Dexamphetamine Sulphate	CNS	CNS stimulant
Atomoxetine	CNS	Central nervous system agent
Bumetanide	DI	Diuretic / antihypertensive
Frusemide	DI	Diuretic
Spironolactone	DI	Diuretic
Ethacrynic Acid	DI	Diuretic
Hydrochlorothiazide	DI	Diuretic
Amiloride Hydrochloride	DI	Diuretic
Indapamide	DI	Diuretic
Triamterene	DI	Diuretic
Chlorthalidone	DI	Diuretic
Desferrioxamine	DxA	Detoxifying agent
Sevelamer	DxA	Detoxifying agent
Acamprosate	DxA	Detoxifying agent
Naltrexone	DxA	Detoxifying agent
Cinacalcet	EA	Endocrine and metabolic agent
Allopurinol	EI	Antigout drug
Cisapride	GP	Gastrointestinal stimulant
Glipizide	HA	Hypoglycaemic agent
Gliclazide	HA	Hypoglycaemic agent
Pioglitazone Hydrochloride	HA	Hypoglycaemic agent
Glibenclamide	HA	Hypoglycaemic agent
Glimepiride	HA	Hypoglycaemic agent
Rosiglitazone	HA	Hypoglycaemic agent
Sitagliptin	HA	Hypoglycaemic agent
Atorvastatin	HL	Hypolipidemic agent
Colestyramine	HL	Hypolipidemic agent
Gemfibrozil	HL	Hypolipidemic agent
Pravastatin	HL	Hypolipidemic agent
Fluvastatin	HL	Hypolipidaemic agent
Simvastatin	HL	Hypolipidemic agent
Fenofibrate	HL	Hypolipideamic agent
Ezetimibe	HL	Hypolipidaemic agent
Rosuvastatin	HL	Hypolipidaemic agent
Danazol	HM	Gonadal hormones
Oxpentifylline	HmT	Hematologic agent
Pegfilgrastim	HP	Hematopoietic stimulator
Tranexamic Acid	HS	Haemostatic Agent
Sildenafil	IA	Impotence agent
Tacrolimus	IM	Immunosupressant
Azathioprine	IM	Immunosuppressant
Sirolimus	IM	Immunosupressant
Fludrocortisone Acetate	MC	Synthetic mineralocorticoid
Carmellose Sodium	MP/LU	Mucoprotectant / Lubricant
Orphenadrine Citrate	MR	Muscle relaxant
Pyridostigmine Bromide	MS	Muscular stimulant
Bromhexine Hydrochloride	Mu	Expectorant / mucolytic agent

Generic	Class Acronym	Therapeutic Class
Homatropine Hydrobromide	MY	Mydriatic
Varenicline	NA	Nicotinic agonist
Baclofen	NB	Neuromuscular blocker / Muscle relaxant / antispasmodic
Clomiphene Citrate	OS	Ovulation stimulant
Flunitrazepam	S	Sedative
Zolpidem	S	Sedative
Zopiclone	S	Sedative
Finasteride	SA	Synthetic antiandrogen
Temazepam	SE	Sedative
Dantrolene Sodium	sMR	Skeletal muscle relaxant
Etanercept	TNF	Antiarthritic
Ivabradine	VA	Vasodilator
Papaverine	VA	Vasodilator
Phenoxybenzamine Hydrochloride	VA	Vasodilator
Isosorbide Mononitrate	VA	Vasodilator
Perhexiline Maleate	VA	Vasodilators
Bosentan	VA	Antihypertensive, vasodilator
Betahistine	VA	Vasodilator
Dihydroergotamine	VP	Vasopressor
Labetalol Hydrochloride	βB	Beta-Blocker
Bisoprolol	βB	Beta-Blocker
Metoprolol	βB	Beta-Blocker
Carvedilol	βB	Beta-Blocker
Propranolol Hydrochloride	βB	Beta-Blocker
Atenolol	βB	Beta-Blocker
Sotalol Hydrochloride	βB	Beta-Blocker
Betaxolol Hydrochloride	βB	Beta-Blocker
Pindolol	βB	Beta-Blocker
Levobunolol	βB	Beta-Blocker
Strontium Ranelate	-	antiosteoporotic agent
Colchicine	-	Antigout drug
Probenecid	-	Antigout drug
Montelukast	-	Anti asthmatic
Modafinil	-	Analeptic / Stimulant
Tamsulosin	-	Anti adrenergic

Supporting information B5: MOEs for hospital-specific compounds.

Table SI 18. MOEs related to hospital–specific compounds at QEII hospital determined in hospital effluent ($MOE_{H_{eff}}$) and the influent of the corresponding STP ($MOE_{STP_{inf}}$) sorted by increasing $MOE_{H_{eff}}$.

Generic	Class Acronym	Therapeutic Class	MOE Heff	MOE STPinf
Ropivacaine	AA	Anaesthetic agent (Local)	1	532
Oxybuprocaine	AA	Anaesthetic agent (Local)	1	594
Piperacillin	AB	Antibiotic	1	757
Isoprenaline	BD	Bronchodilator	2	1 030
Pancuronium	NB	Neuromuscular blocking agent	2	1 122
Tropicamide	MY	Mydriatic	4	2 121
Cisatracurium	NB	Neuromuscular blocking agent	4	2 300
Propofol	AA	Anaesthetic Agent (General)	5	2779
Tazobactam	AB	Antibiotic	5	3 030
Piperacillin	AB	Antibiotic	13	7 599
Cyclopentolate	CM	cycloplegic and mydriatic agent	25	14 845
Suxamethonium	NB	Neuromuscular blocking agent	28	15 213
Mitomycin	AN	Antineoplastic	49	28 641
Bupivacaine	AA	Anaesthetic Agent (Local)	58	33 663
Ketamine	AA	Anaesthetic Agent (General)	70	40 627
Meropenem	AB	Antibiotic	70	40 914
Vecuronium	NB	Neuromuscular blocking agent	110	64 265
Levobupivacaine	AA	Anaesthetic Agent (Local)	116	67 326
Trimipramine	AD	Antidepressant	116	67 326
Thiopentone	AA	Anaesthetic Agent (General)	124	72 135
Dexmedetomidine Hydrochloride	S	Sedative	135	78 546
Sodium Fusidate	AB	Antibiotic	139	80 791
Artemether	AM	Antimalarial	144	84 157
Flumazenil	DxA	Detoxifying agent	208	121 186
Ceftazidime	AB	Antibiotic	233	125 244
Ephedrine	VP	Vasopressor	278	137 212
Abacavir	AV	Antiviral	289	168 314
Nimodipine	VA	Vasodilator	291	169 660
Dopamine	VP	Vasopressor	318	185 084
Levomepromazine	Apsy	Antipsychotic	433	252 471
Emtricitabine	AV	Antiviral	578	336 628
Tenofovir	AV	Antiviral	578	336 628
Ergotamine	Amig	Antimigraine agent	650	378 706
Ergometrine	OA	Oxytocic agent	694	403 953
Neostigmine	MS	Muscular stimulant	771	448 837
Metaraminol tartrate	VP	Vasopressor	867	504 942
Sodium Nitroprusside	Ahyp	Antihypertensive	867	504 942
Pyrazinamide	TB	Antitubercular agent	910	530 189
Lumefantrine	AM	Antimalarial	1 387	807 907
Dicobalt edetate	DxA	Detoxifying agent	1 445	841 569
Ertapenem	AB	Antibiotic	1 445	841 569
Cefoxitin	AB	Antibiotic	1 734	788 971
Ethambutol	AB	Antibiotic	1 820	1 060 377
Dobutamine	VP	Vasopressor	1 942	1 131 069
Mycophenolate Mofetil	IM	Immunosuppressant	2 312	1 346 511
Alfentanil	AG	Analgesic	2 427	1 413 837
Glycopyrrolate	ASp	Antispasmodic	3 468	2 019 767

Generic	Class Acronym	Therapeutic Class	MOE Heff	MOE STPinf
Atracurium	NB	Neuromuscular blocking agent	3 884	2 262 139
Droperidol	Apsy	Antipsychotic agent	6 192	3 606 726
Pralidoxime	DxA	Detoxifying agent	6 935	4 039 533
Diazoxide	VA	Antihypertensive	17 338	10 098 833
Lenograstim	HP	Hematopoietic stimulator	23 073	13 439 511
Aminophylline	BD	Bronchodilator	1 456 350	848 301 953
Adapalene	-	Acne treatment	NA	NA

Table SI 19. MOEs related to hospital–specific compounds at RBWH hospital determined in hospital effluent ($MOE_{H_{eff}}$) and the influent of the corresponding STP ($MOE_{STP_{inf}}$) sorted by increasing $MOE_{H_{eff}}$.

Generic	Class Acronym	Therapeutic Class	MOE Heff	MOE STPinf
Piperacillin	AB	Antibiotic	0.04	8
Vincristine Sulphate	AN	Antineoplastic (cytotoxic)	0.01	0.4
Tazobactam	AB	Antibiotic	0.01	3
Bupivacaine	AA	Anaesthetic Agent (Local)	0.4	69
Ropivacaine	AA	Anaesthetic agent (Local)	0.4	68
Tropicamide	MY	Mydriatic	1	53
Propofol	AA	Anaesthetic Agent (General)	1	125
Remifentanil	AA	Anaesthetic agent (General)	1	134
Oxybuprocaine	AA	Anaesthetic agent (Local)	1	71
Infliximab	ARh	Antirheumatic agent	1	81
Suxamethonium	NB	Neuromuscular blocking agent	2	357
Isoprenaline	BD	Bronchodilator	3	137
Thiopentone	AA	Anaesthetic Agent (General)	4	785
Foscarnet	AV	Antiviral	5	919
Pancuronium	NB	Neuromuscular blocking agent	5	48
Cyclopentolate	CM	Cycloplegic and mydriatic agent	5	540
Carmustine	AN	Antineoplastic	7	380
Vecuronium	NB	Neuromuscular blocking agent	8	1 424
Nimodipine	VA	Vasodilator	9	1 039
Glycopyrrolate	ASp	Antispasmodic	9	1 866
Busulfan	AN	Antineoplastic	10	2 220
Rocuronium	NB	Neuromuscular blocking agent	10	2 070
Ketamine	AA	Anaesthetic Agent (General)	12	1 602
Levobupivacaine	AA	Anaesthetic Agent (Local)	13	2 977
Sodium Phenylbutyrate	-	Orphan drug	15	3 629
Ergometrine	OA	Oxytocic agent	17	3 989
Metaraminol tartrate	VP	Vasopressor	19	2 757
Ephedrine	VP	Vasopressor	21	3 709
Voriconazole	AF	Antifungal Agent	25	4 028
Meropenem	AB	Antibiotic	26	1 732
Cisatracurium	NB	Neuromuscular blocking agent	31	4 801
Neostigmine	MS	Muscular stimulant	32	6 280
Mitomycin	AN	Antineoplastic	48	6 215
Mycophenolate Mofetil	IM	Immunosuppressant	53	3 126
Aztreonam	AB	Antibiotic	57	786
Esmolol	βB	Cardio selective beta 1 -blocker	64	12 670
Artesunate	AM	Antimalarial	76	14 276
Alfentanil	AG	Analgesic	85	8 474
Anagrelide	AN	Antineoplastic	87	16 049
Posaconazole	AF	Antifungal	92	6 798
Atracurium	NB	Neuromuscular blocking agent	98	21 886
Sodium Fusidate	AB	Antibiotic	105	12 752
Amethocaine	AA	Anaesthetic agent (Local)	106	5 157
Pentamidine Isethionate	AB	Antibiotic	107	21 975
Ceftazidime	AB	Antibiotic	113	2 675
Phentolamine Mesylate	Ahyp	Antihypertensive	143	2 236
Dexmedetomidine Hydrochloride	S	Sedative	150	28 594
Dobutamine	VP	Vasopressor	156	8 444

Generic	Class Acronym	Therapeutic Class	MOE Heff	MOE STPinf
Teicoplanin	AB	Antibiotic	160	22 547
Cefoxitin	AB	Antibiotic	168	19 131
Dacarbazine	AN	Antineoplastic	182	33 904
Dactinomycin	AN	Antineoplastic	185	37 672
Levomepromazine	Apsy	Antipsychotic	213	7 853
Dopamine	VP	Vasopressor	222	3 386
Ethambutol	AB	Antibiotic	224	28 808
Lepirudin	Acog	Anticoagulant	231	28 767
Procarbazine	AN	Antineoplastic	249	7 931
Sodium Tetradecylsulphate	SC	Sclerosing agents	254	43 053
Mivacurium	NB	Neuromuscular blocking agent	256	40 112
Flumazenil	DxA	Detoxifying agent	264	34 848
Tenofovir	AV	Antiviral	265	31 466
Capecitabine	AN	Antineoplastic	297	56 236
Caspofungin Acetate	AF	Antifungal Agent	301	19 188
Emtricitabine	AV	Antiviral	315	37 672
Linezolid	AB	Antibiotic	348	16 107
Saquinavir	AV	Antiviral	427	58 205
Abacavir	AV	Antiviral	429	66 317
Acetylcholine Chloride	MY	Mydriatic	447	11 572
Amikacin	AB	Antibiotic	458	71 287
Zidovudine	AV	Antiviral	473	68 064
Levosimendan	CaS	Calcium sensitizers	481	11 392
Thiotepa	AN	Antineoplastic	483	36 941
Pyrazinamide	TB	Antitubercular agent	602	43 447
Lopinavir	AV	Antiviral	629	35 070
Darunavir	AV	Antiviral	727	172 213
Artemether	AM	Antimalarial	753	60 815
Etravirine	AV	Antiviral	817	193739
Droperidol	Apsy	Antipsychotic agent	942	175 103
Tipranavir	AV	Antiviral	954	226 029
Disulfiram	-	Antioxidant	954	193 739
Tigecycline	AB	Antibiotic	1 095	163 888
Ertapenem	AB	Antibiotic	1 156	17 522
Demeclocycline	AB	Antibiotic	1 162	257 095
Primaquine	AM	Antimalarial	1 192	155 882
Enfuvirtide	AV	Antiviral agent	1 271	301 372
Parecoxib Sodium	AI	Anti-inflammatory	1 361	153 936
Docetaxel	AN	Antineoplastic	1 501	36 168
Cidofovir	AV	Antiviral	1 526	258 319
Sodium Clodronate	BP	Bone resorption inhibitor	1 536	154 550
Sodium Nitroprusside	Ahyp	Antihypertensive	2 008	47 253
Ritonavir	AV	Antiviral	2 277	36 605
Metolazone	DI	Diuretic	2 289	5 820
Guanethidine	Ahyp	Antihypertensive	3 093	733 068
Raltegravir	AV	Antiviral	3 633	861 064
Daunorubicin	AN	Antineoplastic antibiotic	4 005	215 755
Dofetilide	AR	Antiarrhythmic	4 768	30 004
Lenograstim	HP	Hematopoietic stimulator	5 076	29 394
Danaparoid	Acog	anticoagulant	5 189	410 962
Pralidoxime	DxA	Detoxifying agent	5 721	516 638
Nitazoxanide	AB	Antibiotic	5 868	1 390 949

Generic	Class Acronym	Therapeutic Class	MOE Heff	MOE STPinf
Midodrine	VP	Vasopressor	6 023	355 330
Colistimethate Sodium	AB	Antibiotic	6 357	53 273
Dicobalt edetate	DxA	Detoxifying agent	6 357	1 506 861
Lomustine	AN	Antineoplastic	6 599	1 5641 22
Flucytosine	AF	Antifungal agent	7 152	122 863
Edrophonium	DxA	Detoxifying agent	7 152	733 068
Lumefantrine	AM	Antimalarial	7 227	583 824
Mycophenolate Sodium	IM	Immunosuppressant	7 382	51 639
Succimer	ChA	Chelating agent	7 629	1 808 234
Lenalidomide	IM	Immunosuppressant	13 622	538 165
Ergotamine	Amig	Antimigraine agent	19 071	4 282 659
Terlipressin	VP	Vasopressor	24 090	1 060 202
Diazoxide	VA	Antihypertensive	38 143	5 424 701
Tetracycline	AB	Antibiotic	106 799	25 315 270
Aminophylline	BD	Bronchodilator	138 500	23 260 585
Sodium Thiosulphate	DxA	Detoxifying agent	143 034	1 027 405
Procaine	AA	Anaesthetic agent (Local)	476 781	61 644 327
Natamycin	AF	Antifungal Agent	640 794	25 315 270
Cocaine	AA	Anaesthetic Agent (Local)	NA	NA
Defibrotide	Acog	Anticoagulant	NA	NA
Perfluorooctane	AA	Anaesthetic agent (General)	NA	NA
Pristinamycin	AB	Antibiotic	NA	NA
Sufentanyl	AG	Analgesic	NA	NA

C. Supporting Information for Chapter 3.

Supporting information C1: Chemicals

Chemical standards of atenolol, propranolol, ciprofloxacin, erythromycin, sulfamethoxazole, cephalexin, praziquantel, perindopril, indomethacin, doxylamine, fluoxetine, dapsone, warfarin, furosemide, naproxen, carbamazepine, metoprolol tartrate, acetaminophen, diclofenac sodium, enrofloxacin, trimethoprim, sertraline hydrochloride, gemfibrozil, venlafaxine hydrochloride, caffeine, roxithromycin, tramadol hydrochloride, ibuprofen, phenytoin (5,5-diphenylhydantoin), sulfadiazine, norfloxacin, and N,N-Diethyl-meta-toluamide (DEET) were purchased from Sigma–Aldrich (Steinheim, Germany), citalopram hydrobromide was purchased from Toronto Research Chemicals (Ontario, Canada), and iopromide was purchased from U.S. Pharmacopeia (Rockville, U.S.A.). All chemical standards were of analytical grade (≥99%).

Isotopically labelled compounds used for the correction of matrix interferences Naproxen-d_3, Furosemide-d_5, Atrazin-d_5, Phenytoin- d_{10}, Sulfamethoxazole-d_4, Propranolol-d_7, Atenolol-d_7, Metoprolol-d_7, Doxylamine-d_5, Sertraline-d_3, Fluoxetine-d_6, Ranitidine-d_6, Citalopram-d_6, Indomethacin-d_4, Perindopril-d_4, Lincomycin-d_3, Erythromycin ^{13}C, Iopromide-d_3, Roxithromycin-d_7, gemfibrozil-d_6, acetaminophen-d_4, tramadol-d_6 hydrochloride, ibuprofen-d_3, venlafaxine-d_6, enrofloxacin-d_5 and sulfadiazine-d_4, were purchased from Toronto Research Chemicals (Ontario, Canada), trimethoprim-^{13}C, carbamazepine-d_{10}, DEET-d_6, caffeine-^{13}C, and diclofenac-d_4 were purchased from Cambridge Isotope Laboratories (Andover, U.S.A.).

Stock solutions of individual standards (1 g L^{-1}) and internal standards were prepared in methanol and stored at −20°C. Stock solutions of norfloxacin and enrofloxacin were renewed monthly because of their limited stability. For the purpose of analyses, a standard mixture in methanol at approx. 20 mg L^{-1} concentration was prepared from the individual stock solutions of the selected analytes. Further dilutions of this mixture were prepared in 25:75 (v/v) of methanol/water and were used as working standard solutions. A mixture of labelled standards at a concentration of 500 µg L^{-1} was prepared by dilution of individual stock solutions in methanol, and was used for internal standard calibration.

Table SI 20. Target analytes with their therapeutic class, molecular weights (MWs), acid dissociation constants (pKas), octanol-water partition coefficients (log K_{OW}s).

Compounds	Therapeutic Class	MW (g mol^{-1})	pKa[a]	log K_{ow}[a]
Acetaminophen	Analgesic	151.16	9.38	0.46
Atenolol	Beta-blocker	266.3	9.6/9.05[b]	-0.03
Caffeine	-	194.2	10.4[d]	-0.07[d]
Carbamazepine	Anticonvulsant	236.27	1; 13.9	2.45
Cephalexin	Antibiotic	347.39	-	0.40
Ciprofloxacin	Antibiotic	331.34	-	0.00
Citalopram	Antidepressant	324.4	9.59[j]	3.74
Dapsone	Antituberculotic and antileprotic	248.3	1.28/2.09[b]	0.77
DEET	Insect repellent	191.28	na	2.18
Diclofenac	Anti-inflammatory	296.15	4.15/4.12[b]	4.6[c]
Doxylamine	Sedative/ Antihistaminic	270.37	8.73[b]	2.37
Enrofloxacin	Antibiotic	359.4	5.94; 8.70[i]	0.7
Erythromycin	Antibiotic	733.95	8.88/8.23[b]	2.48
Fluoxetine	Antidepressant	361.93	-	4.65
Furosemide	Diuretic	330.75	3.5[b]	2.03[b]
Gemfibrozil	Hypolipidemic agent	250.33	4.43	4.77
Ibuprofen	Anti-inflammatory	206.23	4.91	3.97
Indomethacin	Anti-inflammatory	357.8	4.5[b]	4.27[b]
Iopromide	X-ray contrast agent	790.87	0	-2.66
Metoprolol	Beta-blocker	267.36	9.68	1.88
Naproxen	Anti-inflammatory	230.27	4.15[b]	3.18
Norfloxacin	Antibiotic	319.34	6.34; 8.75[j]	-1.03
Perindopril	Antihypertensive	368.468	5.4[b]	-2.42[b]
Phenytoin	Anticonvulsant	252.28	8.33	2.47
Praziquantel	Anthelmintic	312.41	-	2.42
Propranolol	Beta-blocker	259.34	9.24[b]	3.48[b]
Roxithromycin	Antibiotic	837.07	8.8[k]	2.75
Sertraline	Antidepressant	306.24	9.5[j]	5.29
Sulfadiazine	Antibiotic	250.28	1.8; 6.36	-0.09
Sulfamethoxazole	Antibiotic	253.28	1.8/1.84[b]	0.89[b]
Tramadol	Analgesic	263.38	9.44[e]	2.31[f]
Trimethoprim	Antibiotic	290.32	3.2; 7.1	0.91
Venlafaxine	Antidepressant	277.4	9.4[g]	2.91[h]
Warfarin	Anticoagulant	308.33	4.8[b]	2.70[b]

a pKa and log KOW values retrieved from PhysProp Database Demo, Syracuse Research Corporation, 2008 (www.syrres.com/esc/physdemo.htm)
b Reungoat, J. *et al*, (2012). Water Res. 46 (3), 863-872.
c Hansch *et al*, 1995. In: Heller, S.R. (Ed.) Exploring QSAR, American Chemical Society, Washington DC.
d EPI SuiteTM v4.0
e Pospisilova *et al*, 1998, J. Pharm. Biomed, Anal, 18, 777-783.
f Craig, P.N., 1990. In: Hansch, C., Sammes, P.G., Taylor, J.B. (Eds.). Comprehensive Medicinal Chemistry, Vol. 6. Pergamon Press, Oxford.
g Ellingrod *et al*, 1994, Am. J. Hosp. Pharm. 51 (24), 3033-3046.
h Hasemann *et al*, 2007, Electrophoresis 28, 1779–1787.
i Lizondo *et al*, 1997, J. Pharm. Biomed. Anal. 15, 1845-1849.
j The Merck Index
k Huber *et al*, 2003, Environ. Sci. Technol. 37, 1016-1024.

Table SI 21. Determined recoveries for hospital wastewater (HWW) and raw sewage treatment plant influent (STPinf), with their method quantification limits (MQLs). Recoveries are expressed as mean values with their standard deviations (SDs).

Compound	R_1 (HWW) ± SD,%	MDL (HWW), ng L^{-1}	MQL (HWW), ng L^{-1}	R_2 (STPinf.) ± SD,%	MDL (STPinf), ng L^{-1}	MQL (STPinf), ng L^{-1}
Atenolol	72.3 ± 7.9	0.4	1.4	80.5 ± 12.0	0.2	0.6
Tramadol	44.4 ± 9.3	0.6	1.9	56.9 ± 0.3	0.1	0.4
Metoprolol	52.3 ± 6.2	1.0	3.3	86.2 ± 3.2	0.6	1.9
Propranolol	142.8 ± 0.6	0.4	1.5	77.5 ± 2.2	1.8	6.0
Ciprofloxacin	136.9 ± 4.8	4.9	16.2	91.8 ± 13.5	4.7	15.6
Norfloxacin	95.5 ± 20.3	5.5	18.5	133.0 ± 20.0	7.4	24.8
Enrofloxacin	73.6 ± 25.2	1.0	3.5	77.1 ± 6.1	0.9	2.9
Venlafaxine	43.6 ± 9.4	0.1	0.5	60.2 ± 3.7	0.1	0.3
Erythromycin	95.5 ± 12.0	2.3	7.6	76.5 ± 8.0	2.4	8.0
Roxithromycin	41.7 ± 14.8	4.7	15.5	99.3 ± 19.0	1.4	4.7
Sulfamethoxazole	107.2 ± 4.7	1.5	5.1	94.3 ± 2.6	0.7	2.3
Sulfadiazine	75.9 ± 11.3	1.0	3.4	77.8 ± 4.0	1.2	4.2
DEET	71.5 ± 12.8	0.1	0.2	43.1 ± 4.2	0.1	0.3
Phenytoin	77.1 ± 4.5	0.3	0.8	75.3 ± 0.6	0.3	1.0
Carbamazepine	63.5 ± 14.1	0.2	0.7	75.1 ± 1.3	0.1	0.3
Praziquantel	109.8 ± 1.4	0.1	0.2	129.1 ± 2.7	0.0	0.1
Cephalexin	88.7 ± 10.5	2.7	9.0	84.5 ± 7.2	1.7	5.5
Sertraline	68.5 ± 4.1	3.1	10.4	63.8 ± 0.0	2.1	7.2
Citalopram	73.7 ± 8.1	0.3	1.1	87.1 ± 2.1	0.6	1.9
Perindopril	98.6 ± 0.6	0.1	0.3	85.3 ±2 .5	0.0	0.1
Indomethacin	89.4 ± 4.2	0.1	0.3	88.7 ± 1.2	0.1	0.3
Doxylamine	94.2 ± 12.0	0.5	1.6	100.2 ± 2.0	0.2	0.7
Fluoxetine	82.8 ± 4.9	0.1	0.5	78.3 ± 1.2	0.0	0.1
Iopromide	108.5 ± 7.8	4.0	13.5	88.8 ± 4.5	19.8	65.9
Trimethoprim	45.8±1.8	0.7	2.4	85.0 ± 1.3	0.4	1.3
Caffeine	79.1 ± 2.9	4.2	14.0	173.9 ± 10.9	0.5	1.6
Dapsone	38.0 ± 1.4	0.9	2.9	83.3 ± 9.6	0.7	2.2
Gemfibrozil	113.6 ± 10.7	1.1	3.7	84.6 ± 1.2	0.2	0.5
Warfarin	160.5 ± 5.5	0.1	0.2	178.5 ± 10.5	0.1	0.3
Diclofenac	113.0 ± 8.5	1.2	4.0	126.5 ± 3.6	0.2	0.7
Acetaminophen	134.7±9.9	187.3	624.3	110.6 ± 18.9	52.4	174.7
Ibuprofen	94.6 ± 10.6	12.9	42.9	132.9 ± 14.0	3.9	12.9
Furosemide	98.9 ± 9.4	12.5	41.8	69.4 ± 8.7	2.3	7.8
Naproxen	93.0 ± 8.8	129.2	430.6	88.5 ± 8.5	95.9	319.6

Table SI 22. Optimized QqLIT-MS parameters for the analysis of target analytes in the negative ion (NI) mode. SRM-selected reaction monitoring transition. DP-declustering potential, CE-collision energy, CXP-cell exit potential, t_R-retention time.

Compound	Precursor ion, m/z	SRM 1	DP-CE-CXP (V)	SRM 2	DP-CE-CXP (V)	t_R, min
Acetaminophen	150.0	106.9	60-26-7	107.8	60-22-5	6.07
Acetaminophen-d_4	154.0	111.1	60-26-7	120.9	60-42-5	6.07
Ibuprofen	205.0	161.0	52-11-10	-	-	10.59
Ibuprofen-d_3	208.0	163.9	45-10-11	161.3	45-10-5	10.59
Naproxen	229.0	185.0	50-10-13	169.0	50-38-9	9.64
Naproxen-d3	232.0	173.0	40-20-13	188.0	40-10-31	9.63
Gemfibrozil	249.0	121.0	85-20-7	127.0	85-14-5	11.24
Gemfibrozil-d_6	255.1	121.0	60-28-7	133.0	60-14-9	11.24
Diclofenac	293.9	250.0	40-16-1	214.0	40-30-15	10.32
Diclofenac-d_4	298.0	253.9	60-16-1	216.9	60-30-12	10.30
Warfarin	307.0	161.0	85-28-11	250.0	85-32-1	9.80
Furosemide	329.0	284.8	70-22-7	204.8	70-30-11	7.71
Furosemide-d_5	333.9	289.9	75-24-17	205.8	75-32-11	7.69

Table SI 23. Optimized QqLIT-MS parameters for the analysis of target analytes in the positive ion (PI) mode.

Compound	Precursor ion, m/z	SRM 1	DP-CE-CXP (V)	SRM 2	DP-CE-CXP (V)	t_R, min
DEET	192.2	119.1	61-25-8	91.2	61-45-6	10.16
DEET-d_6	198.3	116.1	68-27-8	91.1	68-45-6	10.12
Caffeine	195.1	138.1	71-28-8	110.0	71-32-8	6.32
Caffeine$^{13}C_3$	198.1	140.2	36-29-12	112.1	36-27-12	6.32
Atrazin-d_5	221.1	179.3	71-27-12	101.2	71-35-6	10.04
Carbamazepine	237.2	194.2	61-27-16	193.3	61-47-12	8.65
Carbamazepine-d_{10}	247.2	204.2	81-31-12	202.2	81-50-14	8.58
Dapsone	249.1	108.2	71-31-8	92.1	71-35-6	7.30
Sulfadiazine	251.1	92.1	66-39-6	65.1	66-63-4	6.44
Sulfadiazine-d_4	255.0	160.1	71-23-12	96.2	71-39-6	6.41
Phenytoin	253.1	182.2	61-27-10	104.1	61-51-8	8.42
Phenytoin- d_{10}	263.148	192.1	71-27-14	109.1	71-51-8	8.38
Sulfamethoxazole	254.2	92.1	51-38-8	156	51-23-8	7.54
Sulfamethoxazole-d_4	258.068	96.179	76-37-8	112.178	76-39-6	7.53
Tramadol	264.4	58.1	45-44-8	42.2	45-125-3	6.58
Tramadol-d_6	270.2	64.2	61-39-2	45.2	61-113-8	6.58
Propranolol	260.2	116.2	76-27-8	183.2	76-27-12	7.10
Propranolol-d_7	267.174	123.1	76-27-10	79.2	76-33-6	7.09
Atenolol	267.2	145.3	71-37-12	190.2	71-29-16	5.29
Atenolol d_7	274.2	145.1	71-37-12	79.1	71-33-6	5.28
Metoprolol	268.2	116.2	76-27-8	121.1	76-35-8	6.51
Metoprolol-d_7	275.249	123.1	71-27-10	79.1	71-31-14	6.50
Doxylamine	271.2	182.2	40-24-8	167.2	40-45-7	6.02
Doxylamine-d_5	276.202	187.1	56-25-12	171	56-47-14	5.99
Venlafaxine	278.2	58.1	61-41-10	260.3	61-19-6	6.94
Venlafaxine-d_6	284.3	58.2	61-59-8	266.3	61-19-20	6.93
Trimethoprim	291.2	230.3	86-10-4	261.2	86-37-6	6.07
Trimethoprim$^{13}C_3$	294.2	233.2	96-33-12	126.1	96-34-9	6.07
Sertraline	306.1	159.1	56-39-12	275.1	56-19-18	8.13
Sertraline-d_3	309.1	159	51-35-12	275	51-17-18	8.14
Fluoxetine	310.2	44.1	46-41-6	148.2	46-13-12	7.95
Fluoxetine-d_6	316.261	44.1	56-45-6	154.2	56-13-12	7.94
Praziquantel	313.2	203.2	81-25-18	83.2	81-41-6	10.39
Ranitidine-d_6	321.3	176.2	51-25-16	102.2	51-47-8	5.38
Norfloxacin	320.2	276.2	70-26-14	233.2	70-35-14	6.02
Cephalexin	348.2	158.2	41-15-12	106.1	41-43-6	5.91
Citalopram	325.3	109.1	70-38-4	262.2	70-28-4	7.33
Citalopram-d_6	331.243	109	91-37-8	262.1	91-29-22	7.34
Ciprofloxacin	332.2	314.2	75-34-12	245.2	75-39-15	6.13
Indomethacin	358.1	139	91-27-12	111	91-71-8	11.71
Indomethacin-d_4	362.155	143	81-29-10	115.1	81-77-18	11.68
Enrofloxacin	360.2	316.2	81-29-12	245.2	81-39-20	6.29
Enrofloxacin-d_5	365.2	321.2	86-33-18	245.2	86-35-22	6.29
Perindopril	369.24	172.2	76-29-14	98.1	76-49-6	7.20
Perindopril-d_4	373.244	176.1	76-29-44	102	76-55-8	7.21
Lincomycin-d_3	410.22	129.2	96-39-10	73	96-95-2	5.71
Erythromycin	734.6	158.1	71-41-8	576.4	71-35-8	7.24
Erythromycin $^{13}C_3$	738.575	162	76-45-14	83	76-89-6	7.25
Iopromide	791.88	773.87	120-35-10	572.9	125-54-10	5.44
Iopromide-d_3	795.004	576	96-33-16	561.9	96-41-14	5.43
Roxithromycin	837.6	679.5	96-31-12	158	96-49-12	7.99
Roxithromycin-d_7	844.672	158.1	101-49-12	83.1	101-103-6	7.95

Supporting information C2: UFLC-QqLIT-MS multi-residue method description.

For the analysis in negative ionization (NI) mode, eluent A was a mixture of acetonitrile/methanol (1:1, v/v) and eluent B was 1 mM aqueous solution of ammonium acetate (HPLC grade water) at a flow rate of 1 mL min^{-1}. The elution gradient started with 5% eluent A, increasing to 90% of A in 7 min, held isocratically for 3 min, increased to raising to 100% of A in 2 min and held at 100% of A for 3 min before returning to the initial conditions. The column was re-equilibrated for 5 min before another injection with a total time for chromatographic analysis of 21 min. The analysis in positive ionization (PI) mode was performed using acetonitrile with 0.1% formic acid as eluent A, and HPLC grade water with 0.1% formic acid as eluent B. The elution gradient started with 5% eluent A, increasing to 60% in 5 min, raising to 90% in the following 8 min, further increasing to 100% of A in the next 2 min. Next, the gradient was isocratically held at 100% of A for 2 min before returning to the initial conditions and re-equilibrating the column for 5 min. Chromatographic analysis lasted for 23 min.

The MS parameters were optimized for target pharmaceuticals and pesticides. Settings for source-dependent parameters, common in both NI and PI modes, were determined by Flow Injection Analysis (FIA) and are as follows: curtain gas (CUR), 30V; nitrogen collision gas (CAD) high; source temperature (TEM) was 700 °C, and ion source gases GS1 and GS2 were set at 62 and 55V in NI and PI modes, respectively. Conversely, the ion spray voltages in NI and PI modes were set at -4500 and 5500V, respectively. To achieve higher sensitivity, resolution at the first quadrupole (Q1) is fixed at low while the resolution at the third quadrupole (Q3) was set to unit.

The optimization of compound dependent MS parameters (declustering potential (DP), entrance potential (EP), collision energy (CE) and cell exit potential (CXP)) for each transition was performed by infusing standards of each individual compound at 100 μg L^{-1} to the mass spectrometer. Optimum parameters are summarized Table SI 20 and Table SI21 for the analysis of target analytes in the NI and PI mode, respectively.

D. Supporting Information for Chapter 4

Table SI 24. Micropollutants analysed in studies focusing on pharmaceuticals residues in various water types published between 2009 and 2011.

Study	Tested	Location	Number of Compounds Analysed	Compounds Analysed	Justification for Compounds Selection	Reference
Removal of pharmaceuticals and endocrine disrupting compounds in a water recycling process using reverse osmosis systems	Raw wastewater and reverse osmosis treated wastewater	Australia	11	Carbamazepine; Clofibric acid; Diclofenac; Gemfibrozil; Ibuprofen; Ketoprofen; Naproxen; paracetamol; Phenytoin; Primidone; Salicylic acid.	Compounds cover the physicochemical properties of compounds potentially present in the environment	Al-Rifai *et al.* (2011)
Occurrence and distribution of pharmaceuticals in wastewater from households, livestock farms, hospitals and pharmaceutical manufactures	Municipal wastewater/ Livestock wastewater / Hospital wastewater / pharmaceutical industry wastewater	Korea	24	Acetylsalicylic acid; Caffeine; Carbamazepine; Cefadroxil; Cefradine; Chlortetracycline; Ciprofloxacin; Diclofenac; Enrofloxacin; Erythromycin-H2O; Florfenicol; Ibuprofen; Lincomycin; Mefenamic acid; Naproxen; Oxytetracycline; Penicillin; Sulfamethazine; Sulfamethoxazole; Sulfathiazole; Trimethoprim; Tylosin; Vancomycin.	*Not explicitly mentioned*.	Sim *et al.* (2011)
Analysis of the presence of cardiovascular and analgesic/anti-inflammatory/antipyretic pharmaceuticals in river- and drinking-water of the Madrid Region in Spain	Surface water and drinking water	Spain	25	Codeine; Diclofenac; Ibuprofen; Indomethacin; Ketoprofen; Mefenamic acid; Naproxen; Paracetamol; Phenazone; Propyphenazone; Salicylic acid; Atenolol; Metoprolol tartrate; Nadolol; Propranolol; Sotalol hydrochloride; Bezafibrate; Clofibric acid; Fenofibrate; Frusemide (furosemide); Gemfibrozil; Hydrochlorothiazide; Mevastatin; Pravastatin sodium; Simvastatin.	Compounds belong to the 2 most frequently prescribed and/or therapeutic groups used nationally (cardiovascular and analgesic/anti-inflammatory/antipyretic drugs) Analgesics, anti-inflammatory drugs and antipyretics	Valcárcel *et al.* (2011)
Occurrence of pharmaceutical compounds and Hormones in drinking water	Drinking water	France	51	Androstenedione; Androsterone; Atenolol; Azithromycin; Bezafibrate; Carbamazepine; Diclofenac; Dihydrotestosterone; Epiandrosterone; Epitestosterone; Ethinylestradiol; Etiocholanolone; Fenofibric acid; Fluoxetine; Furosemide; Ibuprofen; Ketoprofen; Levonorgestrel; Lorazepam; Medroxyprogesterone; Megestrol; Metoprolol; Metronidazole; Naproxen; Norethindrone; Norfluoxetine; Oestriol; Oestrone; Oxazepam; Paracetamol; Pravastatine; Progesterone; Propranolol; Roxithromycin; Salicylic acid; Sulphamethoxazole; Testosterone; Tilmicosin; Triclosan; Trimethoprime; Tylosin; 7a-Oestradiol; 17b-Oestradiol; 5-Androstane-3;17-diols 9(x8).	Compounds have been selected based on national consumption ; predicted environmental concentration; ecotoxycological parameters and physicochemical parameters	Vulliet *et al.* (2011)
Fate of b-blocker human pharmaceuticals in surface water: Comparison of measured and simulated concentrations in the Glatt Valley Watershed, Switzerland	Raw and primary treated wastewater Surface water	Switzerland	4	Atenolol; Metoprolol; Propranolol; Sotalol	Compounds belong to a specific drug class (beta-blockers). Consumption information have also been taken into consideration	Alder *et al.* (2010)
HPLC/ UV/ Fluorescence detection of several pharmaceuticals in Sewage treatment plant wastewaters of Jordan	Municipal and hospital wastewater	Jordan	5	Caffeine; Diclofenac; Glimepiride; Ibuprofen; Methotrexate	Compounds are amomg the most consumed pharmaceuticals nationally	Alahmad and Alawi (2010)

Study	Tested	Location	Number of Compounds Analysed	Compounds Analysed	Justification for Compounds Selection	Reference
Occurrence of iodinated X-ray contrast media in indirect potable reuse systems	Secondary and tertiary treated wastewater	Australia	8	Diatrizoic acid; Iodipamide; Iohexol; Iomeprol; Iopamidol; Iopromide; Iothalamic acid; Ioxaglic acid	Compounds belong to a specific drug class (X-ray contrast media) and are known to persist in the environment	Busetti *et al.* (2010)
Winter accumulation of acidic pharmaceuticals in a Swedish river	Surface water	Sweden	5	Bezafibrate; Diclofenac;Ibuprofen; Ketoprofen; Naproxen	Not explicitly mentioned, reference to chemical characteristics of the compounds and representatively of specific drug class (analgesic)	Daneshvar *et al.* (2010a)
Seasonal variations in the occurrence and fate of basic and neutral pharmaceuticals in a Swedish river–lake system.	Surface water	Sweden	5	Acebutolol; Atenolol; Carbamazepine; Metoprolol; Sotalol	Not explicitly mentioned although reference are made to the drug classes covered (beta-blockers and anitepileptic) and to sales volumes within the class investigated	Daneshvar *et al.* (2010b)
Environmental risk assessment of pharmaceuticals in rivers: Relationships between hazard indexes and aquatic macroinvertebrate diversity indexes in the Llobregat River (NE Spain)	Surface water	Spain	29	Atenolol; Azythromycin; Bezafibrate; Carbamazepine; Clofibric Acid; Diclofenac; Erythromycin; Famotidine; Fluoxetine; Gemfibrozil; Ibuprofen; Indomethacine ;Ketoprofen; Lansoprazole; Loratadine ; Mefenamic Acid; Metoprolol; Mevastatin; Naproxen; Ofloxacin; Paracetamol; Paroxetine; Pravastatin ; Propranolol; Propyphenazone; Ranitidine; Sotalol; Sulfamethoxazole; Trimethoprim	Compounds are commonly used pharmaceuticals, Cover a range of drug classes (analgesics and non-steroidal anti-inflammatories (NSAIDs), lipid regulators, psychiatric drugs, anti-histamines, anti-ulcer agents, antibiotics and beta-blockers)	Ginebreda *et al.* (2010)
Pollution by psychoactive pharmaceuticals in the Rivers of Madrid metropolitan area (Spain)	Surface water	Spain	23	7-Aminoflunitrazepam; Alprazolam;Amitriptyline; Carbamazepine; Chlorpromazine; Citalopram; Clomipramine; Diazepam; Flunitrazepam; Fluoxetine; Levomeprazine; Lorazepam; Lormetazepam; Midazolam; Norclomipramine; Nordiazepam; Norfluoxetine; Nortriptiline; Oxazepam; Paroxetine; Sertraline; Tetrazepam; Triazolam; Venlafaxine; α-Hydroxyalprazolam; α-Hydroxytriazolam;	Compounds are highly prescribed highly consumed pharmaceuticals regionally. Techniques of detection for the selected compounds are available. They belong to a category of compounds with specific mode of action (target sites in the central nervous system- psychoactive)	González Alonso *et al.* (2010)
Removal of pharmaceuticals during wastewater treatment and environmental risk assessment using hazard indexes	Influent and effluent of WWTP and surface water	Spain	73	Atenolol; Atorvastatin; Azithromycin; Betaxolol; Bezafibrate; Butalbital; Carazolol; Carbamazepine; Chloramphenicol; Chlortetracycline; Cimetidine; Ciprofloxacin; Clarithromycin; Clenbuterol; Clofibric acid; Codeine; Danofloxacin; Diazepam; Diclofenac; Doxycycline; Enalapril; Enoxacin; Enrofloxacin; Erythromycin; Famotidine; Fenofibrate; Fluoxetine; Furosemide; Gemfibrozil; Glibenclamide; Hydrochlorothiazide; Ibuprofen; Indomethacine; Josamycin; Ketoprofen; Lisinopril; Loratadine; Lorazepam; Mefenamic acid; Metoprolol; Metronidazole; Mevastatin; Nadolol; Naproxen; Nifuroxazide; Norfloxacin; Ofloxacin; Oxytetracycline;paracetamol; Pentobarbital; Phenazone; Phenobarbital; Phenylbutazone; Pindolol; Pravastatin; Propranolol; Propyphenazone; Ranitidine; Roxithromycin; Salbutamol; Salicylic acid; Sotalol; Spiramycin; Sulfadiazine; Sulfamethazine; Sulfamethoxazole; Sulfonamide; Tamoxifen; Tetracycline; Tilmicosin; Timolol; Trimethoprim; Tylosin	Compounds are largely consumed for human health	Gros *et al.* (2010)

Study	Tested	Location	Number of Compounds Analysed	Compounds Analysed	Justification for Compounds Selection	Reference
Assessment of full-scale natural systems for the removal of PPCPs from wastewater in small communities	Wetland or conventionally treated wastewater	Spain	7	Caffeine; Clofibric Acid; Diclofenac; Furosemide; Ibuprofen; Ketoprofen; Naproxen; Salicylic Acid	Compounds are widely used and have been frequently detected in previous studies	Hijosa-Valsero *et al.* (2010)
Occurrence and removal of PPCPs in municipal and hospital wastewaters in Greece	Hospital wastewater	Greece	11	Caffeine; Carbamazepine; Diclofenac; Fenofibrate; Gemfibrozil; Ibuprofen; Naproxen; Paracetamol; Phenazone; Salycilic acid; Triclosan	Compounds were chosen based on high annual consumption and concerns over their possible effects on human and aquatic organisms (previous study)	Kosma *et al.* (2010)
Fate of selected pharmaceuticals and personal care products after secondary wastewater treatment processes in Taiwan	Surface water, hospital and WWTP effluents	Taiwan	20	Ampicillin; Caffeine; Cefazolin; Cephalexin; Cephradine; Chlortetracycline; Clarithromycin; Clofibric Acid; Cloxacillin; Diclofenac; Erythromycin-H_2O; Fenoprofen;Gemfibrozil; Ibuprofen; Ketoprofen; Naproxen; Paracetamol; Sulfamethazine;Sulfamethoxazole; Tetracycline	Drug classes coverage; frequency of detection concentration, stability throughout treatment process	Lin *et al.* (2010)
Loadings, trends, comparisons, and fate of achiral and chiral pharmaceuticals in wastewaters from urban tertiary and rural aerated lagoon treatments	Surface water	Canada	16	Atenolol; Carbamazepine; Celecoxib; Citalopram; Clarithromycin; Codeine; Diclofenac; Erythromycin; Gemfibrozil; Metoprolol; Naproxen; Paroxetine; Propranolol; Sotalol; Temazepam; Triclosan	*Not explicitly mentioned.*	MacLeod and Wong (2010)
Screening of pharmaceuticals and endocrine disrupting compounds in water supplies of Cyprus	Groundwater, influent and tertiary-treated effluent of WWTP, raw and finished surface water, household potable water	Cyprus	16	Atenolol; Atorvastatin; Caffeine; Carbamazepine; Diazepam; Phenytoin; Fluoxetine; Meprobamate; Primidone; Sulfamethoxazole; Trimethoprim; Diclofenac; Gemfibrozil; Ibuprofen; Naproxen; Triclosan	*Not explicitly mentioned.*	Makris and Snyder (2010)
Antidepressants and their metabolites in municipal wastewater, and downstream exposure in an urban watershed	Influent and effluent of WWTP, surface water and drinking water	Canada	9	Bupropion ; Citalopram ; Desmethyl citalopram ; Desmethyl sertaline; Fluoxetine ; Norfluoxetine ; Paroxetine ;Venlafaxine	Compounds belong to a specific drug class (antidepressant)	Metcalfe *et al.* (2010)
Occurrence and fate of micropollutants in the Vidy bay of lake Geneva, Switzerland. Part ii: micropollutant removal between wastewater and Raw drinking water	Raw wastewater, treated wastewater and raw drinking water	Switzerland	37	Acipimox; Atenolol; Azithromycin; Bezafibrate; Carbamazepine; Ciprofloxacin; Clarithromycin; Clindamycin; Clofibric acid; Diatrizoic;Diclofenac; Fenofibrate; Gabapentin; Gemfibrozil; Ibuprofen; Iohexol; Iomeprol; Iopamidol; Iopromide; Iothalamic acid; Ketoprofen; Mefenamic acid; Metoprolol; Metronidazole; Nadolol; Naproxen; Norfloxacin; Ofloxacin; Paracetamol; Pravastatin; Primidone; Propranolol; Simvastatin; Sotalol; Sulfadimethoxine; Sulfamethoxazole; Trimethoprim	The criteria for the selection of compounds include annual sales, metabolism, removal rates by WWTP, analytical feasibility and results from a prequel study.	Morasch *et al.* (2010)
Modelling of hospital wastewater pollution by pharmaceuticals: first results of mediflux study carried out in three French hospitals	Hospital wastewater	France	13	cyclophosphamide; ifosfamide; 5-fluorouracil; propofol; iomeprol, iobitridol; gadolinium; atenolol; ketoprofen; prednisolone; methylprednisolone; sulfamethoxazole; ciprofloxacin	The criteria for the selection of compounds include location/site specificity, annual consumption, Maximum recommended therapeutic dose, metabolism, analytical capabilities, therapeutic classes.	Mullot *et al.* (2010)

Study	Tested	Location	Number of Compounds Analysed	Compounds Analysed	Justification for Compounds Selection	Reference
Concentrations and mass loadings of cardiovascular pharmaceuticals in healthcare facility wastewaters	Healthcare facilities wastewaters	USA	19	Clonidine; Hydrochlorothiazide; Furosemide; Triamterene; Fluocinonide; Propranolol; Metoprolol; Atenolol; Amlodipine; Verapamil; Norverapamil; Diltiazem; Desmethyl diltiazem; Enalapril; Valsartan; Simvastatin; Atorvastatin; Gemfibrozi.	Compounds belong to a specific drug class	Nagarnaik *et al.* (2010)
Determining the fraction of pharmaceutical residues in wastewater originating from a hospital	Hospital wastewater	Australia	59	Atenolol; Acetylsalicylic acid; Atorvastatin; Caffeine; Carbamazepine; Cephalexin; Chlortetracycline; Chlroamphenicol; Ciprofloxacin; Citalopram; Codeine; Cyclophosphamide; Dapsone; DEET; Desmethyl Citalopram; Desmethyl Diazepam; Diazepam; Diclofenac; Doxylamine; Enrofloxacin; Erythromycin; Fluoxetine; Fluvastatin; Furosemide; Gabapentin; Gemfibrozil; Hydrochlorthiazide; Ibuprofen; Ifosfamide; Indomethacin; Iopromide; Lincomycin; Metoprolol; Naproxen; Norfloxacin; Oxazepam; Oxycodone; Oxytetracycline; Paracetamol; Phenytoin; Praziquantel; Propranolol; Ranitidine; Roxithromycin; Salicylic acid; Sertraline; Simvastatin; Sulfasalazine; Sulphadiazine; Sulphamethoxazole; Sulphathiazole; Temazepam; Tetracycline; Tramadol; Triclosan; Trimethoprim; Tylosin; Venlafaxine; Warfarin	Compounds are dissolved pollutant which cannot be eliminated in conventional wastewater treatment	Ort *et al.* (2010)
Antiviral drugs in wastewater and surface waters: a new pharmaceutical class of environmental relevance?	Raw and treated wastewater, surface water	Germany	9	Acyclovir; abacavir; lamivudine; nevirapine; oseltamivir; penciclovir; ribavirin; stavudine; zidovudine	Compounds belong to a specific drug class (antiviral)	Prasse *et al.* (2010)
Pharmaceutical formulation facilities as sources of opioids and other pharmaceuticals to wastewater treatment plant effluents	Raw wastewater receiving pharmaceutical industry wastewater	USA	7	Butalbital ; carisoprodol ; diazepam; metaxalone; methadone oxycodone; phendimetrazine	Compounds produced by industries discharging to WWTP	Phillips *et al.* (2010)
Dynamics and attenuation of acidic pharmaceuticals along a river stretch	Surface water	Germany	4	Bezafibrate; Clofibric acid; Diclofenac; Naproxen	*Not explicitly mentioned*	Radke *et al.* (2010)

Study	Tested	Location	Number of Compounds Analysed	Compounds Analysed	Justification for Compounds Selection	Reference
Occurrence of emerging pollutants in urban wastewater and their removal through biological treatment followed by ozonation	Influent and effluent of the secondary clarifier of a municipal WWTP	Spain	66	Atenolol; Azithromycin; Bezafibrate; Caffeine; Carbamazepine; Carbamazepine Epoxide; Cefotaxime; Celestolide; Ciprofloxacin; Citalopram Hydrobromide; Clarithromycin; Clofibric Acid; Clorophene; Codeine; Cotinine; Diazepam; Diclofenac; Erythromycin; Ethylhexyl Methoxycinnamate; Famotidine; Fenofibrate; Fenofibric Acid; Fenoprofen; Fluoxetine; Furosemide; Gemfibrozil; Hydrochlorothiazide; Ibuprofen; Indomethacin; Isoproturon; Ketoprofen; Ketorolac; Lansoprazole; Lincomycin; Loratadine; Mefenamic Acid; Mepivacaine; Methylprednisolone 6-Alpha Sodium Succinate; Metoprolol; Metronidazole; Naproxen; Norfloxacin; Ofloxacin; Omeprazole; Paracetamol; Paraxanthine; Paroxetine; Pravastatin; Primidone; Propanolol; Propyphenazone; Ranitidine; Salbutamol; Salicylic Acid; Sotalol; Sulfamethoxazole; Sulfapyridine; Terbutaline; Triclosan; Trimethoprim; Venlafaxine;4-Aminoantipyrine (4-Aa); 4-Dimethylaminoantipyrine (4-Daa); 4-Methylaminoantipyrine (4-Maa);N-Acetyl-4-Amino-Antipiryne (4-Aaa);N-Formyl-4-Amino-Antipiryne (4-Faa)	*Not explicitly mentioned*	Rosal *et al.* (2010)
Surface Water Concentrations and Loading Budgets of Pharmaceuticals and Other Domestic-Use Chemicals in an Urban Watershed (Washington, DC, USA)	Surface water	USA	6	Carbamazepine; Diclofenac;Ibuprofen; Ketoprofen; Naproxen; Triclosan	Compounds were detected in previous studies in the same location.	Shala and Foster (2010)
Antidepressant Pharmaceuticals in Two U.S. Effluent-Impacted Streams: Occurrence and Fate in Water and Sediment, and Selective Uptake in Fish Neural Tissue	Surface water	USA	10	Bupropion; Citalopram; Duloxetine;Fluoxetine; Fluvoxamine; Norfluoxetine (Degradate); Norsertraline (Degradate); Paroxetine; Sertraline; Venlafaxine	Compounds belong to a specific drug class (antidepressant)and are among the most sold pharmaceuticals in the US	Shultz *et al.* (2010)
Occurrence and removal of pharmaceuticals, caffeine and DEET in wastewater treatment plants of Beijing, China	Influent and treated effluent from municipal WWTP	China	13	Chloramphenicol; nalidixic acid; trimethoprim; bezafibrate; clofibric acid; gemfibrozi; diclofenac; indomethacin; ketoprofen; mefenamic acid; metoprolol; carbamazepine; caffeine	*Not explicitly mentioned but compounds are covering different classes*	Sui *et al.* (2010)
Relating environmental concentrations of pharmaceuticals to consumption: A mass balance approach for the river Rhine	Surface water	The Netherlands	20	Amidotrizoinic acid; Anhydro-erythromycin A; Atenolol; Carbamazepine; Clarithromycin; Clindamycin; Ibuprofen Diclofenac; Iohexol; Iomeprol; Iopamidol; Iopromide; Ioxitalamic acid; Metoprolol; Pentoxifylline Bezafibrate;Roxithromycin; Sotalol; Sulfamethoxazole; Trimethoprim	*Not explicitly mentioned*	Ter Laak *et al.* (2010)

Study	Tested	Location	Number of Compounds Analysed	Compounds Analysed	Justification for Compounds Selection	Reference
Occurrence of emerging contaminants, priority substances (2008/105/CE) and heavy metals in treated wastewater and groundwater at Depurbaix facility (Barcelona, Spain).	Municipal wastewater, advanced treated wastewater and groundwater	Spain	82	Amitriptyline; Amoxicillin; Antipyrine; Atenolol; Azithromycin; Bezafibrate; Caffeine; Caffeine C13 ; Carbamazepine; Carb-Epoxide; Cefotaxime; Ciprofloxacin; Citalopram; Clarithromycin; Clofibric Acid; Clomipramine; Clotrimazole; Codeine; Cotinine; Cyclophosphamide; Diatrizoate; Diazepam; Diclofenac; Erythromycin; Famotidine; Fenofibrate; Fenofibric Acid; Fenoprofen; Fluoxetine; Furosemide; Gemfibrozil; Hydrochlorothiazide; Ibuprofen; Ifosfamide; Indomethacine; Iopamidol; Iopromide; Ketoprofen; Ketorolac; Lansoprazole; Lincomycin; Loratadine; Mefenamic Acid; Mepivacaine; Methylprednisolone; Metoprolol; Metronidazole; Mevastatin; N-Acetyl-4-Amino-Antipiryne (4-Aaa); Nadolol; Naproxen; N-Formyl-4-Amino-Antipiryne (4-Faa); Nicotine; Norfloxacin; Ofloxacin; Omeprazole; Paracetamol; Paraxanthine; Paroxetine; Phenacethin; Pravastatin; Primidone; Propanolol Hydrochloride; Propyphenazone; Ranitidine; Salbutamol; Salicilic Acid; Simvastatin; Sotalol; Sulfadiazine; Sulfamethazine; Sulfamethoxazole; Sulfapyridine; Sulfathiazole; Tamoxifen; Terbutaline; Tetracycline; Trimethoprim; Velafaxime;4-Amino-Antipiryne (4-Aa); 4-Dimethylaminoantipiryne (4-Daa); 4maa; 6-Alpha Sodium Succinate	Metabolism of pharmaceuticals, known persistence in the environment, know environmental risk (EDCs)	Teijon *et al.* (2010)
Optimization and validation of a hydrophilic interaction liquid chromatography–tandem mass spectrometry method for the determination of 13 top-prescribed pharmaceuticals in influent wastewater	Influent of WWTP	Belgium	13	Atenolol; Bisoprolol; Citalopram; Fluoxetine; Metformin; Metoprolol; Nebivolol;Omeprazole; Pantoprazole; Paroxetine; Ranitidine; Tramadol; Venlafaxine	Top sold prescription in Belgium	van Nuijs *et al.* (2010)
Spatiotemporal distribution of pharmaceuticals in the Douro River estuary (Portugal)	Surface water	Portugal	5+ 2 metabolites	Carbamazepine; Diazepam; Fenofibrate; Fenofibric Acid; Propranolol; Sulfamethoxazole;Trimethoprim	Coverage of therapeutic classes with distinct physico-chemical properties, environmental behaviour and persistence, previous knowledge on substances; national consumption	Madureira *et al.* (2010)
A field study on 8 pharmaceuticals and 1 pesticide in Belgium: Removal rates in waste water treatment plants and occurrence in surface water	Influent and effluent of WWTP receiving industrial effluent, surface water	Belgium	8	Cinnarizine; Domperidone; Flubendazole; Itraconazole; Ketoconazole; Miconazole; Pipamperone; Rabeprazole	High PEC values; produced in belgium	Van De Steene *et al.* (2010)
Occurrence of endocrine disrupting compounds, pharmaceuticals, and personal care products in the Han River (Seoul, South Korea)	Surface water	Korea	23	Atenolol; Atorvastatin; Atrazine; Caffeine; Carbamazepine; Diazepam; Diclofenac; Dilantin; Estradiol; Estrone; Ethinylestradiol; Fluoxetine; Gemfibrozil; Ibuprofen; Iopromide; Meprobamate; Naproxen; Primidone; Progesterone; Sulfamethoxazole; Testosterone; Triclosan; Trimethoprim	*Not explicitly mentioned. Primary focus on one specific class (EDCs)*	Yoon *et al.* (2010)

Study	Tested	Location	Number of Compounds Analysed	Compounds Analysed	Justification for Compounds Selection	Reference
Occurrence and a screening-level risk assessment of human pharmaceuticals in the Pearl river system, South China	Surface water	China	14	Carbamazepine; Clofibric acid; Diclofenac; Fenoprofen; Gemfibrozil; Ibuprofen; Indomethacin; Ketoprofen; Meclofenamic acid; Mefenamic acid; Naproxen; Primidone;Salicylic acid; Tolfenamic acid	*Not explicitly mentioned*	Zhao *et al.* (2010)
The occurrence of illicit and therapeutic pharmaceuticals in wastewater effluent and surface waters in Nebraska	Effluent of WWTP, surface water	USA	18	Azithromycin; Caffeine; Carbamazepine; Cotinine; D-Amphetamine; DEET; Diphenhydramine; Methamphetamine;Paracetamol; Sulfachloropyridazine; Sulfadimethoxine; Sulfamerazine; Sulfamethazine; Sulfamethiazole; Sulfamethoxazole; Sulfathiazole; Thiabendazole; Virginiamycin;7-Dimethylxanthine	*Not explicitly mentioned*	Bartelt-Hunt *et al.* (2009)
Pharmaceuticals and endocrine disrupting compounds in U.S drinking water	Drinking water	USA	20	Atenolol; Atorvastatin; Carbamazepine; Diazepam; Diclofenac; Enalapril; Fluoxetine; Gemfibrozil; Meprobamate; Naproxen; Norfluoxetine; o-Hydroxy atorvastatin; Phenytoin; p-Hydroxy atorvastatin; Risperidone; Simvastatin; Simvastatin; Sulfamethoxazole; Triclosan; Trimethoprim	Volume of use; Toxicity; Occurrence and public interest; Drug class; Availability of analytical standards	Benotti *et al.* (2009)
Analysis of pharmaceuticals in indirect potable reuse systems using solid-phase extraction and liquid chromatography–tandem mass spectrometry	Drinking water	Australia	18	Atorvastatin; Bezafibrate; Carbamazepine; Clofibric; Cyclophosphamide; Diazepam; Diclofenac; Fluoxetine; Gemfibrozil; Ibuprofen; Ifosfamide; Indomethacin; Ketoprofen; Morphine; Naproxen; Paracetamol; Phenytoin; Warfarin	No screening method but compounds covering multiple drug classes (lipid lowering agents, analgesics, non-steroidal anti-inflammatory drugs (NSAIDs), anticoagulants, antipyretics, cytostatic, antiepileptic, antidepressants and tranquilizers)	Busetti *et al.* (2009)
An affordable method for the simultaneous determination of the most studied pharmaceutical compounds as wastewater and surface water pollutants	Influent and effluent of WWTP, Surface water	Spain	17	Caffeine; Carbamazepine; Clofibric Acid; Diclofenac; 17a-Ethinylestradiol; 17b-Estradiol; Estriol; Estrone; Gemfibrozil; Ibuprofen; Ketoprofen; Naproxen;Paracetamol; Propranolol; Salicylic Acid; Sulfamethoxazole; Trimethoprim	Common compounds to four reviews listing pharmaceuticals based on European consumption criteria, behaviour during wastewater treatment and frequency of detection in wastewater samples	Camacho-Muñoz *et al.* (2009)
Contamination of surface, ground, and drinking water from pharmaceutical production	Surface, ground and drinking water	India	12	Cetirizine; Ciprofloxacin; Citalopram; Enalapril; Enoxacin; Enrofloxacin; Lomefloxacin; Metoprolol; Norfloxacin; Ofloxacin; Terbinafine; Trimethoprim	*Not explicitly mentioned.* Pharmaceutical produced by pharmaceutical companies in the area investigated.	Fick *et al.* (2009)
Screening of antibiotics in surface and wastewater samples by ultra-high-pressure liquid chromatography coupled to hybrid quadrupole time-of-flight mass spectrometry	Surface and wastewater sample	Spain	42	Amoxicillin; Ampicillin; Azithromycin; Cefaclor; Cefotaxime; Ceftazidime; Ceftriaxone; Cefuroxime; Cephadrine; Cephalexin; Chlortetracycline; Ciprofloxacin; Clarithromycin; Clindamycine; Cloxacillin; Dicloxacillin;Doxycycline; Enrofloxacin; Erythromycin; Flumequine; Lyncomycin; Marbofloxacine; Moxifloxacine; Nalidixic acid; Norfloxacine; Ofloxacin; Oxacillin acid; Oxolinic acid; Oxytetracycline; Pefloxacine; Penicillin G; Pipemidic acid; Piperacillin; Roxithromycin; Sarafloxacine; Sulfadiazine; Sulfamethazine; Sulfamethoxazole; Sulfathiazole; Tetracycline; Trimethoprim	Compounds belong to a specific drug class (antibiotics)	Ibáñez *et al.* (2009)

Study	Tested	Location	Number of Compounds Analysed	Compounds Analysed	Justification for Compounds Selection	Reference
The removal of pharmaceuticals, personal care products, endocrine disruptors and illicit drugs during wastewater treatment and its impact on the quality of receiving waters	Influent and effluent of WWTP, Surface water	UK	37	5-Aminosalicylic; Amitriptyline; Aspirin; Atenolol; Bendroflumethiazide; Bezafibrate; Carbamazepine; Chloramphenicol; Cimetidine; Clofibric; Codeine; Diclofenac; Digoxigenin; Digoxin; Diltiazem; Erythromycin-H2O; Furosemide; Gabapentin; Ibuprofen; Ketoprofen; Mefenamic acid; Metoprolol; Metronidazole; Naproxen; Paracetamol; Pravastatin; Propranolol; Ranitidine; Salbutamol; Salicylic acid; Simvastatin; Sulfamethoxazole; Sulfapyridine; Sulfasalazine; Tramadol; Trimethoprim; Valsartan	*Not explicitly mentioned*	Kasprzyk-Hordern *et al.* (2009)
The occurrence of antihistamines in sewage waters and in recipient rivers	Influent and effluent of WWTP	Finland	6	Acrivastine; Cetirizine; Desloratadine; Ebastine; Fexofenadine; Loratadine	Compounds belonging to a specific drug class (antihistamines)	Kosonen and Kronberg (2009)
Determination of pharmaceutical compounds in hospital effluents and their contribution to wastewater treatment works	Hospital wastewater	Norway	40	Acetylcysteine; Amitriptyline; Atenolol; Atorvastatin; Bezafibrate; Carbamazepine; Carisoprodol; Citalopram; Clofibric acid; Clotrimazole; Diclofenac; Doxazosin; Fenoprofen; Fluoxetine; Flurbiprofen; Furosemide; Gemfibrozil; Glucuronated paracetamol; Ibuprofen; Indomethacine; Ketoprofen; Mefenamic acids; Metformin; Metoprolol; Naproxen; Nifedepine; Nimesulide; Nortriptyline; Papaverine; Paroxetine; Phenacetin; Phenazone; Pravastatin; Propranolol; Sertraline; Simvastatin; Spiramycin; Tamoxifen; Warfarin	*Not explicitly mentioned.* Compounds are covering a range of therapeutic classes	Langford and Thomas, 2009
Occurrence of pharmaceuticals in Taiwan's surface waters: Impact of waste streams from hospitals and pharmaceutical production facilities	wastewater and surface water	Taiwan	21	Ampicillin; Chlortetracydine; Diclofenac; Erythromycin-H2O; 17α-Ethinylestradiol; 17β-Estradiol; Estrone; Gemfibrozil; Ibuprofen; Ketoprofen; Naproxen; Oxytetracycline;Paracetamol; Paracetamol; Penicillin; Propranolol; Sulfadimethoxine; Sulfamethazine; Sulfamethoxazole; Sulfamonomethoxine; Tetracycline; Tylosin	Commonly used human and veterinary pharmaceuticals	Lin and Tsai. (2009)
Preliminary screening of small-scale domestic wastewater treatment systems for removal of pharmaceutical and personal care products	Effluent of onsite household secondary wastewater treatment systems	Denmark	10	Caffeine; Ca-ibuprofen; Carbamazepine; Diclofenac; Furosemide; Ibuprofen; Ketoprofen; Naproxen; OH-ibuprofen;Salicylic acid	*Not explicitly mentioned*	Matamoros *et al.* (2009)
Environmental exposure of pharmaceuticals and musk fragrances in the Somes River before and after upgrading the municipal wastewater treatment plant Cluj-Napoca, Romania	Surface water	Romania	7	Caffeine; Carbamazepine; Cyclophosphamide; Galaxolide; Ibuprofen; Pentoxifillin; Tonalide	Compound were selected based on consumption at the regional scale, reported aquatic toxicity, and the suitability of the gas chromatography/mass spectrometry (GC/MS) method for the determination of the compounds at trace levels	Moldovan *et al.* (2009)
Fate and distribution of pharmaceuticals in wastewater and sewage sludge of the conventional activated sludge (CAS) and advanced membrane bioreactor (MBR) treatment	Influent and effluent of full-scale MBR and CAS systems	Spain	26	Atenolol; Bezafibrate; Carbamazepine; Diclofenac; Erythromycin; Famotidine; Fluoxetine; Gemfibrozil; Glibenclamide; Hydrochlorothiazide; Ibuprofen; Indomethacin; Ketoprofen; Loratadine; Mefenamic acid; Metoprolol; Naproxen; Ofloxacin;Paracetamol; Pravastatin; Propranolol; Propyphenazone; Ranitidine; Sotalol; Sulfamethoxazole; Trimethoprim	Compounds are representative of various therapeutic groups; Variety of physicochemical properties.	Radjenovic *et al.* (2009)

Study	Tested	Location	Number of Compounds Analysed	Compounds Analysed	Justification for Compounds Selection	Reference
Multi-residue analytical method for the determination of emerging pollutants in water by solid-phase extraction and liquid chromatography–tandem mass spectrometry	Drinking water, surface water and wastewater	Spain	15	Atenolol; Bezafibrate; Carbamazepine; Clofibric acid; Diclofenac; Fenoprofen; Ibuprofen; Indomethacine; Ketoprofen; Naproxen; Phenazone; Propanolol; Propyphenazone; Salbutamol; Salicylic acid	Compounds are representative of various therapeutic groups	Rodil *et al.* (2009)
Occurrence of pharmaceutically active compounds during 1-year period in wastewaters from four wastewater treatment plants in Seville (Spain)	Influent and effluent of WWTP	Spain	6	Caffeine; Carbamazepine; Diclofenac; Ibuprofen; Ketoprofen; Naproxen	*Not explicitly mentioned*	Santos *et al.* (2009)
The occurrence of antibiotics in an urban watershed:from wastewater to drinking water	Hospital effluents, influent and effluent of WWTPs, surface water and a drinking water	Australia	28	Amoxicillin; Bacitracin; Cefaclor; Cephalexin; Chlortetracycline; Ciprofloxacin; Clindamycin; Cloxacillin; Doxycycline; Enrofloxacin; Erythromycin; Erythromycin-H20; Lincomycin; Monensin; Nalidixic; Norfloxacin; Oleandomycin; Oxytetracycline; Penicillin G; Penicillin V; Roxithromycin; Salinomycin; Sulfamethoxazole; Sulfasalazine; Sulfathiazole; Tetracycline; Trimethoprim; Tylosin	Compounds belong to a specific drug class (*i.e.* antibiotics) and listed as key contaminants nationally by the therapeutic and goods administration, TGA)	Watkinson *et al.* (2009)
Mass flow of X-ray contrasts media and cytostatics in hospital wastewater	Hospital wastewater	Germany	9	Diatrizoate; Difluorodeoxyuridine (Dfdu); 5-Fluorouracil; Gemcitabine; Iohexol; Iomeprol; Iopamidol; Iopromide; Ioxitalamic acid	Compounds belong to specific therapeutic classes (X-ray contrast media and Cytostatic)	Weissbrodt *et al.* (2009)
Pharmaceutical residues in wastewater treatment works effluents and their impact on receiving river water	Influent and effluent of WWTP, Surface water	UK	10	Carbamazepine; Diclofenac; Indomethacine; Meberverine; Meclofenamic Acid; Monensin; Propranolol; Sulfamethoxazole; Tamoxifen; Thioridazine	Compound selected based on their high risk characterisation ratio, quantity of chemicals used per year, reported occurrence worldwide, and availability of an analytical method	Zhou *et al.* (2009)
Occurrence and removal of pharmaceuticals in a municipal sewage treatment system in the south of Sweden	Influent and effluent of WWTP.	Sweden	13	Ciprofloxacin; Clofibric acid; Diclofenac; 17α-Ethinylestradiol; 17β-Estradiol; Estrone; Fluoxetine; Ibuprofen;4-Isobutylacetophenone; Naproxen; Norfloxacin; Norfluoxetine; Ofloxacin	Compounds listed as priority compounds nationally and represent a range of physico chemical properties	Zorita *et al.* (2009)

Table SI 25. Top 20 pharmaceuticals used in 2004 in the UK, France and Australia.

	United Kingdom *Watts et al., 2007* 2004				France *Besse et al., 2008* 2004				Australia Khan and Ongerst, 2004 2004			
Rank	**Name**	**Drug class**	**mass amount (AI in kg)**	**g per inhabitant**	**Name**	**Drug class**	**mass amount (AI in kg)**	**g per inhabitant**	**Name**	**Drug class**	**mass amount (AI in kg)**	**g per inhabitant**
1	**Paracetamol**	Analgesic	3,534,737	59.075	**Paracetamol**	Analgesic	3,303,077	53.796	**Paracetamol**	Analgesic	295,882	14.720
2	**Metformin**	Antidiabtetic	497,753	8.319	**Metformin**	Antidiabtetic	716,858	11.675	**Metformin**	Antidiabtetic	90,878	4.521
3	**Ibuprofen**	Analgesic	330,292	5.520	**Troxerutin**	Phlebotropic	444,339	7.237	**Lactulose**	Laxative	88,099	4.383
4	**Aspirin**	Analgesic	177,623	2.969	**Aspirin**	Analgesic	396,212	6.453	**Amoxycillin**	Antibiotic	46,204	2.299
5	**Amoxycillin**	Antibiotic	141,287	2.361	**Diosmin**	Phlebotropic	373,544	6.084	**Ranitidine**	H2 Antagonist	33,724	1.678
6	**Valproci acid**	Anticonvulsant	72,953	1.219	**Amoxycillin**	Antibiotic	333,223	5.427	**Cephalexin**	Antibiotic	25,408	1.264
7	**Mesalazine**	Anti Inflammatory	65,088	1.088	**Ibuprofen**	Analgesic	240,024	3.909	**Naproxen**	Anti Inflammatory	22,850	1.137
8	**Sulfasalazine**	Anti Inflammatory	61,414	1.026	**Carbocistein**	Mucolytic	232,308	3.784	**Valproic acid**	Antiepileptic	20,889	1.039
9	**Flucloxacillin**	Antibiotic	57,551	0.962	**Valproci acid**	Antiepileptic	112,162	1.827	**Aspirin**	Analgesic	20,389	1.014
10	**Carbamazepine**	Antiepileptic	52,245	0.873	**Acetylcystein**	Mucolytic	96,759	1.576	**Gemfibrozil**	Hypolipidemic agent	20,042	0.997
11	**Atenolol**	Beta blocker	49,547	0.828	**Fenofibrate**	Lipid regulating	85,670	1.395	**Allopurinol**	Enzyme inhibitor	19,168	0.954
12	**Erythromycin**	Antibiotic	48,654	0.813	**Allopurinol**	Enzyme inhibitor	54,247	0.884	**Sulfasalazine**	Anti Inflammatory	17,998	0.895
13	**Gabapentin**	Anticonvulsant	48,468	0.810	**Dextropropoxyphene**	Analgesic	51,963	0.846	**Ibuprofen**	Analgesic	14,196	0.706
14	**Ranitidine**	H2 Antagonist	48,087	0.804	**Buflomedil**	Anti-ischaemic	50,968	0.830	**Chlorothiazide**	Diuretic	12,181	0.606
15	**Codeine**	Analgesic	42,198	0.705	**Naftidrofuryl**	Anti-ischaemic	45,523	0.741	**Quinine**	Antimalarial	11,670	0.581
16	**Povidone-Iodine**	Antibacterial	37,935	0.634	**Benfluorex**	Lipid regulating	40,730	0.663	**Erythromycin**	Antibiotic	10,971	0.546
17	**Salicylic acid**	Kerotic agent	36,573	0.611	**Pristinamycin**	Antibiotic	39,855	0.649	**Cefaclor**	Antibiotic	10,463	0.521
18	**Diclofenac**	Anti Inflammatory	35,361	0.591	**Naproxen**	Anti Inflammatory	37,332	0.608	**Carbamazepine**	Antiepileptic	9,975	0.496
19	**Naproxen**	Anti Inflammatory	33,580	0.561	**Metronidazole**	Antiprotozoal	36,545	0.595	**Verapamil**	Calcium channel blockers	9,786	0.487
20	**Dextro-propoxyphene**	Analgesic	32,820	0.549	**Carbamazepine**	Anti-epileptic	33,514	0.546	**Moclobemide**	Antidepressant	9,457	0.470

GLOSSARY

ABR:	Antibiotic resistant bacteria
ADI:	Acceptable daily intake
AF:	Assessment factor
API:	Pharmaceutically active ingredient
AWTP:	Advanced Water Treatment plant
CAB:	Caboolture Hospital
CDS:	Calibration Dichotomous Susceptibility
CEC:	Critical environmental concentration
CLSI:	Clinical Laboratory Standard Institute
CR:	Concentration ratio
DDmin:	Minimum daily dose
DNA:	Deoxyribonucleic acid
DWEL:	Drinking water equivalent level
EC_{50}:	Median effective concentration
EIC:	Expected Introduction concentration.
EMA:	European Medicines Agency
EPHC:	The Environment Protection and Heritage Council
ESBL:	Extended spectrum beta-lactamase
FPM:	Fish plasma model
$F_{ss}PC$:	Fish steady state plasma concentration
GU:	Griffith University
HTPC:	Human therapeutic plasma concentration
HWW:	Hospital wastewater
IPS:	Ipswich hospital
LC_{50}:	Median Lethal concentration
LOAEL:	Lowest observed adverse effect levels
LOEC:	Lowest observable effect concentration
LOQ:	Limit of quantification
MDR:	Multi drug resistant
MEC:	Measured environmental concentration
MEEC:	Maximum expected environmental concentration
MIC:	Minimum inhibitory concentration
MLVA:	Multi-locus variable number tandem repeat analysis
mPEC:	marketing data-based Predicted environmental concentration.
MRSA:	Methicillin resistant Staphylococcus aureus
MTD:	Minimum therapeutic dose
NHMRC:	National Health and Medical Research Council
NOAEL:	No observed adverse effect levels
NRMMC:	Natural Resource Management Ministerial Council
OTC:	Over the counter
PA:	Princess Alexandra
PBS:	Phosphate-buffered saline
PC:	The Prince Charles Hospital
PCR:	Polymerase chain reaction
PEC:	Predicted environmental concentration.
PNEC:	Predicted no effect concentration
QEII:	Queen Elizabeth II Jubilee
QSAR:	Quantitative structure activity relationship
QSPR:	Quantitative Structure Property Relationships
QUU:	Queensland Urban Utilities
RAPD-PCR:	Random amplified polymorphism-PCR
RBWH:	The Royal Brisbane and Women's Hospital
RQ:	Risk quotient
SEQ:	South East Queensland
STP:	Sewage treatment plant
STP:	Sewage treatment plant
STPinf:	(Raw) Influent of Sewage treatment plant
UPGMA:	Unweighted pair-group method with arithmetic averages
UQ:	Queensland University
USC:	University of the Sunshine Coast
uspA:	Universal stress protein A
UWSRA:	Urban Water Security Research Alliance
VRSA:	Vancomycin resistant Satphylococcus aureus
WWTP:	Waste water treatment plant.

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